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11th European Conference of Apidology

7-10 September 2026
BOLOGNA, ITALY

EurBee11

Like foraging bees
We seek novelty, we need cooperation and we profit from diversity

Abstract Book

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Like foraging bees
We seek novelty, we need cooperation and we profit from diversity

Abstract Book

Edited by Antonio Nanetti, Piotr Medrzycki and Antonella Pedroni
Coordinated by Anna Ricci

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WELCOME TO THE EURBEE11 CONFERENCE!

On behalf of the Local Organising Committee

Antonio Nanetti
CREA Research Centre for Agriculture and Environment
President of EurBee 11

Fabio Sgolastra
Department of Agricultural and Food Sciences – University of Bologna
Vice-coordinator of EurBee 11

Dear colleague,

It is a great pleasure and honour for us to welcome you to Bologna for EurBee 11 - the 11th European Conference of Apidology.

EurBee conferences are held every two years, and over time they have become major occasions to bring together bee scientists from Europe and beyond. EurBee 1 was held in Udine in 2004, and since then our Conference has travelled across many European countries. After a long journey, it now returns to Italy.

In those early years, the programme revolved largely around the managed honey bee. Apidology has since become a far more complex science, closely connected with other scientific and technological fields and increasingly attentive to wild bees and to the ecological questions that surround them. We have tried to make EurBee 11 reflect this shift. The Conference is built around ten scientific topics, which we reformulated from the traditional ones to match where our discipline stands today: the new challenges and threats we face, and the growing weight of research on wild bees. For the first time in the history of EurBee, the programme also includes a topic devoted to *Ethics in bee science*. It has attracted only a few contributions, but we are convinced it will matter a great deal in the years to come. In addition, EurBee 11 adopts a Code of Conduct, as a way of stating plainly the respectful, welcoming and inclusive spirit we want this Conference to have.

This edition carries another first. For the first time, two apidological institutions of global standing, born in the same city and grown along parallel paths, have joined forces to organise the Conference: the apidology group of the CREA Research Centre for Agriculture and Environment and the Department of Agricultural and Food Sciences of the University of Bologna.

The response has gone beyond our expectations. Over 500 contributions have been received, approximately 200 of them chosen for oral presentation, by authors not only from across Europe, but from all continents. Each morning and afternoon opens with a plenary keynote lecture on a subject of particular relevance, given by a distinguished specialist and followed by four parallel oral sessions. The one exception is the second afternoon, which we have set aside for a social visit of the city.

All of this is gathered in the motto we chose for EurBee 11: *Like foraging bees. We seek novelty, we need cooperation and we profit from diversity*. As in a colony, knowledge moves forward only when different minds and disciplines work together, and the best discoveries come precisely from that variety of backgrounds and points of view.

We are sure you will enjoy Bologna, the city home to the most ancient University of Europe. Its convivial, easy-going spirit, together with the art, the music, the culture, and the food the city offers in abundance, makes it the perfect place to reward long days of intellectual work with evenings you will remember.

Finally, we warmly thank everyone who has made EurBee 11 possible: the institutions, the many people who worked behind the scenes, our sponsors and collaborators, the EurBee members who elected our venue for this Conference, and all EurBee 11 participants, whose commitment keeps this community alive.

Benvenuti a Bologna!

CODE OF CONDUCT

1. Our commitment

The 11th European Conference of Apidology (EurBee 11) is committed to providing a safe, welcoming, and intellectually stimulating environment for all participants. This commitment is grounded in the values enshrined in the **Universal Declaration of Human Rights** and in the **European Charter for Researchers**, which uphold the dignity, freedom, and equal worth of every person, and call for integrity, transparency, and respect in all scientific activity.

We believe that the best science emerges from communities where every voice is heard and every participant feels valued. This Code of Conduct reflects that belief.

It applies to all EurBee 11 participants — attendees, speakers, poster presenters, session chairs, organisers, volunteers, sponsors, and venue staff — and covers all conference activities: plenary and parallel sessions, poster sessions, workshops, social events, field excursions, and any other activities associated with the conference.

2. Our shared values

By registering for and participating in EurBee 11, we each commit to upholding the following principles:

2.1. Inclusion and respect for all

We welcome and celebrate the diversity of our community. Every participant deserves to be treated with courtesy, dignity and respect, regardless of age, career stage, disability, ethnicity, gender identity or expression, nationality, physical appearance, race, religion, socioeconomic background, sexual orientation or any other personal characteristic.

We actively contribute to a conference atmosphere in which all participants — particularly those from historically underrepresented groups — feel genuinely included and heard. We pay thoughtful attention to accessibility and help create conditions that allow everyone to engage fully.

2.2. Scientific integrity and open dialogue

In the spirit of the European Charter for Researchers, we commit to the highest standards of scientific honesty and integrity. We present our work truthfully, acknowledge the contributions of others, and engage critically but respectfully with ideas that differ from our own.

We welcome diverse scientific perspectives and career stages. We encourage questions, discussion and healthy debate. We listen as carefully as we speak.

2.3. Professionalism in all interactions

We communicate in ways that are constructive, thoughtful, and appropriate to a professional scientific gathering — in sessions, at social events, in written communications, and on social media. We extend this standard of professionalism to every person we encounter at the conference, regardless of their role.

Conference presentations and discussions are scientific spaces. Participants are asked to keep contributions focused on scientific content and to refrain from using the conference platform to promote personal political, religious, ideological positions or commercial products and services (unless specifically authorised under the conference's sponsorship plan).

2.4. Responsibility toward our community

We each bear a share of responsibility for the quality of our shared environment. If we witness behaviour that makes others feel unsafe or excluded, we are encouraged to act — whether by directly and calmly addressing the situation if we feel comfortable doing so, or by alerting a conference organiser.

2.5. Compliance with local rules and laws

We respect the rules of all conference venues and abide by applicable local laws and regulations at all times.

3. Behaviours that harm our community

While we prefer to lead with what we aspire to, we also name clearly the behaviours that are incompatible with these values, so that all participants know what is not acceptable and feel confident reporting it.
The following behaviours will not be tolerated at EurBee 11:

- **Deliberate exclusion** of individuals from scientific conversations, networking, or social activities on discriminatory grounds;
- **Disruptive behaviour** during presentations, discussions, or other conference activities;
- **Harassment or discrimination** of any kind, including on the basis of any of the characteristics listed above;
- **Intimidation, threats, or bullying**, whether verbal, physical, or online;
- **Misrepresentation** of one's own work or the work of others, or any form of scientific misconduct;
- **Sexual harassment**, including unwelcome advances, inappropriate physical contact, or sexually suggestive remarks, images or jokes.

4. Reporting a concern

We ask every participant to help us maintain the standards described here. If you experience or witness behaviour that violates this Code of Conduct, please report it — your report matters, and it helps protect others. You can reach out to the EurBee11 Local Organising Committee[1] at any time during the conference, either directly (e.g. via the Registration Desk) or by email.
All reports will be treated seriously, handled with discretion, and investigated promptly. The identity of the person reporting will be protected to the greatest extent possible. **Retaliation against anyone who raises a concern in good faith is itself a violation of this Code of Conduct and will be treated as such.**
If you are ever in immediate danger, please contact local emergency services immediately (European emergency number: 112)

4.1 How we respond

The EurBee 11 Organising Committee will review all reported concerns and take appropriate action. Our goal is always to restore safety and inclusion for those affected. Depending on the severity and nature of the incident, responses may include:

- A private conversation with the person whose behaviour was reported
- A formal warning
- Removal from one or more conference activities

5. Acknowledgement

This Code of Conduct draws inspiration from established models in the scientific community and from the principles of the **Universal Declaration of Human Rights** (United Nations, 1948) and the **European Charter for Researchers** (European Commission, 2025, doi: 10.2777/5214248), both of which affirm the right of every person to participate in scientific and public life with dignity and without discrimination.
We are grateful to everyone who helps make EurBee 11 a conference where great science and genuine humanity go hand in hand.

[1]Antonio Nanetti *antonio.nanetti@crea.gov.it*
Fabio Sgolastra *fabio.sgolastra2@unibo.it*
Daniele Alberoni *daniele.alberoni@unibo.it*
Cecilia Costa *cecilia.costa@crea.gov.it*
Luca Fontanesi *luca.fontanesi@unibo.it*
Manuela Giovanetti *manuela.giovanetti@crea.gov.it*
Serena Magagnoli *serena.magagnoli4@unibo.it*
Piotr Medrzycki *piotr.medrzycki@crea.gov.it*

LOCAL ORGANISING COMMITTEE

NAME	COUNTRY	INSTITUTION
Antonio Nanetti	IT	CREA Research Centre for Agriculture and Environment, Bologna
Fabio Sgolastra	IT	University of Bologna
Daniele Alberoni	IT	University of Bologna
Cecilia Costa	IT	CREA Research Centre for Agriculture and Environment, Bologna
Luca Fontanesi	IT	University of Bologna
Manuela Giovanetti	IT	CREA Research Centre for Agriculture and Environment, Bologna
Serena Magagnoli	IT	University of Bologna
Piotr Medrzycki	IT	CREA Research Centre for Agriculture and Environment, Bologna

SCIENTIFIC BOARD

NAME	COUNTRY	INSTITUTION
Petr Bogusch	CZ	University of Hradec Králové
Gennaro Di Prisco	IT	National Research Council, Naples
Edy Fantinato	IT	Ca' Foscari University of Venice
Anna Gajda	PL	Institute of Veterinary Medicine, Warsaw
Manuela Giovanetti	IT	CREA Research Centre for Agriculture and Environment, Bologna
Fabio Manfredini	IT	'La Statale' University of Milan
Francesco Nazzi	IT	University of Udine
Maria Alice Pinto	PT	Instituto Politécnico de Bragança
Soledad Sagastume De Andrés	ES	Centro de Investigación Apícola y Agroambiental, Marchamalo
Fabio Sgolastra	IT	University of Bologna
Thomas Tscheulin	GR	University of the Aegean, Mytilene
Iratxe Zarraonaindia	ES	University of the Basque Country, Bilbao

Organisers:

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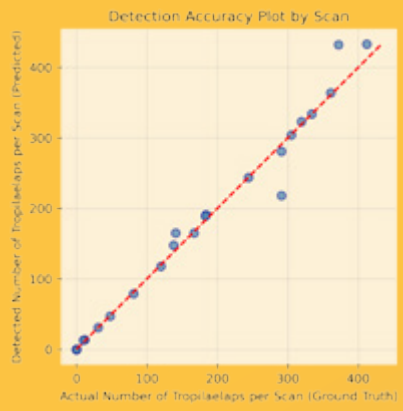
TROPI ALARM

AI-powered non-invasive monitoring of Tropilaelaps mites in honey bee colonies

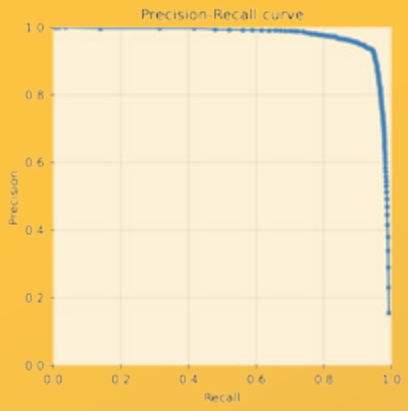
Western apiculture is currently facing a new threat, posed by the westward spread of the parasitic mite Tropilaelaps mercedesae. The rapid reproductive cycle of this mite and the severe damage it causes to developing brood make early detection and monitoring essential for effective management and biosecurity. Until now, identifying Tropilaelaps has relied on colony inspection and expert examination, which are not always feasible. Tropi Alarm changes this: it is a scanner that automatically detects and quantifies Tropilaelaps mercedesae mites fallen onto the sticky sheets placed on hive bottom trays — making automated detection of Tropilaelaps presence possible for the first time.



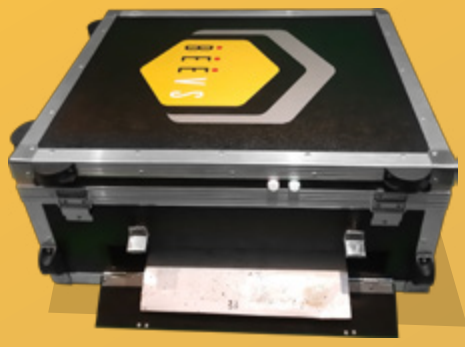
Photo of Irakli Janashia, researcher at Agricoltural University of Georgia, during the collection phase of the sticky sheets for the neural network training scans.



Each point is one sticky monitoring sheet of the test set: the horizontal axis shows the number of Tropilaelaps mites actually present on the sheet (ground truth), the vertical axis the number detected by the model. Points fall along the ideal 1:1 line, showing that detected counts closely match the true counts across the full range of infestation levels. Tropilaelaps presence was detected in 100% of the infested scans. The 16 control sheets — collected from an apiary in Italy where Varroa and hive debris are present but Tropilaelaps is absent — all sit at the origin: no false alarm was raised on a single Varroa-and-debris sheet.



This curve summarises detection reliability on the test set at the level of individual mites as the model's confidence threshold is varied. Recall (horizontal axis) measures how many of the real Tropilaelaps mites are found; precision (vertical axis) how many of the flagged objects are truly Tropilaelaps rather than debris. The model keeps high precision while recovering the large majority of mites (area under the curve ≈ 0.97; best F1 ≈ 0.94 at 0.93 precision and 0.94 recall), meaning the mites it flags are reliably real — the same reliability seen on the control sheets, now measured mite by mites.





Example of a high-resolution image and the recognition result of tropilaelaps and varroa mite.

Tropi Alarm is the evolution of the Bee Varroa Scanner (BeeVS), developed by Apisfero A.P.S. for the automated detection of Varroa destructor. Because Tropilaelaps is smaller than Varroa, the scanner now acquires images at higher resolution; on top of this, a completely new detection algorithm, dedicated to Tropilaelaps, runs alongside the existing Varroa algorithm, which remains fully effective in the new version. The Tropilaelaps model is trained, validated and tested on images of sticky sheets collected from two sources: infested colonies in Georgia, where the parasite is currently present, and colonies in Italy, where it is absent. The Georgian sheets provide naturally fallen Tropilaelaps mites, while the Italian sheets contribute Tropilaelaps-free material carrying Varroa and hive debris. High-resolution scans are systematically annotated, and the dataset captures the variability of field monitoring — mite appearance orientation, debris composition and illumination. By making non-invasive monitoring practical, Tropi Alarm enables beekeepers and authorities to detect Tropilaelaps early and intervene promptly should it appear in areas currently free of the parasite.

Tropi Alarm is part of the GENAPIS.IT.4 project, coordinated by CREA with the support of the associations FAI – Federazione Apicoltori Italiani e Miele in Cooperativa..
The GENAPIS.IT project is funded by the Ministry of Agriculture, Food Sovereignty, and Forests as part of the implementation of EU Regulation 2021/2115.

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*ITSAP-Institut de l'abeille. (2023). Piégeage des fondatrices au printemps: Attractivité et sélectivité de dispositifs de piégeage – Dynamique de capture.
Rein, C. (2025). Bericht über die Versuche zu selektiven Fallen zum Fang der Asiatischen Hornisse (Vespa velutina nigrithorax) im Frühjahr 2025 in Baden-Württemberg. Landesanstalt für Bienenkunde, Universität Hohenheim.

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PROGRAMME OVERVIEW

7 SEPTEMBER

- **16:30-21:00** Registration and Information Desk
- **17:30-19:30** Opening Ceremony
- **19:30-21:00** Welcome Aperitif

8 SEPTEMBER

- **8:30-18:00** Registration and Information Desk
- **9:00-9:45 Keynote:** Fanny Mondet 'Honey bee health in the context of biological invasions: the case of *Varroa destructor*'
- **9:45-10:45** Parallel Sessions
  - **Symposium 1** Welfare in bees and ONEHEALTH
  - **Symposium 2** Technological applications for the bee science & Bee-based monitoring networks
  - **Symposium 3** Plasticity and genomic variation to overcome threats
  - **Symposium 4** Microbiome and nutrition
- **10:45-11:15** Coffee Break
- **11:15-13:00** Parallel Sessions
  - **Symposium 1** Welfare in bees and ONEHEALTH
  - **Symposium 2** Technological applications for the bee science & Bee-based monitoring networks
  - **Symposium 3** Plasticity and genomic variation to overcome threats
  - **Symposium 4** Microbiome and nutrition
- **13:00-14:30** Lunch Break
- **14:30-15:15 Keynote:** Domenica Auteri 'Safeguarding bees in the EU: Regulated risk assessment of pesticides and evolving policy'
- **15:15-16:00** Parallel Sessions
  - **Symposium 5** Welfare in bees and ONEHEALTH
  - **Symposium 6** Climate change
  - **Symposium 7** Decline of bee populations and policies
  - **Symposium 8** Microbiome and nutrition
- **16:00-16:30** Coffee Break
- **16:30-17:45** Parallel Sessions
  - **Symposium 5** Welfare in bees and ONEHEALTH
  - **Symposium 6** Climate change
  - **Symposium 7** Decline of bee populations and policies
  - **Symposium 8** Microbiome and nutrition
- **17:45-18:30** EurBee General Assembly

9 SEPTEMBER

- **8:30-16:00** Registration and Information Desk
- **9:00-9:45 Keynote:** Björn K. Klatt 'The 4th agricultural revolution – Rise of the bees?'
- **9:45-10:45** Parallel Sessions
  - **Symposium 9** Welfare in bees and ONEHEALTH
  - **Symposium 10** Technological applications for the bee science & Bee-based monitoring networks
  - **Symposium 11** Plasticity and genomic variation to overcome threats
  - **Symposium 12** Agroecology
- **10:45-11:15** Coffee Break
- **11:15-13:00** Parallel Sessions
  - **Symposium 9** Welfare in bees and ONEHEALTH
  - **Symposium 10** Technological applications for the bee science & Bee-based monitoring networks

- **Symposium 11** Plasticity and genomic variation to overcome threats
- **Symposium 12** Agroecology

- **13:00-14:30** Lunch Break

- **14:30-15:00** Poster session, presenting authors at their poster

- **15:00-15:45 Keynote:** Jordi Bosch 'Solitary bees in a toxic world'

- **15:45-16:00** Group Photo

- **Afternoon:** Guided Tour of Bologna (exact time and arrangements will be announced during the Conference)

10 SEPTEMBER

- **8:30-13:00** Registration and Information Desk

- **9:00-9:45 Keynote:** Janez Prešern '(Dis)Enchantment in digitalization in beekeeping'

- **9:45-10:45** Parallel Sessions
  - **Symposium 13** Welfare in bees and ONEHEALTH
  - **Symposium 14** Technological applications for the bee science & Bee-based monitoring networks
  - **Symposium 15** Plasticity and genomic variation to overcome threats
  - **Symposium 16** Relationship between managed and wild bees

- **10:45-11:15** Coffee Break

- **11:15-13:00** Parallel Sessions
  - **Symposium 13** Welfare in bees and ONEHEALTH
  - **Symposium 14** Technological applications for the bee science & Bee-based monitoring networks
  - **Symposium 15** Plasticity and genomic variation to overcome threats
  - **Symposium 16** Relationship between managed and wild bees

- **13:00-14:30** Lunch Break

- **14:30-15:15 Keynote:** Oliver Schweiger 'How climate change threatens wild bees in manifold ways'

- **15:15-16:00** Parallel Sessions
  - **Symposium 17** Welfare in bees and ONEHEALTH
  - **Symposium 18** Climate change
  - **Symposium 19** Decline of bee populations and policies
  - **Symposium 20** Bee products, ecosystemic services and circular economy

- **16:00-16:30** Coffee Break and Poster session, presenting authors at their poster

- **16:30-17:45** Parallel Sessions
  - **Symposium 17** Welfare in bees and ONEHEALTH
  - **Symposium 18** Climate change
  - **Symposium 19** Decline of bee populations and policies
  - **Symposium 20** Bee products, ecosystemic services and circular economy

- **17:45-18:00** Closing Ceremony

- **20:00-24:00** Gala Dinner



ABSTRACTS



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Keynote speakers



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NAME	COUNTRY	INSTITUTION
Domenica Auteri	IT	EFSA, Parma
Jordi Bosch	ES	CREAF, Centre for Ecological Research and Forestry Applications, Barcelona
Björn K. Klatt	SE	Halmstad University
Fanny Mondet	FR	INRAE, Avignon
Janez Prešern	SI	Agricultural Institute of Slovenia, Ljubljana
Oliver Schweiger	DE	Department Community Ecology, Helmholtz Centre for Environmental Research, Halle



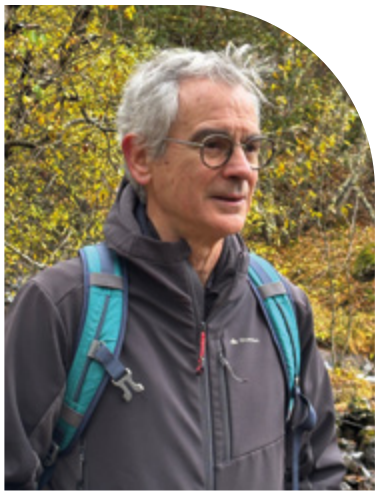
DOMENICA AUTERI

European Food Safety Authority (EFSA), Parma, Italy

She is a biologist with a post-graduate degree in (eco)toxicology. She has been working in the field of environmental risk assessment of pesticides for more than 30 years. She joined the European Food Safety Authority (EFSA) in 2008 as ecotoxicologist and, since 2011, she has been leading the team responsible for the evaluation of the risk of pesticides to non-target organisms and for the development of guidance documents. Among her activities, she has worked on the review of the risk assessment for bees of three neonicotinoids and fipronil, the development of the joint ECHA-EFSA guidance document to identify endocrine disruptors, the recent revision of EFSA guidance documents for Birds and Mammals and for Bees. She is currently leading strategically important, high-budget projects to advance the environmental risk assessment of pesticides, including pollinators. Finally, she is leading the revision of terrestrial ecotoxicology guidance document, which includes non-target arthropods, in-soil organisms and terrestrial non-target plants and the development of an approach for evaluating indirect effects of pesticides.

Keynote: 'Safeguarding bees in the EU: Regulated risk assessment of pesticides and evolving policy'

Pesticides are regulated products undergoing comprehensive pre–marketing risk assessments to ensure their use does not pose unacceptable risks to human health or environment. Within the European Union, this process is governed by Regulation 1107/2009, which explicitly requires an evaluation of risks to bees and establishes that pesticides cannot be approved if they pose unacceptable risks. To perform this evaluation, EFSA developed a guidance document integrating methodologies to addresses multiple exposure pathways (contact, dietary), diverse effect type, different life stages and exposure scenarios. A pivotal aspect of the guidance was the establishment of specific protection goals: EU risk managers agreed on a maximum tolerable effect of <10% reduction in honeybee colony size. The 1107/2009 also consider reassessments when new scientific or monitoring evidence emerges. This mechanism led to the re-evaluation of neonicotinoids, resulting in a partial ban in 2013 and a complete ban in 2018. Post–authorisation use is governed by the Sustainable Use Directive, promoting Integrated Pest Management and reducing pesticide pressure. Complementary initiatives, including the EU Pollinator Initiative and new provisions under the Nature Restoration Regulation, strengthen pollinator monitoring and support long–term habitat restoration. Specifically, to implement the pollinator target, the EU adopted a Regulation, which establishes a science-based, standardised EU-wide monitoring for pollinator diversity and abundance.



JORDI BOSCH

CREAF, Centre for Ecological Research and Forestry Applications, Barcelona, Spain

Jordi Bosch is a researcher at the Centre for Ecological Research and Forestry Applications (CREAF) in Bellaterra (Barcelona, Spain). He works on solitary bee ecology. His research has enhanced the establishment of *Osmia cornuta* and *Osmia lignaria* as managed pollinators of orchard crops in Europe and North America, respectively. He has also investigated parental investment strategies and the life cycle ecophysiology of Osmia and Megachile. In the last years, his research has focussed on the sublethal effects of pesticides on solitary bee populations, and on the interactions between pesticide exposure and other stressors such as climate change and suboptimal nutrition. Jordi also works on pollinator community ecology in both natural and agricultural environments. He is studying how local and landscape factors affect taxonomic and functional diversity in pollinator communities and the structure of plant-pollinator and host-parasite interaction networks, as well as the consequences on pollination services.

Keynote: 'Solitary bees in a toxic world'

Pesticide use is one of the main drivers of bee declines. However, our knowledge on the effects of pesticide exposure on bees comes mostly from studies with the highly social western honey bee, *Apis mellifera*, even though most bee species worldwide are solitary. Social and solitary bees experience different routes and levels of exposure and may differ in sensitivity to specific pesticides and vulnerability at the population level. Therefore, to better understand the effects of pesticide exposure on the full range of bee diversity, ecotoxicological studies on solitary bees are needed. Importantly, due to fundamental differences in behaviour and life history traits, some of the ecotoxicological methodologies used with honey bees cannot be readily applied to solitary bees. This presentation will provide an overview of the ecotoxicology program currently being developed at CREAF to improve our understanding of the impact of pesticide applications on solitary bees (*Osmia* spp.). In the last years, we have been conducting laboratory, semi-field and field experiments focussing on sublethal effects, exposure to combinations of pesticides and interactions between pesticides and other stressors, such as climate change and suboptimal diet. The results of these studies have important implications for pesticide risk assessment.



BJÖRN K. KLATT

Halmstad University, School of Business, Innovation and Sustainability, Biology & Environmental Sciences, Halmstad, Sweden

Björn Klatt is a biologist and agricultural scientist interested in a framework of multiple topics related to bees and pollination. He is investigating aspects of how bee pollination benefits agricultural production but also how anthropogenic stressors, mostly land-use change, pesticides and climate change, alone and in interaction, affect bee individual fitness, communities, populations, biodiversity and pollination. Further, his research is addressing solutions to overcome these threats, often in the frameworks of agroecology and integrated pest and pollinator management, for instance how aquatic and terrestrial landscape elements support biodiversity, and how new strategies and tools can mitigate human impacts. This often requires multidisciplinary approaches as well as considering both animal and plant perspectives and not least the perspectives of farmers and authorities. Following his studies and diploma in Biology at multiple universities in Germany as well as the Museum of Natural History in Berlin and a nature reserve in Namibia, Björn Klatt conducted a PhD in agricultural sciences at the agroecology group at Göttingen university. He continued his scientific journey to Lund university in Sweden and finally to Halmstad university, where he is currently holding a position as associate professor in biology.

Keynote: 'The 4th agricultural revolution – Rise of the bees?'

Bees and pollination are essential parts of agricultural production. However, during three so-called revolutions, agriculture went through periods of development and intensification which increasingly led to negative effects on natural ecosystems. Particularly, the third 'Green Revolution' was and is fostering strong declines of biodiversity, alone and in interaction with a changing climate. Also, bees and pollination are declining, although the importance of crops that benefit from pollination is increasing and thereby agriculture is undermining its own functionality. Hence, a transformative change of agricultural production systems is needed to maintain food security and simultaneously regain biodiversity and ecosystem services – a fourth agricultural revolution. The concept of agroecology can, if applied on large scales, become a game changer for the transformative change of agriculture. However, emerging climate change will challenge even nature-based systems such as agroecology, providing potential threats to its functionality. In my presentation, I will discuss the role of bees to contribute to a fourth agricultural revolution as a system-wide change based on the concepts of agroecology. Further, I will argue for how the plasticity of different bee-systems provide the potential to adapt to the challenges to solve under climate change and potentially even to counteract its impacts on agricultural production.



FANNY MONDET

INRAE, UR 406 Abeilles et Environnement, Avignon, France

Fanny Mondet is a researcher at the French National Institute for Agriculture, Food and Environment and co-leads the Bees and Environment research group. Her research centers on honey bee pathology, with a particular focus on host–parasite interactions between honey bees and *Varroa destructor*, one of the most detrimental threats to global apiculture. Combining chemical ecology and behavioral ecology approaches, she investigates the mechanistic basis of collective defense responses in honey bee colonies confronted with invasive parasites. Her work aims to elucidate the evolutionary and physiological processes underlying natural resistance to *Varroa*, bridging fundamental and applied research. By identifying key drivers of colony resilience, her research contributes to the development of sustainable and innovative strategies to mitigate parasite impact and support beekeepers in managing this major biological threat.

Keynote: 'Honey bee health in the context of biological invasions: the case of *Varroa destructor*'

The honey bee, *Apis mellifera*, is an iconic example of a socially living species of major economic importance that is increasingly threatened by invasive species in the context of globalization. The host shift of the parasitic mite *Varroa destructor* from *Apis cerana* to *Apis mellifera* has disrupted the evolutionary balance of the host–parasite relationship, severely compromising honey bee health and challenging beekeeping worldwide. Understanding the key biological processes underlying interactions between honey bees and this devastating parasite is essential for developing sustainable strategies to control *Varroa* and safeguard both honey bee health and the beekeeping sector. In this presentation, I will describe how recent advances in our understanding of *Varroa* reproductive biology and of the mechanisms by which honey bees detect and respond to infestation within the colony open new avenues for improved parasite management. I will place particular emphasis on our latest findings regarding social immunity processes, especially hygienic behavior. Using tools from chemical ecology, we provide new insights into collective defense mechanisms operating at both the individual and colony (superorganism) levels. These findings contribute to the development of integrated pest management strategies for *Varroa* and support the transition toward more sustainable beekeeping practices.



JANEZ PREŠERN

Agricultural institute of Slovenia, Department of animal production, Ljubljana, Slovenia

Janez Prešern is the leader of the Apiculture group and head of the Department of Animal Production at the Agricultural Institute of Slovenia. As a biologist with a strong physiology background, he utilises his broad knowledge to combine the efforts of colleagues from various disciplines in efficient bee science. This work is based on two tiers: i) selection in honey bees with supporting activities and ii) feed, food and nutrition, which are linked to the performance of honey bee colonies and other pollinators and the effect of colony density. He has led several high-profile international and domestic projects, including the EEA & Norway Grants Fund for Regional Cooperation BeeConSel and ARIS SimDRONE. Within Horizon Europe project BEE-GUARDS, he leads a work package dedicated to the collection of data through various sensors, augmenting it with data from other sources, organising it, and finally piping it to the modellers for decision.

Keynote: '(Dis)Enchantment in digitalization in beekeeping'

Technological advances are crucial in addressing agricultural challenges. The so-called 'digital transition', enshrined in EU strategy and aligned with the 'Green Deal', is being implemented through various funding initiatives across daily life, government administration, industry and agriculture including beekeeping. In the apiculture technology market, products are available that promise complete hands-off colony monitoring and pre-emptive diagnosis. However, the functionality of these devices is often unclear. Users have specific expectations when investing in such technology, including the usefulness of data and the intended recipients, whether beekeepers or scientists. Furthermore, the performance of these devices in the field is a critical consideration. To address these challenges, we conducted an analysis of online services and products, coupled with a survey of users to gather feedback. The analysis revealed that many platforms offer more than beekeepers require as guidance for their decisions. Additionally, there is a dependency on the service after purchasing a device. This raises the question of whether the push towards digitalisation in the sector is excessive, creating a dependency on a different type of service and incurring additional costs. Open science, encouraged through various funding schemes and community-based development, offers a potential solution to some of these challenges.



OLIVER SCHWEIGER

Helmholtz Centre for Environmental Research – UFZ, Department Community Ecology, Leipzig, Germany

Oliver Schweiger is the head of the Working Group 'Macroecology for Animal Communities', Department Community Ecology, at the Helmholtz Centre for Environmental Research – UFZ. His research covers multiple aspects of biodiversity focusing on the structure, dynamics and functions of ecological systems across a variety of temporal and spatial scales. In particular, he seeks to understand the effects of systematic variations in environmental conditions such as that of global change on functional, phylogenetic and taxonomic aspects of species assemblages as well as on species interactions and the provisioning of ecosystem services. He works at several disciplinary levels such as population genetics and dynamics, community ecology, ecosystem functioning, and macroecology. He is particularly interested in the effects of global change on pollinators across Europe and assesses their future risks but also options for risk mitigation and pollinator restoration.

Keynote: 'How climate change threatens wild bees in manifold ways'

Climate change is one of the most important pressures for wild pollinators, particularly contributing to considerable concerns about their current declines. There are multiple ways how climate change can impact pollinators and a deeper understanding of these manifold mechanisms is indispensable for successfully mitigating the respective risks and designing adequate restoration actions. Here, I provide an overview on different modes of action of climate change. Based on my research in multiple EU-funded projects, I show that direct impacts of continuous climate change lead to considerable range retractions of wild bees, which is further exacerbated in the future, ultimately causing shifts in functional bee assemblage composition and diversity hot and cold spots across Europe. In addition to continuous change, heat waves can also lead to considerable population declines and even local extinctions of wild bees, depending on species traits. I also show indirect effects of climate change such as negative impacts on plant-pollinator networks via extinction cascades, increased bee virus pressure, or options for pathogen invasion. Finally, I provide evidence for land-based options to mitigate both direct and indirect effects of climate change on wild bees, which are particularly relevant for the current implementation of the EU Nature Restoration Regulation.

Oral communications



Agroecology

ROAD TRIP WITH BEES: EFFECTS OF TRANSPORTATION ON HONEY BEE HEALTH, BEHAVIOUR AND POLLINATION EFFICIENCY IN FINLAND

OC1

Anna-Maria Borshagovski[1], Sakari Raiskio[2]

[1]Finnish Beekeepers' Association, Helsinki, Finland.
[2]Natural Resources Institute Finland, Helsinki, Finland

Abstract

Pollination is a crucial factor in food production with a great economic impact around the globe. In Finland, pollination service is rare despite its potential to boost crop yields while giving beekeepers another source of income. Beekeepers' concern about the effects of transportation on honeybees is one factor hindering pollination service as studies on the subject are lacking in Finnish conditions.

In a pilot study of the project *Honeybee pollination as a part of security of supply (MehiVarma)*, we tested how moving honeybees for pollination affects honeybee health, behaviour and pollination efficiency. Six colonies with sister queens were assigned into control and experimental groups. We moved experimental colonies seven times within a month, transportation times varying between 10-120 minutes. After each transportation the following day, we collected data regarding colony strength, aggression, swarming tendencies, pathogens and honey yield.

Moving the colonies did not affect colony strength nor amount of brood, as well as the aggression levels nor swarming tendencies. The experimental colonies collected the same amount of nectar as the control colonies. Transportation did not increase the prevalence of European foulbrood found in half of the colonies prior to the experiment, and no American foulbrood, deformed wing virus, acute bee paralysis virus and sacbrood virus were found before and after the experiment.

Based on this pilot study, transporting honeybee colonies does not affect their-well being and pollination efficiency on a local scale pollination service in Finnish conditions. The more crucial factors affecting honeybee well-being are most likely beekeeping practices and sufficient ventilation and temperature during transportation. These findings suggest that honeybee well-being is influenced more by beekeeping practices and transport conditions than by the act of relocation itself.

OC 2

FROM SOIL TO NECTAR: HOW POTASSIUM AND PHOSPHORUS MODULATE HONEYBEE ATTRACTIVENESS

Arnon Dag

Agricultural Research Organization, Gilat, Israel

Abstract

Fertilization is a cornerstone of modern agriculture, traditionally optimized to meet the direct nutritional demands of the crop. However, the influence of soil nutrient management on the critical interface of pollination activity remains largely unexplored. This study identifies two contrasting fertilization effects on honeybee (*Apis mellifera*) attractiveness in major fruit-tree crops.

In avocado, we identified high potassium concentrations in nectar (~4,000 ppm), which significantly reduces its attractiveness to honeybees. Controlled experiments with sugar solutions confirmed this deterrent effect, while field trials on container-grown trees demonstrated that reducing potassium applications successfully increased both bee activity and subsequent fruit set and yield.

Conversely, in almond, we found that increased phosphorus fertilization during the summer improves carbohydrate conversion into nectar the following spring. This enrichment leads to higher bee recruitment, facilitating improved cross-pollination and yields.

Our findings suggest that crop nutrition plays a pivotal role in modulating floral attractiveness, highlighting the need for "pollination-aware" fertilization strategies to optimize fruit set and yield in commercial orchards

COMMERCIAL POLLINATOR ACTIVITY DRIVES PREMATURE FRUIT LOSS IN SWEET CHERRIES AND IMPACTS FRUIT YIELDS AT HARVEST

OC 3

Jane Devlin[1], Alison Karley[2], Dominic Williams[2], Roslyn Henry[1], Fabio Manfredini[3]

[1]University of Aberdeen, Aberdeen, United Kingdom
[2]James Hutton Institute, Dundee, United Kingdom
[3]Università di Milano, Milan, Italy

Abstract

In Scotland sweet cherry undergo a natural phenomenon known as June drop, a process by which immature fruit are abscised from the tree. June drop is unpredictable and can dramatically affect fruit yields. The aim of this study was to assess the role of insect pollinators (species and density) in fruit loss during June drop. Twelve time-lapse cameras were deployed in the spring of two consecutive years within two pollinator treatments containing either *Osmia* spp. or commercial *Bombus terrestris*. Higher pollinator foraging activity was found to reduce fruit drop and increase fruit retained until harvest. Additionally, pollinator exclusion treatments in both years indicated that pollination is a determining factor of the June drop phenomenon. Open pollinated flower clusters lost 83.2% of all set fruit in 2024 and 70.1% in 2025, compared to control flowers that were made inaccessible to pollinators – which lost 100% of all developing fruit in both years. Furthermore, the diameter of developing fruit was indicative of impending fruit loss, with fruit below 6 mm having a 100% chance of loss. Finally, cherry flowers exposed to the higher foraging activity exhibited by *B. terrestris* were less likely to be impacted by June drop. Our results show that by maintaining higher numbers of foraging pollinators in cherry orchards farmers can reduce loss and improve yield stability. Furthermore, cherry fruit size can be used as an early predictor of upcoming fruit loss and yield. We are currently in the process of analysing RNA sequencing data from individual styles collected from cherry flowers that were hand-pollinated and then sampled at multiple time-points: 24, 48, 72, 96, 120 and 144 hours after pollination. These results will provide insights on what key molecular pathways are triggered by this process, with the potential to identify biomarkers for the early detection of successful pollination.

OC 4

ASSESSING THE EFFECTIVENESS OF MITIGATION MEASURES IN VINEYARDS AND APPLE ORCHARDS BY A MULTI-ENDPOINT APPROACH APPLIED ON WILD POLLINATORS (ÆM-POLLYPROJECT)

Agata Di Noi[1], Tommaso Campani[1], Ilaria Caliani[1], Laura Giovanetti[1], Elena Johnson Gittins[2], Mariana P. Mazzei[3], Lisa Cabiddu[4], Lucia Lenzi[4], Francesco Lami[4], Fabio Sgolastra[4], Silvia Casini[1]

[1]Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy
[2]Department of Toxicology, Wageningen University and Research, Netherlands
[3]Instituto de Investigaciones en Ciencias Agrarias de Rosario, IICAR-CONICET-Universidad Nacional de Rosario, Zavalla, Santa Fe, Argentina
[4]Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

Abstract

The honey bee colony losses reported worldwide over recent decades have drawn public attention, yet they mask a broader crisis: the decline in abundance and diversity of wild pollinators. EU Green Deal promotes initiatives aimed at reversing this trend through mitigation measures for pollinators, however critical data gaps persist regarding pollinator status, decline drivers, and ecosystem impacts, underscoring the need to assess the effectiveness of these measures. The ÆM-POLLY project developed and validated an innovative integrated monitoring protocol to assess whether Green Deal agricultural practices are effective. This protocol combines endpoints for presence, abundance, and diversity of wild pollinators with health indicators at sub-individual, individual, and population levels. These endpoints,unprecedented for wild pollinators, detect biological alterations from key pressures: pesticide-induced chemical stress, climate change, food/water shortages, habitat loss, and diseases. The protocol was applied in Emilia-Romagna and Tuscany (Italy) during spring and summer 2024. In each region, two macro-areas were selected, containing three ecosystem types: (I) natural ecosystems; (II) agroecosystems with mitigation measures, featuring flower strips and reduced pesticide use; and (III) agroecosystems without measures. Apple orchards were selected in Emilia-Romagna, vineyards in Tuscany. Orchards showed significantly higher butterfly abundance in agroecosystems with mitigation measures; vineyards exhibited increased taxonomic diversity of butterflies and hoverflies. Significant trends were detected for bee risk of contamination, across ecosystems and regions. Natural areas showed similarly low risk levels to areas with mitigation measures and differed from areas without measures. Biomarkers were successfully applied to *Apis mellifera, Bombus terrestris, Sphaerophoria scripta*. Mitigation measures enhanced pollinator health by stabilizing neurotoxic enzymatic pathways, reducing genotoxic damage, and boosting macronutrient sequestration. In this project, agroecological mitigation measures were found to provide a robust physiological "shield," effectively decoupling agricultural production from high agrochemical risk and aligning the chemical profile of managed sites with that of natural environments.

OC 5

PAPILIOTREMA TERRESTRIS PT22AV, NOVELTY YEAST BIOCONTROL AGENT, BRIDGING SUSTAINABLE FLORAL DISEASE CONTROL WITH POLLINATOR HEALTH, ATTRACTIVENESS AND ENTOMOVECTORING

Bruno Folchi[1], Ivan Portillo[1], Piotr Medrzycki[2], Manuel Roppo Valente[3], Davide Palmieri[4]

[1]AgroVentures srl, Latina, Italy
[2]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[3]Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università di Torino, Torino, Italy
[4]University of Molise, Campobasso, Italy

Abstract

The flowering stage of many crops represents a critical window for pathogen infection while also coinciding with intense pollinator activity, making the use of conventional chemical fungicides problematic due to their potential negative effects on beneficial insects. The basidiomycetous yeast *Papiliotrema terrestris* strain PT22AV has recently emerged as a promising biocontrol agent capable of suppressing multiple plant pathogens while maintaining compatibility with pollinators. This study investigates the potential of PT22AV as a disease management tool during flowering in major pathosystems, including strawberry grey mould and apple fire blight, with a particular focus on its interaction with honey bees. Monitoring PT22AV populations on strawberry plants during flowering showed that the yeast rapidly colonizes floral tissues, especially the receptacle and nectary regions, which are key infection sites for several pathogens. This rapid colonization underpins its antagonistic activity, primarily driven by competition for nutrients and space, a mechanism that limits pathogen establishment on plant surfaces. Repeated applications of PT22AV throughout the flowering period significantly reduced strawberry grey mould incidence, achieving up to 80% control. Laboratory experiments demonstrated that PT22AV exhibits a favorable biosafety profile and high compatibility with pollinators, with PT22AV-amended nectar being more attractive to honey bee foragers than control nectar. Field trials further showed that PT22AV applications increased the duration of bee visits on flowers by approximately 25–31%, suggesting a potential improvement in pollination efficiency. Overall, these findings position PT22AV as a valuable component of integrated disease management strategies aimed at reducing or replacing chemical fungicides during flowering, while simultaneously supporting crop protection and pollination services, with high potential for its entomovectoring application.

OC 6

THE ROLE OF CULTIVAR INTERACTIONS WITH ARTHROPODS IN INTEGRATED PEST AND POLLINATOR MANAGEMENT STRATEGIES FOR APPLE ORCHARDS

Francesco Lami[1], Melissa Venturi[1], Lisa Cabiddu[1], Lucia Lenzi[1], Mariana P. Mazzei[1,2], Diletta Missere[1], Brunella Morandi[1], Fabio Sgolastra[1]

[1]Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy
[2]Instituto de Investigaciones en Ciencias Agrarias de Rosario, IICAR-CONICET- Universidad Nacional de Rosario, Rosario, Argentina

Abstract

The diversification of floral resources is a pillar of pollinator conservation in agroecosystems, potentially also supporting biological control agents in Integrated Pest and Pollinator Management (IPPM) frameworks. Given the necessity to reduce pesticide use for IPPM systems to work, employed crop cultivars should ideally be not only highly attractive and dependent on pollinators, but also highly resistant to pests. Cultivar selection should thus be informed by a correct understanding of trade-offs and synergies between plant traits related to pollination and pest susceptibility.

In the present study, we set 3 pairs of experimental apple orchards (agroecological vs. conventional management) in Northern Italy with the aim of i) assessing the effects of pollinator-friendly agroecological measures (cultivar diversification, flowering cover crops) on beneficial arthropods, and ii) investigating the interaction of individual apple cultivars with pollinators and pests, highlighting trade-offs and synergies between functions.

The tested pollinator-friendly measures, and especially the use of cover crops, were confirmed as useful multifunctional tools capable of increasing the abundance of pollinators and predatory arthropods. The 9 tested cultivars differed in terms of both pollinator dependence and pest resistance, with some important trade-offs between one and the other. Cultivars such as the commercially important 'Pink Lady®', which would especially benefit from increased pollination service, may be unfit for a fully agroecological management due to pest susceptibility. Cultivars such as 'Abbondanza' and 'Rossa Precoce' are more promising in this regard.

These results highlight that the management of non-crop vegetation is an important tool to support beneficial arthropods in apple orchards, but also that cultivar selection is a critical element for a truly successful IPPM system; given the observed trade-offs with pollination traits, pest-resistant cultivars should be prioritized. Our results may find application in better informing the planning of biodiversity-friendly orchards.

PERVASIVE PESTICIDE CONTAMINATION OF CROP AND WILD FLOWERS IN APPLE AGROECOSYSTEMS

OC 7

Lucia Lenzi[1], Mariana P. Mazzei[2], Claudia Arcidiacono[3], Agnieszka Bednarska[4], Marta Bonforte[3], Jordi Bosch[5], Lisa Cabiddu[1], Roberto Catania[3], Costanza Geppert[6], Barbara Jaśkowiec[7], Francesco Lami[8], Lorenzo Marini[6], Gaetana Mazzeo[3], Alison O'Reilly[9], Giovanni Pirrello[3], Inés Prieto Ruiz[10], Michele Ricupero[3], Laura Roquer-Beni[5], Maj Rundlöf[10], Lucia Zappalà[3], Elżbieta Ziółkowska[7], Fabio Sgolastra[1]

[1]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy. [2]Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET), Universidad Nacional de Rosario, Rosario, Argentina. [3]Department of Agriculture, Food and Environment, University of Catania, Catania, Italy. [4]Institute of Nature Conservation, Polish Academy of Sciences, Krakow, Poland. [5]CREAF-Centre de Recerca Ecològica i Aplicacions Forestals, Universitat Autònoma de Barcelona, Bellaterra, Spain. [6]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Legnaro, Italy. [7]Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Krakow, Poland. [8]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy. [9]Department of Earth and Environmental Sciences, Lund University, Lund, Sweden. [10]Department of Biology, Lund University, Lund, Sweden

Abstract

Pesticides are among the main drivers of pollinator decline, alongside loss of food and nesting resources mediated by land use change due to intensive agriculture. Flower plantings in agricultural landscapes have been proposed to diversify and increase food and nesting resources for pollinators across Europe. However, such flowers and wildflowers may, just as crop flowers, be contaminated by pesticides due to drift and thus becoming a route of pesticide exposure for pollinators. The aim of this study was to assess pesticide risk to pollinators through flowers of cultivated apple trees, *Malus domestica*, and wildflowers growing inside and outside apple orchards surrounded by varying proportion of semi-natural areas. The study included 27 apple orchards across four European countries in a latitudinal and longitudinal gradient: Italy, Spain, Poland and Sweden. Samples of apple flowers were collected in spring while wildflowers were collected both during (spring) and after (summer) apple bloom, within apple rows and at the distances of 0-5 and 5-10 m of the orchards' edges, totalling 170 samples. Samples were analysed for residues of 775 pesticides, of which 90 were detected and only 9% of samples without any detections. A risk index was calculated by summing concentrations after division by the substances' *Apis mellifera* acute LD50. The number of detected pesticides was lower in summer compared to spring, in wildflowers at 5-10 m compared to 0-5 m or inside the orchard and decreased with increasing proportion of semi-natural areas. The pesticide concentration and risk index did not differ between types of flower samples or seasons. However, they decreased with higher proportion of semi-natural areas. Overall, the results confirm a widespread pesticide contamination of flower resources in agroecosystem, which was consistent across the European countries involved, and highlight the importance of preserving semi-natural areas to mitigate pesticide risk for pollinators.

OC 8

POLLINATOR-SAFE BIOCONTROL: ASSESSING THE COMPATIBILITY OF *PAPILIOTREMA TERRESTRIS* STRAIN PT22AV WITH HONEY BEES UNDER PROLONGED COLONY EXPOSURE

Antonio Nanetti[1], Manuel Roppo Valente[2], Vittorio Capano[1], Bruno Folchi[3], Davide Palmieri[4], Piotr Medrzycki[1]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]Department of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy
[3]Agroventures, Latina, Italy
[4]Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy

Abstract

The wild yeast-type *Papiliotrema terrestris* strain PT22AV, developed by AgroVentures, represents a promising biocontrol agent against plant-pathogenic fungi and nematodes. As environmental application of such agents requires comprehensive safety evaluation, particular attention must be given to potential impacts on non-target organisms, including pollinators. Prior GLP-compliant acute oral and dermal toxicity studies on honey bees confirmed the safety of PT22AV, with LD_{50} values exceeding 1×10^7 CFU per bee, far above estimated field exposure levels.

This study assessed the long-term colony-level compatibility of PT22AV under worst-case chronic exposure conditions. Twenty *Apis mellifera ligustica* colonies were randomly assigned to treatment and control groups (n = 10 each) during spring 2022. Over a 21-week period, treatment colonies were fed weekly with 1 kg sugar solution supplemented with *P. terrestris* (2.5×10^{10} CFU; 5.0 g PT22AV), while control colonies received unsupplemented sugar solution. Colony health parameters—including adult population size, brood area, honey stores, and survival—were monitored at 3–4-week intervals until hibernation.

Linear mixed-effects model analysis showed no significant differences between treated and control colonies across all measured parameters. Adult population dynamics, brood development, brood-to-adult ratios, and honey production remained unaffected by sustained high-dose exposure. Additionally, colony survival and queen health indicators— mortality, supersedure, and abnormal laying behaviour—did not differ significantly between groups.

These results demonstrate a high level of biocompatibility between PT22AV and honey bee colonies, even under prolonged exposure conditions unlikely to occur in field applications. Taken together with previously generated acute toxicity data in honey bees, these findings support a favourable environmental safety profile for PT22AV and its safe integration into pollinator-compatible biocontrol strategies, including bee-vectoring concepts for crop disease control. In addition, the absence of adverse effects under sustained exposure provides a rationale to further explore potential probiotic roles of PT22AV in honey bee gut health.

BEE-WARE THE BERRY: HOW LOWBUSH BLUEBERRY POLLINATION SHAPES COLONY HEALTH AND PERFORMANCE IN BUMBLE BEES

OC 9

Ana María Quiroga Arcila[1], Frédéric McCune[1], Mathilde L. Tissier[2], Pierre Giovenazzo[1], Valérie Fournier[1]

[1]Université Laval, Quebec, Canada
[2]University of Strasbourg, Strasbourg, France

Abstract

Lowbush blueberries (*Vaccinium angustifolium*) rely heavily on insect pollination, and the use of commercial pollinators is increasingly common in Canada. Among these, *Bombus impatiens* is a primary managed species. The use of commercial colonies raises ecological concern, including pathogen spillover and genetic introgression. However, little is known about the impact of lowbush blueberry pollination on bumble bee health and performance. This study assessed how lowbush blueberry pollination affects colony health (parasite loads) and performance (mass gain, number of individuals, and reproductive outputs) in *B. impatiens*. Colonies delivered for pollination were compared with those maintained outside blueberry fields and others placed in blueberry fields (1.5 multi-hives/ha) during the summers of 2022 and 2023. Parameters measured *included Nosema* spp. and *Crithidia* spp. prevalence, pesticide residues, colony and multi-hive weight gain, and colony composition by caste. Results show that colonies used for pollination frequently arrived already infected, with up to 56% individuals with *Crithidia* spp., however, pathogen loads decreased during pollination. Contrary to expectations, blueberry pollination had minimal impact on colony performance, which varied more between years than between habitats. These findings highlight the importance of monitoring pathogen loads at arrival and suggest that, under the studied conditions, lowbush blueberry pollination poses limited short-term risks to *B. impatiens* colony performance.

OC 10

BEE-HIND COLONY COUNTS: EXPLORING POLLINATOR DENSITIES AND LANDSCAPE SYNERGIES IN LOWBUSH BLUEBERRY PRODUCTION

Ana María Quiroga Arcila, Frédéric McCune, Pierre Giovenazzo, Valérie Fournier

Université Laval, Quebec, Canada

Abstract

Quebec is the leading producer of lowbush blueberries (*Vaccinium angustifolium*) in Canada. Growers increasingly rely on commercial pollinators, primarily honeybees (*Apis mellifera*) and bumblebees (*Bombus impatiens*). However, optimal colony densities for effective pollination remain unclear. This study examines how varying densities of commercial pollinators and surrounding landscape composition influence pollination quality in lowbush blueberries. Twelve fields in the Saguenay–Lac-Saint-Jean region were surveyed in 2022 and 2023, using four treatments: no commercial pollinators (control), one bumblebee density (6 colonies/ha), and two honey bee densities (2.5 and 5 hives/ha). Pollinator activity was measured via forager densities, flower visitation rates, and flower handling time. Pollination quality was assessed through seed set, fruit set, fruit drop, berry weight, and yield. Landscape composition metrics included the percentage of mixed forest, coniferous forest, blueberry fields, and nearby managed colonies. Results showed that increasing honey bee densities did not increase observed honey bee activity within fields; higher stocking densities were associated with reduced flower visitation rates by both managed and wild pollinators, suggesting competition for floral resources. Honey bee density was positively influenced by the number of colonies in the surrounding landscape. No significant differences in pollination quality metrics were observed among treatments. This study is the first in Canada to integrate foraging behavior, pollination outcomes, and landscape structure in lowbush blueberry systems. Findings highlight the influence of landscape structure on pollinator activity, the potential for density-driven competition, the critical role of wild pollinators in yield gain, and the importance of regional coordination between blueberry growers and beekeepers when managing commercial pollinator densities.

LABORATORY AND PROOF-OF-CONCEPT FIELD STUDIES: FLUPYRADIFURONE EXPOSURE TESTS DEFINE A KEY PROTOCOL FOR EUROPEAN LARGE-SCALE PESTICIDE EVALUATIONS IN BEES

OC 11

Annelise Rosa-Fontana[1,2], Antonio Pérez-Pérez[2], Dirk C. de Graaf[3], Lina De Smet[3], Raquel Martín-Hernández[2], Mariano Higes[2]

[1]Instituto Politécnico de Bragança, Bragança, Portugal
[2]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha, Marchamalo, Spain
[3]Ghent University, Ghent, Belgium

Abstract

This study aims to develop and validate a standardized lab-to-field pipeline for assessing pesticide impacts on honey bee colony resilience, providing a robust methodological framework for the EU project BETTER-B. For this proof-of-concept, a pilot study was implemented at the Centre for Beekeeping and Agri-environmental Research (CIAPA), Spain, within a low-complexity agricultural buffer. Eight honey bee colonies were established and divided into two groups: Control and flupyradifurone (FPF)-exposed. For both groups, the feeding solution consisted of a 50% (w/v) aqueous sugar syrup, with the FPF-exposed group receiving 36 ppm. The experimental FPF concentration (36 ppm) was previously established through laboratory assays, ensuring a field-realistic dose that avoids acute mortality while enabling the assessment of long-term resilience. These ready-prepared solutions were offered in 250 ml plastic bags placed directly inside the hives, on top of the brood frames. To ensure continuous *ad libitum* exposure, bags were monitored daily to verify complete consumption before replacement, while a coloring agent was added to the syrup to track food uptake and its distribution within the colonies. Results showed no significant increase in acute bee mortality ($p > 0.05$), validating the refined concentration. Short- and long-term impacts were quantified using the 'sixths method' at days 0, 10, 21, 31, 42, and 200, assessing total brood, adult bees, honey, and pollen stores. These assessments demonstrated no significant deviations compared to controls, confirming the protocol's capacity to track colony development and resource administration. To ensure internal validity, a double analytical control was implemented: APIStrips monitored background pesticide influx, and honey sampling screened for pesticide residues. This monitoring confirmed outcomes were directly linked to controlled FPF exposure. This pilot study successfully validates the BETTER-B methodological pipeline, establishing a standardized framework to evaluate honey bee resilience as a metric for future pollinator protection policies across Europe.

Bee products, ecosystemic services and circular economy

IMPACTS OF BEE VENOM HARVESTING ON AGGRESSIVE BEHAVIOR AND COLONY PERFORMANCE ON *APIS MELLIFERA ANATOLIACA*

OC 12

Melis S. Bahar, Banu Yucel

Ege University, Izmir, Turkey

Abstract

Bee venom is a valuable beekeeping product that has a wide range of therapeutic applications. In recent years, there has been a significant increase in demand for bee venom. Nevertheless, the potential impacts of bee venom production on colony behaviour and development remain a significant concern from the perspective of sustainable beekeeping. In addition, the conservation of genetic resources is of particular importance in terms of biodiversity. The objective of this study was to investigate the correlation between bee venom production in colonies and aggression behaviour, as well as colony development, utilising the native honeybee *Apis mellifera anatoliaca* ecotype. The Anatolian honeybee, which is used as the source material, is characterised by its high capacity for adaptation. The present study hypothesises that aggressive behaviour plays a role in the underlying mechanism of sustainable venom production, which relies on the triggering of stinging behaviour. In terms of the development of aggressive behavior, the Anatolian honeybee exhibits a higher tendency toward aggression compared to the Italian, Carniolan, and Caucasian honeybees. Thanks to these characteristics, the Anatolian honeybee shows promising potential for honeybee venom production. A range of parameters were evaluated, including the amount of bee venom collected during the study period, the colonies' aggressive behaviour, and indicators of colony development. The results indicate that bee venom production has measurable effects on colony behavior and development. This study conducted with the Anatolian honeybee is not only the first to reveal the effects of venom production on ecotypes, but also provides a characterization of the venom production process in colonies. In conclusion, while resolving beekeepers' potential concerns regarding colony performance in bee venom production, the study highlights the importance of local genetic resources for sustainable venom production by providing management tips for colonies.

OC 13

COMBINING MULTIPLE DNA-BASED ASSAYS TO UNCOVER ADULTERATED HONEY

Samuele Bovo, Anisa Ribani, Valeria Taurisano, Maria Letizia Calabri, Giuseppina Schiavo, Valerio Joe Utzeri, Luca Fontanesi

Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

Abstract

One of the major challenges facing the global beekeeping sector is the widespread presence of honey that does not meet regulatory standards, as evidenced by numerous cases of adulteration. The most common type of fraud involves the addition of sugar syrups to dilute honey. Other fraudulent practices include mislabeling the geographical, botanical, or entomological origin of honey. Because honey fraud is complex and requires multiple types of information to be properly identified, a multi-analytical strategy is necessary. In this study, we developed and employed a combination of DNA-based analytical methods to examine authentic, suspicious, and adulterated honey samples from different countries. The applied methodologies included: i) two targeted DNA-based assays to detect the honey bee mitochondrial DNA lineages that produced the investigated honey; ii) targeted DNA-based assays to detect the varieties/lines of the botanical species from which the honey was prevalently produced; iii) DNA metabarcoding methods to disclose the botanical profiles of honey using seven plant derived universal primer pairs targeting six different gene regions *(matK, psbA-trnH, rbcl, trnL_UAA, ITS2 and 18S)*; iv) DNA metabarcoding methods to define the plant-sucking insect fingerprints of honey using two mitochondrial DNA gene targets (COX1 and CYTB); v) definition of honey microbiota profiles using two 16S primer pairs; vi) three whole DNA sequencing approaches based on different next-generation sequencing techniques. The combined results of these complementary approaches enabled the precise classification of honey samples as either authentic or adulterated. These findings underscore the significance of employing multi-level DNA-based analytical approaches to capture relevant DNA fingerprints of honey which are useful to highlight various types of honey adulterations. Ongoing activities are directed towards the construction of comprehensive reference sequence databases useful to enhance the identification of counterfeit honey by applying various DNA-based assays.

CURE OR POISON? QUANTIFICATION OF HEAVY METALS AND MICROPLASTICS IN THAI BEE PROPOLIS

OC 14

Evada Chotiaroonrat[1], Sanchai Naree[2], Christopher Mayack[3], Alexis L. Beaurepaire[4,5], James D. Ellis[6], Guntima Suwannapong[1]

[1]Biological Science Program, Department of Biology, Faculty of Science, Burapha University, Chonburi, Thailand
[2]Pharmacology and Biopharmaceutical Department, Faculty of Pharmaceutical Sciences, Burapha University, Chonburi, Thailand
[3]Department of Biology, William Paterson University, Wayne, United States
[4]Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[5]Bee Research Centre, Agroscope, Bern, Switzerland
[6]Entomology and Nematology Department, University of Florida, Gainesville, United States

Abstract

Environmental pollution has become a global issue, with contaminants such as heavy metals and microplastics (MPs) entering ecosystems and food chains. These pollutants accumulate in air, soil, water, and plants, and can even be detected in bee products, raising concerns for pollinator health and human consumers. Among these products, propolis is a resinous hive material collected by foraging bees from several plant species and is well-known for its antimicrobial, antioxidant, and immunomodulatory properties. Propolis reflects the environmental conditions of the habitats where bees forage, making it an important topic for both health-related and ecological research. Our study aimed to assess heavy metal content (cadmium, Cd, and lead, Pb) in propolis of the western honey bee (*Apis mellifera*) and stingless bee (*Tetragonula pagdeni*) collected from different regions of Thailand. MPs were identified using a stereomicroscope and a micro-Fourier transform infrared (FTIR) spectroscopy. Additionally, the biological activities of propolis, including total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (EC50) were analyzed. The results showed that Cd and Pb were below the limit of detection (LOD = 0.0019 and 0.0344 mg/mL, respectively) in all samples, whereas polypropylene (PP) fiber and polyethylene (PE) fragment MPs were observed with a primarily blue color. Propolis exhibited wide variation in TPC (18.33 ± 1.36 to 116.56 ± 5.83 mg GAE/g), TFC (4.68 ± 0.10 to 113.63 ± 5.86 mg QE/g), and EC50 (30.61 ± 0.43 to 1.01 ± 0.11 mg/mL). These findings demonstrate that propolis remains a valuable natural product with strong bioactive properties. At the same time, its contamination with microplastics highlights its role as a bioindicator of pollution, making it an excellent tool for addressing concerns of environmental contamination, pollinator decline, and food safety.

OC 15

COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY IN DIFFERENT HONEY TYPES FROM SPAIN

Sonia Harbane, María José Pérez-Alvarez, Julia Carballo-Rodríguez, Yasmine Saker, María Carmen Seijo Coello, Olga Escuredo

Universidade de Vigo, Ourense, Spain

Abstract

Honey has been valued for its therapeutic properties, with a particular emphasis on its antimicrobial effects. The capacity of honey to promote healing and inhibit microbial growth is closely tied to its unique physicochemical composition. This composition is very complex and shaped by several factors, including botanical origin, the metabolic activity of the honey bees, and environmental factors such as climate. This study evaluated the antimicrobial activity of different honey types produced in Spain against a Gram-positive bacterium (*Staphylococcus aureus* CECT 239), a Gram-negative bacterium (*Escherichia coli* LGC), and a yeast strain (*Candida albicans* CECT 1394). In addition, physicochemical parameters including color, humidity, pH, and total phenolic content were determined. Hierarchical cluster analysis was applied to classify honey samples according to their antimicrobial activity, phenolic content, and moisture. The results showed that most honey samples exhibited moderate to strong activity (13–27 mm) against *S. aureus* and weak to moderate activity (≤12–17 mm) against *E. coli*, whereas *C. albicans* was the most resistant microorganism, with most samples showing weak activity. *Castanea*, *Arbutus* and honeydew honeys demonstrated the highest antimicrobial potential. Statistical analysis revealed that the botanical origin significantly influences the antimicrobial activity of honey, indicating its important role in bioactive composition and biological potential of honey.

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BEES, FLORA AND THE TERRITORY. MELISSOPALINOLOGICAL AND PHYSICO-CHEMICAL CHARACTERISATION OF *MYRACRODRUON URUNDEUVA* HONEY FROM BOLIVIA

OC 16

Erica Holzer[1], Carla Gianoncelli[2], Daniela Lupi[1]

[1]Department of Food, Environmental and Nutritional Science (DeFENS), University of Milan, Milano, Italy
[2]Fondazione Fojanini di Studi Superiori, Sondrio, Italy

Abstract

Monofloral honeys are essential for understanding the interactions between bees, floral resources, and landscape structure. *Myracrodruon urundeuva* (Anacardiaceae) is a significant tree species in South American dry ecosystems. However, the production of honey from this species is not well understood outside of Brazil. This study analysed honey samples collected in the Chuquisaca department (Bolivia), exploring the link between pollen composition, geographical context, and product characteristics.

A multidisciplinary approach was adopted, integrating melissopalinological, physicochemical and microbiological analyses, as well as calculating biodiversity indices (Shannon and Simpson) and performing multivariate statistical analyses (HCA and PCA). Geographical distances between apiaries were considered to evaluate the relationship between spatial proximity and pollen similarity. The results revealed a significant presence of *M. urundeuva* pollen, enabling monofloral and marginally monofloral honeys to be identified. Multivariate analyses suggest that the variability between samples is primarily quantitative. Once the effect of the dominant species is accounted for, the secondary pollen component appears relatively homogeneous and is not strictly structured by geographical distance. Physico-chemically, the honeys display dark amber colouration correlated with botanical dominance, while microbiological analyses confirm excellent hygiene and sanitary standards.

In conclusion, the study highlights that *M. urundeuva* honey reflects the species' ecological importance and marked pollen over-representation rather than clear spatial differentiation. The study confirms the value of melissopalinology as a complementary tool for studying bee-flora-territory interactions in poorly investigated dry ecosystems and provides valuable data to enhance local beekeeping resources in Bolivia.

OC 17

AUTOMATED AI-POWERED MELISSOPALYNOLOGY FOR ROUTINE HONEY ANALYSIS IN PUBLIC AGRIFOOD CONTROL LABORATORIES

Iratxe Perales[1], Asma Ghorab[1], Naoufal Amrani[1], Ana María Ruano Ramos[2], Paloma Delgado Fernández[2], Salud Serrano Jiménez[3], Inmaculada Rodríguez[3]

[1]MICROFY SYSTEMS SL, Barcelona, Spain
[2]Agri-food Arbitration Laboratory of the Spanish Ministry of Agriculture, Fisheries and Food, Madrid, Spain
[3]Department of Food Science and Technology, University of Cordoba, Córdoba, Spain

Abstract

Honey characterization in EU relies heavily on melissopalynological analysis to verify botanical and geographical origin, both essential for quality assessment, fraud screening and regulatory enforcement.

Despite being an official and well-established method, pollen analysis is still performed manually using light microscopy, requiring highly specialized expertise and intensive labour.

Public control laboratories face a critical shortage of trained melissopalynologists and increasing sample volumes driven by reinforced honey origin regulations, which often leads into limited inspection capacity.

This work presents Honey.AI, an automated, AI-powered microscopy system developed to support routine honey characterization and fraud screening in public laboratories.

The system combines a robotized digital microscope with deep learning models for autonomous pollen detection, classification, and quantification, reducing dependency on expert operators while preserving analytical rigor.

Honey.AI was validated within the InnoBuyer (Horizon Europe) innovation procurement framework through a co-creation pilot between Microfy Systems, the Agri-Food Arbitration Laboratory of Spain (LAA), and a palynologist from the University of Córdoba (UCO). During the project, specific custom datasets were specifically adapted to LAA's official sample preparation protocol (heating and fixation with gelatine), and AI models were retrained accordingly. A total of 54 Spanish honey samples, covering the full set of relevant monofloral types, were analysed in parallel using Honey.AI and traditional manual counting by LAA's expert. Results demonstrate a classification accuracy comparable to expert manual melissopalynology, with reduced analysis time and high reproducibility. Independent review by UCO confirmed the reliability of pollen species identification for Spanish honeys. The system, besides pollen analysis, also enables pollen richness calculation and specific percentages of unknown pollen, supporting geographical origin screening.

Honey.AI represents a novel, affordable and scalable alternative solution to support routine honey control in institutional laboratories, increasing inspection capacity, reducing bottlenecks, and supporting harmonised enforcement of EU authenticity regulations, required to meet just-released EU breakfast directives.

BEEBREAD EXERTS ACTIVITY AGAINST BIOFILM FORMATION AND ERADICATION OF *STAPHYLOCOCCUS AUREUS* AND *ACINETOBACTER BAUMANNII*

OC 18

Ioannis Psimoulis[1], Nikos Asoutis-Didaras[1], Katerina Karatasou[2], Dimitris Mossialos[1]

[1]Department of Biochemistry & Biotechnology, University of Thessaly, Larissa, Greece
[2]Apicultural Centre of Larissa, Federation of Greek Beekeepers Associations, Larissa, Greece

Abstract

Biofilm forming antibiotic resistant bacteria is a major health issue worldwide. Therefore, studies demonstrating biofilm inhibition and eradication by natural products are increasingly important. Bee products are extensively studied for their in vitro antimicrobial activity. Nevertheless, research on their antibiofilm properties is rather scarce. In our study we tested the antibiofilm activity of eight bee bread samples (seven multifloral and one unifloral) against two major nosocomial pathogens, *Staphylococcus aureus* and *Acinetobacter baumannii*. We have demonstrated for the first time that bee bread collected in Greece is effective in preventing bacterial biofilm formation as well as eradicating already formed biofilm. Inhibition of biofilm formation depends on bee bread concentration and on the tested pathogen. Higher inhibition rates were observed against *A. baumannii* (70.8% - 87.3%) compared to *S. aureus* (50.10% - 90.80%). Regarding eradication, the best results were observed at concentrations greater than the minimal inhibition concentration (MIC). For example, one sample demonstrated the best eradication activity (84.1%) against *S. aureus* at concentration twice the MIC. Overall, higher eradication activity has been observed against *S. aureus* compared to *A. baumannii*. Considering that all tested bee bread samples demonstrated significant antibiofilm activity, against two major pathogenic bacteria, we suggest that bee bread is a promising source of antibiofilm agents.

OC 19

MULTIPLE METABARCODING ASSAYS GENERATE BOTANICAL AND ENTOMOLOGICAL DNA FINGERPRINTS TO DETERMINE THE GEOGRAPHIC ORIGIN OF TURKISH HONEY

Anisa Ribani[1], Ayça Özkan Koca[2], Valeria Taurisano[1], Samuele Bovo[1], Luca Fontanesi[1]

[1]Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy
[2]Department of Gastronomy and Culinary Arts, Maltepe University, Istanbul, Turkey

Abstract

Honey is among the most frequently adulterated animal-derived food products, with fraud commonly involving the addition of inexpensive syrups and incorrect labeling of its botanical, entomological, or geographical origin. Türkiye plays a significant role in the global honey market as the second-largest producer, with annual production exceeding 100,000 metric tons. Ensuring and verifying the geographic origin of Turkish honey is therefore essential for guaranteeing its authenticity.

To address this, we developed a series of metabarcoding assays based on next-generation sequencing (NGS) to analyze 48 Turkish honey samples. This approach enabled the characterization of both botanical and entomological components, allowing the establishment of geographic fingerprints. The samples were collected from diverse regions across Türkiye, each accompanied by detailed geographic and ecological metadata, as well as declarations indicating whether the honey was monofloral or polyfloral and whether honeydew was present.

Botanical composition was determined using DNA metabarcoding of informative chloroplast regions, combined with a bioinformatics pipeline referencing a database of over 150,000 plant species. This analysis identified the dominant plant taxa in each sample, encompassing more than 1,000 plant groups or species, thereby providing insights into both botanical and geographic origins.

Entomological profiling relied on two mitochondrial DNA markers targeting hemipteran insects, capturing information about plant-sucking species inhabiting the agricultural and forest environments where the honey was produced. These insects, often associated with honeydew, display host-plant specificity linked to distinct ecosystems.

By integrating botanical and entomological metabarcoding data, we established a comprehensive framework for verifying the geographic origin of Turkish honey, offering a powerful tool for its authentication.

Climate change

OC 20

HONEY BEE WINTER BROOD BREAK IN THE NORTHERN HEMISPHERE: CASE STUDIES TO DEFINE THE PHENOMENON

Sergio Albertazzi[1], Irakli Janashia[2], Marin Kovačić[3], Aleksandar Uzunov[4,5,6], Goran Aleksovski[7], Cecilia Costa[1]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]Agricultural University of Georgia, Tbilisi, Georgia
[3]Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia
[4]Ss. Cyril and Methodius University in Skopje Faculty of Agricultural Sciences and Food, Institute of Animal Biotechnology, Skopje, North Macedonia
[5]Macedonian Academy of Sciences and Arts Research Center for Environment and Materials, Skopje, North Macedonia
[6]State Key Laboratory of Resource Insects, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, China
[7]Ss. Cyril and Methodius University in Skopje Faculty of Agricultural Sciences and Food, Institute of Animal Biotechnology, Skopje, North Macedonia

Abstract

In honey bee (*Apis mellifera*) colonies, a natural winter brood break can interrupt reproduction of parasitic mites such as *Varroa* and *Tropilaelaps*, which rely exclusively on capped brood to reproduce. Depending on local climate, broodless periods may last from weeks to several months. Global warming is progressively shortening the window during which colonies naturally interrupt brood rearing. In response, many beekeepers cage or ring queens to force brood breaks. Selectively breeding queens with a natural tendency for winter brood break could offer more resilient, chemical-free alternatives. Beekeepers report variability in this trait within individual apiaries, yet scientific evidence to characterise the phenomenon remains scarce.

This study investigated variability in winter brood break across four apiaries (73 colonies total) in south-eastern Europe and the South Caucasus: Macedonia, Croatia, Italy, and Georgia. Colonies were inspected monthly from November to February during winter 2024–2025. Brood breaks occurred between November and January, with all colonies showing brood presence again by February. In Croatia and Macedonia, all colonies within each apiary were simultaneously broodless at certain points. In Italy and Georgia, 12% and 65% of colonies respectively retained brood at every inspection. Peak brood levels during the observation period reached 5 frames in Italy, 3 in Macedonia and Georgia, and 2 in Croatia.

In the Italian apiary, monitoring continued into winter 2025–2026, where the proportion of colonies without any brood break rose to 43%. Among 8 colonies tracked across both winters, 6 showed consistent behaviour. These results are compared with data on varroa infestation estimated by mite fall after winter treatment. Brood break timing appears linked to outdoor temperature trends: a hypothesis to be further explored using digital sensors deployed in the Italian apiary, which could also enable automatic detection of broodless periods, offering a non-invasive monitoring tool for both researchers and beekeepers.

EXPLORING THE LINKS BETWEEN CLIMATE CHANGE AND EMERGING VIRAL DISEASE IN HONEY BEES

OC 21

Giles E. Budge[1], Steve P. Rushton[1], Janez Prešern[2], Hollie Pufal[1]

[1]Newcastle University, Newcastle, United Kingdom
[2]Agricultural Institute of Slovenia, Ljubljana, Slovenia

Abstract

Honey bees are central point foragers that depend completely on access to honey and pollen to fulfil their nutritional needs. Extreme weather events are an increasingly common feature of climate change, and can cause foraging cessation in honey bee colonies. In tandem, viral diseases have been reemerging across honey bee populations in Europe, North America and Asia. It is unclear whether malnutrition induced by foraging cessation can increase susceptibility to emerging viral disease. We constructed a "rain machine" to deliver a controlled foraging cessation event to one colony, whilst another control colony was able to forage freely, with eggs from the same queen nursed in each colony. We then assessed the susceptibility of adult honey bees that were nursed during the foraging cessation using chronic bee paralysis virus as a model for the additional stress induced by an emerging pathogen. Finally, we used data from IoT enabled in-hive sensors deployed across an extensive apiary network to monitor which meteorological parameters drive foraging cessation in honey bees across Europe and beyond. Our data highlight the wider risk climate change poses to honey bee health by providing a link between emerging weather patterns and susceptibility to emerging viral disease.

OC 22

ENZYMATIC STUDY OF THE EFFECT OF UV RADIATION ON MEDITERRANEAN HONEYBEE SUBSPECIES

Fernando Doblado[1,2], Soledad Sagastume[1], Giovanni Cilia[3], Banan Al-Shagour[4], Marion Zammit Mangion[5], Sami Alarsan[4], Mustafa Necati Muz[6], Dilek Muz[6], Mariano Higes[1], Antonio Nanetti[3], Raquel Martín-Hernández[1]

[1]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Centro de Investigación Apícola y Agroambiental de Marchamalo, (CIAPA), Marchamalo, Spain
[2]Facultad de Veterinaria de la Universidad Complutense de Madrid (UCM), Madrid, Spain
[3]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[4]NARC-National Agriculture Research Center, Amman, Jordan
[5]Department of Physiology and Biochemistry, University of Malta, Msida, Malta
[6]Department of Parasitology, Faculty of Veterinary Medicine, University of Namik Kemal, Tekirdag, Turkey

Abstract

Climate change is linked to the depletion of the ozone layer, which increases amount of ultraviolet (UV) radiation reaching the Earth's surface. The light near the Earth's surface is enriched by UV-A, which has also been linked to oxidative stress. UV radiation promotes photochemical reactions that lead to the formation of reactive oxygen species (ROS). Nowadays, there are not many studies investigating the effect of UV radiation on bees. For this reason, two objectives were proposed on this study: (i) to investigate how the activity of various antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and glutathione-S-transferase (GST), and one enzyme in detoxification, carboxylesterase (CbE), are affected by exposure to UV radiation, and (ii) to study the possible variability on this enzymatic activity in 5 different subspecies of *Apis mellifera* in the Mediterranean basin. The experiments involved placing 15-day-old adult worker bees (3 cages of 33 bees each) in UV-transparent acrylic cages, exposing them to UVA light (54 W, 395 nm) for four exposure durations (30 minutes, 1 hour, 2 hours and 4 hours). Furthermore, the same protocol was followed for the control groups, which were exposed for the same durations but in darkness. For the analyses, the bees were dissected and enzymatic assays were performed on the abdomens. Our results show that there are slight changes in enzymatic activity in the 30-minute, 1-hour and 2-hour groups comparing with control groups. However, significant differences were found when compared with the group exposed for 4 hours in almost all enzymes, which may indicate that prolonged exposure to UV radiation could affect the biochemistry of the bee. This is the first study about UVA response relating to time of exposure.
These results are under the project 2011-MEDIBEES, which is part of the PRIMA programme supported by the European Union.

STUDY OF THE ENZYMATIC ACTIVITY IN FIVE SUBSPECIES OF MEDITERRANEAN HONEYBEE SUBSPECIES

OC 23

Fernando Doblado[1,2], Soledad Sagastume[1], Giovanni Cilia[3], Dalida Darazy[4], Marion Zammit Mangion[5], Chadi Hosri[4], Mustafa Necati Muz[6], Dilek Muz[6], Mariano Higes[1], Antonio Nanetti[3], Raquel Martín-Hernández[1]

[1]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Centro de Investigación Apícola y Agroambiental de Marchamalo, (CIAPA), Marchamalo, Spain
[2]Facultad de Veterinaria de la Universidad Complutense de Madrid (UCM), Madrid, Spain
[3]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[4]Faculty of Agriculture, Lebanese University (LU), Dekweneh, Lebanon
[5]Department of Physiology and Biochemistry, University of Malta, Msida, Malta
[6]Department of Parasitology, Faculty of Veterinary Medicine, University of Namik Kemal, Tekirdag, Turkey

Abstract

Climate change is one of the main sources of stress for the honey bee. Rising temperatures and changes in rainfall patterns are causing alterations to the conditions to which bee species and subspecies are adapted. Temperature and humidity are two variables that, when combined, can alter the level of stress experienced, even acting as independent stress factor. Following this line, the aim of this study was to investigate the enzymatic activity on different subspecies of honey bees that were exposed to different conditions of temperature and relative humidity. The cellular stress-related markers selected were (i) four key enzymes in oxidative metabolism: catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and glutathione-S-transferase (GST), (ii) one enzyme in detoxification: carboxylesterase (CbE), and (iii) neurotransmitter-related Acetylcholinesterase (AChE). The study was conducted on five Mediterranean subspecies, *A. mellifera iberiensis* and *A. m. ligustica*, *A. m. anatoliaca*, *A. m. ruttneri*, and *A. m. syriaca*. Newly emerged bees were exposed to 12 combinations of temperature (35, 40 or 45 °C) and relative humidity (15%, 30%, 50% or 75%) for two hours. For the analysis, each bee was dissected: the abdomen was used for all the enzymatic assays (removing the digestive tract to avoid interference), and the head for AChE. The results showed that whilst the enzymatic activity of some enzymes, such as CbE and SOD was primarily affected by temperature, others, such as CAT, were primarily affected by relative humidity. Furthermore, significant differences were found within the five subspecies responding differently to exposure to the various conditions. Studies of this kind shed light on how cellular stress may respond differently depending on such adaptations and their potential consequences in the face of climate change.

These results are under the project 2011-MEDIBEES, which is part of the PRIMA programme supported by the European Union.

OC 24

DISTURBANCE LEGACIES IN MOUNTAIN FORESTS SHAPE POLLINATOR COMMUNITIES AND TRAIT DISTRIBUTIONS THROUGH RESOURCE AVAILABILITY

Kilian Frühholz[1,2], David Liebler[2], Sina Appeltauer[2], Sara D. Leonhardt[3], Michael Maroschek[1,2], Rupert Seidl[1,2], Sebastian König[1,2]

[1]Berchtesgaden National Park, Berchtesgaden, Germany
[2]Ecosystem Dynamics and Forest Management Group, TUM School of Life Sciences, Technical University of Munich, Freising, Germany
[3]Plant-Insect Interactions, TUM School of Life Sciences, Technical University of Munich, Freising, Germany

Abstract

Climate change is changing disturbance regimes in European mountain forests. Insect and wind related disturbances, e.g. bark beetle events and wind throws are expected to become more frequent, while snow-related disturbances, e.g. avalanches, will likely decrease. Disturbed forests with open canopy can provide lots of resources for pollinators such as herbal plant species and larval habitat, the occurrence of which is, in turn, affected by the frequency of disturbance agents. Bark beetle disturbances provide large amounts of standing deadwood, avalanches expose bare soil, and wind throws produce lying deadwood and locally expose bare soil at root plates, while management of disturbed forests, i.e. salvage logging, reduces the amount of deadwood.

We compared pollinator diversity and traits in forests disturbed by bark beetle, wind throw and avalanches in the German Alps and compared bark beetle sites without deadwood removal with salvage logged bark beetle sites. We studied the effects of disturbance legacies, i.e. bare soil, deadwood, temperature, light availability and floral richness, on taxonomic, phylogenetic and functional diversity and traits of bees and hoverflies.

Bee diversity was highest on the most open and warm sites while the diversity of hoverflies depended mostly on resources like deadwood and bare soil. Hoverfly diversity showed a maximum diversity between 20 – 30 m³/ha of deadwood. Furthermore, resource availability correlated with specific pollinator traits, e.g., ground nesting bees profited from high availability of bare soil.

Our results show the importance of protecting natural processes in forests. Retaining natural legacies of forest disturbances is particularly important in the face of climate change and changing habitats to protect disturbed forests as valuable pollinator habitats.

CLIMATE AND GEOGRAPHY SHAPE NUTRITIONAL QUALITY OF CORBICULAR POLLEN: INSIGHTS FROM ITALIAN BIOMONITORING

OC 25

Alessandra Giacomelli[1], Daniele Alberoni[2], Chiara Braglia[2], Emanuele Mele[2], Luca Rubbini[2], Anabella R. Nicolli[3], Simone Tosi[4], Amanda Dettori[4], Diana Di Gioia[2]

[1]UNAAPI Unione Nazionale Associazioni Apicoltori Italiani, Florence, Italy
[2]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy
[3]CONICET, Consejo Nacional de Investigaciones Científicas y Técnicas, Mar del Plata, Argentina
[4]Department of Agricultural Sciences, University of Turin, Turin, Italy

Abstract

Corbicular pollen is the main source of proteins and lipids for honey bees, yet its nutritional quality is highly variable and remains poorly understood at large spatial scales. Here, we present results from a nationwide biomonitoring network across Italy, designed to disentangle how geographic and climatic factors shape protein–lipid relationships within pollen of the same botanical genus.
During the 2025 season, environmental parameters (temperature, rainfall, and relative humidity) were continuously recorded across 12 Italian regions spanning contrasting agro-climatic conditions. In parallel, corbicular pollen was collected from multiple apiaries at bimonthly intervals. Samples were sorted by color to minimize botanical heterogeneity, and their origin was determined through integrated palynological analysis and full-length 18S rRNA gene sequencing, enabling robust genus-level assignment. Total protein and lipid contents were quantified and linked to environmental variables.
Marked variability in nutrient composition was detected within the same genus across regions (ANOVA, $p < 0.001$), highlighting strong environmental control. Strikingly, *Hedera* (ivy), a key late-season resource in Italy, showed pronounced shifts in protein–lipid balance among sites. Winter climate emerged as a major driver: January temperature was positively correlated with lipid content ($r = 0.62$, $p < 0.01$), while precipitation was negatively associated with protein levels ($r = -0.55$, $p < 0.05$). These results reveal a previously underappreciated carry-over effect of pre-flowering climatic conditions on pollen nutritional quality.
Overall, protein and lipid contents were only weakly coupled, indicating independent environmental regulation. This study provides the first evidence at a national scale that climatic conditions months before blossoming can shape pollen quality, with important implications for predicting nutritional landscapes and managing pollinator health under climate change.

OC 26

GENOMIC VARIATION IN MONTANE BUMBLEBEES IN SCANDINAVIA: HIGH LEVELS OF INTRASPECIFIC DIVERSITY DESPITE POPULATION VULNERABILITY

Yuanzhen Liu[1], Anna Olsson[1], Tuuli Larva[1], Aoife Cantwell-Jones[2], Richard J. Gill[2], Björn Cederberg[3], Matthew T. Webster[1]

[1]Uppsala University, Uppsala, Sweden
[2]Imperial College, London, United Kingdom
[3]Swedish University of Agricultural Sciences, Uppsala, Sweden

Abstract

Populations of many bumblebee species are declining, with distributions shifting northwards to track suitable climates. Climate change is considered a major contributing factor. Arctic species are particularly vulnerable as they cannot shift further north, making assessment of their population viability important. Analysis of levels of whole-genome variation is a powerful way to analyse population declines and fragmentation. Here, we use genome sequencing to analyse genetic variation in seven species of bumblebee from the Scandinavian mountains, including two classified as vulnerable. We sequenced 333 samples from across the ranges of these species in Sweden. Estimates of effective population size (NE) vary from ~55,000 for species with restricted high alpine distributions to 220,000 for more widespread species. Population fragmentation is generally very low or undetectable over large distances in the mountains, suggesting an absence of barriers to gene flow. The relatively high NE and low population structure indicate that none of the species are at immediate risk of negative genetic effects caused by high levels of genetic drift. However, reconstruction of historical fluctuations in NE indicates that the arctic specialist species *Bombus hyperboreus* has experienced population declines since the last ice age and we detected one highly inbred diploid male of this species close to the southern limit of its range, potentially indicating elevated genetic load. Although the levels of genetic variation in montane bumblebee populations are currently relatively high, their ranges are predicted to shrink drastically due to the effects of climate change and monitoring is essential to detect future population declines.

CARRY-OVER EFFECTS OF CYCLIC THERMAL STRESS DURING PUPATION IN BUMBLE BEES

OC 27

Maximilian M. Mandlinger, Christoph Kurze

Universität Regensburg, Regensburg, Germany

Abstract

Extreme weather events are increasing in frequency and intensity due to climate change, posing a significant threat to global biodiversity. While the decline of crucial pollinators like bumble bees is well-documented, the stage-specific impacts of environmental stressors, including thermal stress during pupation, remain poorly understood. In this study, we utilized an *in vitro* rearing protocol to investigate the developmental consequences of cyclic thermal stress. With a 30% reduction in emergence success under extreme heat stress peaking at 38°C, our findings also reveal significant carry-over effects. Notably, there was an increase in deferred adult mortality (HR = 2.00), where wing deformations served as a strong predictor of premature mortality (HR = 2.38). These findings suggest that thermal fluctuations during early life stages have cascading implications for colony fitness. This research underscores the critical need for stage-specific data to bridge the gap between individual physiological stress and colony-level resilience in a changing climate.

OC 28

ASSESSING HONEY COMPOSITION AND FLORAL RESOURCE VARIABILITY UNDER CHANGING ENVIRONMENTAL CONDITIONS IN SPAIN

Javier Pérez-Hernández[1], María J. Ramos-Román[1], Olga Escuredo[2], María Carmen Seijo Coello[2], María Shantal Rodríguez-Flores[2], Jose-Antonio López-Pérez[1], Carlos García-Villarrubia[1], Amelia V. González-Porto[1]

[1]IRIAF-CIAPA, Marchamalo, Guadalajara, Spain
[2]Universidad de Vigo, Ourense, Spain

Abstract

The honey production sector faces several challenges that threaten the long term sustainability of beekeeping, an activity of high ecological importance. Adulteration, fraud, and misleading labelling continue to undermine consumer confidence, making the accurate determination of honey's geographical and botanical origin essential for ensuring authenticity, supporting local producers, and protecting the future of the sector. In addition, climate change is increasingly altering nectar availability in Spain. Extreme weather events such as droughts, heat waves, and irregular rainfall are likely modifying floral resources, leading to notable shifts in honey yields. These environmental pressures affect bee colonies, and their foraging activity. In this study, ten honey samples from different Spanish regions were classified and characterized during several years to identify spatial and temporal variability. A broad set of physicochemical, pollen, and compositional analyses was conducted to better understand the factors influencing honey quality and authenticity.

Research on the nectaries of major nectar producing plants in Spain revealed fluctuations in nectar volume and pollen production across periods. These differences appear strongly linked to climatic conditions. The study also examined how sugar concentration in nectar responds to temperature, humidity, and rainfall, showing clear meteorological influences on carbohydrate accumulation. Protein content associated with pollen was found to vary with climatic context, indicating that nutritional quality is sensitive to environmental stress. Furthermore, changes in pollen grain size were documented, suggesting that certain morphological traits adapt in response to shifting weather patterns.The nectar analyses were carried out by taking samples from the most representative plant species in each region. In the case of pollen, samples were taken from honey and plants.

These findings reveal how environmental factors influence floral resources and honey composition, aiding honey authentication, beekeeping practices, and monitoring climate change impacts on productivity.

These results are under the programme IRIAF-FEDER.

DISCLOSING PHENOLOGICAL INTERACTIONS BETWEEN PLANTS AND INSECTS THROUGH ENVIRONMENTAL DNA ANALYSIS FROM HONEY SAMPLES COLLECTED THROUGHOUT THE SEASON

OC 29

Anisa Ribani, Valeria Taurisano, Samuele Bovo, Maria Letizia Calabri, Giuseppina Schiavo, Valerio Joe Utzeri, Luca Fontanesi

Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

Abstract

Honey contains DNA traces from all organisms that directly or indirectly contributed to its production and that have been encountered by honey bees during their foraging activities. This honey-derived environmental DNA (eDNA) can provide insights into the ecological, climatic and seasonal changes of all these organisms. Botanical fingerprints present in honey primarily derive from the pollen content, while entomological fingerprints, excluding those from the honey bees, are primarily constituted by plant-sucking insects that produce honeydew. Honeydew is collected by honey bees as a sweet feed source and all honey samples may be derived from a fraction of this component. In this study, we investigated the botanical and entomological DNA fingerprints from honey samples collected from three different apiaries at three different time points throughout the beekeeping season (end of June, end of July and end of October). The three apiaries were located in three different agroecological spots in the province of Bologna, Italy. From each apiary, honey samples were collected from three different colonies at all three time points. Metabarcoding analysis was based on using five different metabarcoding systems, including three plant universal primer pairs (*matK*, *rbcl* and *trnL_UAA*) and two universal entomological primer pairs targeting the COX1 and CYTB mitochondrial DNA genes, specifically designed to capture hemipters. Next generation sequencing data analysis assigned approximately 50% of produced reads to botanical or insect taxa. Complementary information was obtained through the multiple barcoding analyses applied. Differences between apiaries and within apiaries across the three sampling time points were observed with more frequently recurrent entomological profiles providing interesting information on the different botanical and entomological associations. These findings demonstrate that honey-derived eDNA can be useful to dissect phenological interactions between plants and their pests, specifically their plant-sucking insects, potentially capturing ecological and climate-derived changes.

OC 30

ECO-PHYSIOLOGICAL RESILIENCE OF MEDITERRANEAN HONEY BEE SUBSPECIES TO CLIMATE CHANGE

Soledad Sagastume[1], Giovanni Cilia[2], Banan Al-Shagour[3], Asmaa Anwer Eissa[4], Dalida Darazy[5], Dilek Muz[6], Sami Alarsan[7], Fernando Doblado[1], Marion Zammit Mangion[8], Thomas Galea[8], Noureddine Adjlane[9], Soumeya Kaouche[10], Mustafa Necati Muz[6], Chadi Hosri[5], Antonio Nanetti[2], Mariano Higes[1], Raquel Martín-Hernández[1]

[1]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Centro de Investigación Apícola y Agroambiental de Marchamalo, (CIAPA), Marchamalo, Spain
[2]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[3]NARC-National Agriculture Research Center, Amman, Jordan
[4]Beekeeping Departament Plant protection research institute, Ministry of Agriculture, Dokki, Egypt
[5]Lebanese University, Faculty of Agriculture, Dekweneh, Lebanon
[6]Department of Parasitology, Faculty of Veterinary Medicine, University of Namik Kemal, Tekirdag, Turkey
[7]NARC-National Agriculture Research Center, Amman, Jordan, Amman, Jordan
[8]Department of Physiology and Biochemistry, University of Malta, Msida, Malta
[9]Department of Agronomy, Faculty of Sciences, University of Boumerdes, Boumerdes, Algeria
[10]Department of Agronomy, Faculty of Sciences, University of Boumerdes, Algeria

Abstract

The Mediterranean region is a recognised climate change hotspot, experiencing increases in annual surface temperatures and declines in precipitation. A concerning aspect of these changing environmental conditions for bees is their resilience to changes in temperature and humidity. The ability to adapt to those new conditions, or eco-physiological resilience, is the aim of this work. To do this, different honey bee subspecies were studied focusing on thermal tolerance to cold and heat, survival rate with controlled food intake, and body water loss. Firstly, our results presented the thermal tolerance limits of *A. m. iberiensis*, *A. m. ligustica* and *A. m. syriaca*. They established general limits of thermal stress close to 40ºC, hyperthermia at 41,5ºC, and intolerance at 45ºC. *A. ligustica* showed the lowest thermotolerance to heat (41ºC). As for survival, different patterns of seven subspecies were observed living under six different experimental conditions of temperature (35, 40 and 45ºC) at 75% of RH, and humidity (15, 30, and 50%) at 35ºC. Here, the African *A. m. sahariensis* was the most resilient to 45ºC and the Maltese *A. m. ruttneri* the most intolerant to low humidity (15%). Regarding humidity changes at 35ºC, 50% seemed to be a common comfortable condition, being lower levels of food intake a good marker to stablish comfort. Finally, the BWL ability was studied as an adaptative trait in nine subspecies exposed to nine conditions combining temperature (35, 40 and 45ºC) and RH (10, 30 and 50%) during 2 hours. Statistically differences were found, being common T35ºC H50%, T40ºC H25% and T45 H10%. These studies showed different eco-physiological resilience in Mediterranean *A. mellifera* subspecies showing their potential consequences in the face of climate change.

QUEEN INTRODUCTION SUCCESS IN HONEY BEE (*APIS MELLIFERA CARNICA*, POLLMANN 1879) COLONIES: A FIELD STUDY FROM 2025

OC 31

Maja I. Smodiš Škerl, Jernej Bubnič, Ajda Moškrič

Agricultural Institute of Slovenia, Ljubljana, Slovenia

Abstract

Successful queen introduction is essential for colony stability, uninterrupted brood production, and normal colony development. Despite its practical importance, the success of queen introduction may vary depending on colony condition, management practices, environmental circumstances, and queen-related traits. This study evaluated queen introduction success in honey bee colonies during the 2025 season and examined the effects of selected technological, biological, and environmental factors, with special attention to queen body mass.

The analysis was based on structured field reports collected from beekeepers after queen introduction. In total, 490 queens from 32 breeding stations with clearly defined outcomes were included. Overall, 88.6% of queens were successfully accepted, confirming a high level of practical success under field conditions. Acceptance rates remained high regardless of hive type, introduction method, and colony type, and none of these factors showed a statistically significant effect. Similarly, no significant differences were detected among weather categories. However, the lowest acceptance rates were observed during periods of hot weather and after nectar flow under hot conditions, suggesting that environmental stress may still reduce the likelihood of successful introduction in practice. In the subsample of queens with measured body mass, accepted queens were on average heavier than rejected queens (214.1 ± 17.4 mg vs. 205.0 ± 17.9 mg), although this difference was not statistically significant. This trend suggests that queen body mass may still be biologically relevant and should be examined further using larger datasets.

Future monitoring should include more detailed and standardized data collection from both beekeepers and queen breeders. In practice, queen introduction should be planned during periods without pronounced heat stress, while additional caution is advisable during very hot weather. More comprehensive field records and larger datasets will improve understanding of the factors influencing queen acceptance and support even more reliable queen introduction outcomes in beekeeping.

OC 32

THE SEARCH FOR GENETIC MARKERS ASSOCIATED WITH THERMAL TOLERANCE WITHIN SEVERAL EUROPEAN HONEYBEE POPULATIONS

Kamiar Torabi[1], Raquel Martín-Hernández[2], Ana R. Lopes[3], Maria Alice Pinto[3], Mariana C. Ribeiro Elói[4], Nuno Capela[4], Fernando Fleites-Ayil[5], Robert J. Paxton[5], Camilla Pedrelli[6], Giovanni Formato[6], Trudy van den Bosch[7], Séverine D. Kotrschal[7], Linn F. Groeneveld[8], Bjørn Dahle[8], Tudor N. Ternar[9], Alexandru-Ioan Giurgiu[9], Fernando Doblado[2], Soledad Sagastume[2], Matthew T. Webster[10], Luc Peelman[1], Ellen Danneels[1], Dirk C. de Graaf[1], Lina De Smet[1]

[1]Universiteit Gent, Gent, Belgium
[2]Centro de Investigación Apicola y Agroambiental (CIAPA), Marchamalo, Spain
[3]Instituto Politécnico de Bragança, Bragança, Portugal
[4]Universidade de Coimbra, Coimbra, Portugal
[5]Martin-Luther-Universität Halle-Wittenberg, Halle (Saale), Germany
[6]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma, Italy
[7]Wageningen University and Research, Wageningen, Netherlands
[8]Norges Birøkterlag, Kløfta, Norway
[9]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Cluj Napoca, Romania
[10]Uppsala University, Uppsala, Sweden

Abstract

One of the research tasks within the European Better-B project is the quest for genetic markers associated with thermal tolerance. This would allow future bee breeding programmes to consider resilience towards the effects of climate change, such as extremely high lor low temperatures. The first phase consisted of selecting three bee colonies in nine different locations across Europe to perform simple phenotypic assays on heat and cold tolerance. These assays were performed on recently eclosed drones and worker bees and on forager bees. Even though differences between bees of different locations and different bee categories were detected, the most remarkable result was the big spread of thermal tolerance observed between individuals of the same location and category. Therefore, instead of further on comparing different bee colonies with different averages in heat or cold tolerance values, genetic variation was studied in haploid drones from the same colony that showed an extremely high or low thermal tolerance value.

Subsequently, more phenotypic assays on heat and cold tolerance were performed on colonies that showed the highest spread. Heat tolerance assays were performed on drones from a Norwegian and Belgian colony. Cold tolerance assays were performed on drones from a Spanish and Portuguese colony. In all four cases, the six surviving most tolerant and least tolerant drones were selected to perform high throughput RNA sequencing on the transcriptome of their individual heads and abdomens. In this way, differentially expressed genes (DEGs) were identified. From the same assays, the legs of the fifty most and least tolerant drones were pooled for DNA sequencing, to validate whether the identified DEGs result from single nucleotide polymorphism (SNPs) based on allele counts in the genome. These results will also be presented.

ADAPTATION IN HONEYBEES: REGULATORY PATHWAYS AT THE CENTER OF XERIC ENVIRONMENT

OC 33

Carlos A. Yadró García[1,2,3], Dora Henriques[1], Mustafa Necati Muz[4], Dilek Muz[5], Alireza Arab[6], Asmaa A. Eissa[7], Banan Al-Shagour[8], Nizar Haddad[9], Wisam Obeidat[10], Chadi Hosri[11], Fouad Lamghari[12], Khawla M. Alyammahi[12], José A. Rufino[13], Antonio Nanetti[14], Raquel Martín-Hernández[15], Maria Alice Pinto[1]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal. [2]Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Porto, Portugal. [3]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Porto, Portugal. [4]Department of Parasitology, Faculty of Veterinary Medicine, University of Namik Kemal, Tekirdag, Turkey. [5]Department of Virology, Faculty of Veterinary Medicine, University of Namik Kemal, Tekirdag, Turkey. [6]Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Tehran, Iran. [7]Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. [8]Bee Research Directorate, National Agriculture Research Center, Amman, Jordan. [9]National Agriculture Research Center, Amman, Jordan. [10]University of Jordan, Amman, Jordan. [11]Lebanese University, Faculty of Agriculture, Beirut, Lebanon. [12]Fujairah Research Centre, Fujairah, United Arab Emirates. [13]CeDRI, SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal. [14]CREA Research Centre for Agriculture and Environment, Bologna, Italy. [15]Centro de Investigación Apícola y Agroambiental (CIAPA). IRIAF. Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Marchamalo, Spain.

Abstract

The Middle East harbours six *Apis mellifera* subspecies, adapted to extreme arid and desert conditions, making it a compelling system for dissecting the genomic basis of adaptation across environmental gradients. We assembled a collection of 514 drones samples organised into two latitudinal parallel transects extending from Egypt to Central Anatolia and from the Arabian peninsula to northeastern Türkiye. Transect 1 (T1; N=341) included 66 *A. m. lamarckii* from Egypt, 227 *A. m. syriaca* from Jordan and Lebanon, 21 *A. m. meda* from southeastern Türkiye, and 27 A. m. anatoliaca from Anatolia. Transect 2 (T2; N=173) included 23 *A. m. jemenitica* from Oman and the UAE, 75 *A. m. meda* from Iran, and 75 *A. m. caucasia* from northeastern Türkiye. Whole genomes were resequenced for all individuals, and bioclimatic variables were obtained from WorldClim. Within each transect, three genotype–environment association methods (Samβada, pRDA, and LFMM) were combined with an outlier-detection approach (PCAdapt) to identify environmentally associated SNPs which were subsequently annotated. No common SNPs were identified in the intersection of the four methods in any of the transects. However the intersection of annotated genes for each method identified 1091 genes in T1 and 332 in T2 (204 genes shared between transects). These candidate genes were submitted to enrichment analysis. Enriched functional categories consistently included endocrine, neuroendocrine, and neurotransmitter signalling; transcriptional regulation (including HOX genes); transmembrane and ionic transport; and neurogenesis and synaptic development. In T1, 186 candidate genes (including *Hbg3, Glut1,nlgn1, nlgn2, nlgn5, nmdar2 and titin*) contained 392 missense variants. In T2, 41 genes (*including rg, nlgn1, nrx-1, axo, odorant receptor 4-like and glucose dehydrogenase*) contained 73 missense variants. Together, these results point to a complex ensemble of regulatory and sensory pathways that collectively underpin environmental adaptation in honey bee subspecies shaped by long-term exposure to arid conditions.

OC 34

WHY DO SOME BUMBLEBEES THRIVE WHILE OTHERS DECLINE? ANATOLIA CASE: A GENOMIC INVESTIGATION INTO CLIMATE CHANGE ADAPTATION

Gonca Yeşilyurt[1], Yusuf U. Çınar[1], Tuana Öğretici[1], Onur Obut[1], Mehmet A. Balcı[1], Selahattin B. Çay[1], Çiğdem Özenirler[2], Vahap Eldem[3], Fatih Dikmen[3]

[1]Institute of Graduate Studies in Sciences, Istanbul University, Istanbul, Turkey
[2]Department of Biology, Hacettepe University, Ankara, Turkey
[3]Department of Biology, Istanbul University, Istanbul, Turkey

Abstract

Climate change is driving a global decline in pollinators, yet the genomic basis of species-specific resilience remains poorly understood. Bumblebees (genus *Bombus*) offer a powerful model system to address this gap, as closely related species exhibit starkly divergent climate trajectories. Türkiye, a global hotspot for Bombus diversity, hosts species ranging from projected winners *(B. haematurus, B. argillaceus, B. niveatus)* to vulnerable losers *(B. fragrans, B. lapidarius)* and data-deficient unknowns *(B. armeniacus, B. zonatus)*, making it an ideal arena for comparative genomic investigation. This study aimed to identify the genomic signatures, including positively selected genes and evolving gene families, that underpin these divergent adaptive trajectories across nine species. To this end, we (1) generated high-quality de novo assemblies for five Turkish species (*B. haematurus, B. argillaceus, B. armeniacus, B. fragrans, B. zonatus*), (2) incorporated our previously sequenced genomes of B. lapidarius and B. niveatus, and (3) integrated these with publicly available genomes of *B. terrestris* and *B. pascuorum*, enabling broad phylogenetic comparison across species with contrasting conservation statuses.

Genome sizes ranged from 254.5 Mb to 331.1 Mb, with assembly quality metrics comparable to the *B. terrestris* reference genome (392.9 Mb), confirming reliability across all target species. Orthologous gene sets were constructed across all nine taxa, and genome-wide positive selection analysis (dN/dS; $\omega > 1$, p-adj < 0.05, BEB > 0.95) identified 265 to 426 positively selected genes (PSGs) per species. Functional enrichment of these PSGs revealed targeted adaptation in biological processes related to cellular metabolism, protein modification, and stress response, with prominent terms including "regulation of metabolic process" and "protein phosphorylation." Gene family dynamics assessed via CAFE5 uncovered significant lineage-specific expansions and contractions linked to differing environmental pressures. Together, these findings illuminate the genomic architecture that facilitates or constrains climate adaptation, providing a mechanistic foundation for evidence-based conservation strategies in threatened *Bombus* populations.

THE FUTURE OF MEDITERRANEAN BEEKEEPING - RESULTS FROM A DETAILED SURVEY

OC 35

Marion Zammit Mangion[1], Antonio Nanetti[2], Sandro Lanfranco[3], Noureddine Adjlane[4], Banan Al-Shagour[5], Asmaa A. Eissa[6], Giovanni Cilia[2], Ahmad Daour[7], Claudia Garrido[8], Dora Henriques[9], Chadi Hosri[10], Mustafa Necati Muz[11], Wisam Obeidat[12], Maria Alice Pinto[9], Thomas Galea[13], Soledad Sagastume[14], Soumeya Kaouche[4], Raquel Martín-Hernández[14]

[1]Department of Physiology and Biochemistry, University of Malta, Msida, Malta
[2]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[3]Dept of Biology, University of Malta, Msida, Malta
[4]Department of Agronomy, Faculty of Sciences, University of Boumerdes, Boumerdès, Algeria
[5]National Agricultural Research Center (NARC), Amman, Jordan
[6]Plant Protection Research Institute, Giemeza, Agricultural Research Center, Dokki, Giza, Egypt.
[7]Jordanian Beekeepers Union, Amman, Jordan
[8]BeeSafe, Bielefeld, Germany
[9]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[10]Lebanese University, Faculty of Agriculture (UL), Dekweneh, Lebanon
[11]University of Namik Kemal (UNK), Faculty of Veterinary Medicine, Tekirdağ, Turkey
[12]University of Jordan, Amman, Jordan
[13]Breeds of Origin Conservancy 63, Triq Nerik Sant, Zebbug, Malta
[14]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF) de Castilla-La Mancha, Centro de Investigación Apícola Y Agroambiental (CIAPA) de Marchamalo, Marchamalo, Spain

Abstract

The Mediterranean region is a climate change hotspot that is expected to challenge the apiculture industry's future. As part of MEDIBEES, we conducted a survey representing 1301 beekeepers, 122,145 colonies and nine native subspecies from nine countries across the Mediterranean and neighbouring regions to establish baseline characteristics and better understand beekeeping practices and vulnerabilities. We show that Mediterranean beekeeping is characterised by low productivity and high losses. Through our data we identify regions where the industry is characterised by boom-bust cycles and extended loss periods ($X2$ (22) = 200.39, $p < 0.001$), that in south Mediterranean countries are present both in winter and summer. Losses across the Mediterranean are attributed to high *Varroa* (reported by a range of 58.6-100.0% of participating beekeepers) and *Nosema* infestations, lack of forage (reported by 22.5%-76% of beekeepers) and low reported melliferous plant diversity that in turn require a strong reliance on supplementary feeding. Our data indicates that 1) abiotic and biotic stressors are acting independently and interactively increasing colony deaths 2) countries already geographically exposed to high aridity and low precipitation are experiencing mortality patterns that climate models predict for the wider region. Research-based management proposals and mitigating steps are presented.

Decline of bee populations and policies

WILD *APIS MELLIFERA* ENDANGERED IN THE EU: HOW DID WE GET HERE AND WHAT'S NEXT?

OC 36

Jovana Bila Dubaić[1,2], Keith A. Browne[3], Raffaele Dall'Olio[2,4], Pilar De la Rúa[5], Paolo Fontana[6], Patrick L. Kohl[7], Per Kryger[8], Grace P. McCormack[9], Andrzej Oleksa[10], Maria Alice Pinto[11], Fabrice Requier[12], Steve Rogenstein[13], Benjamin Rutschmann[14], Arrigo Moro[2,15]

[1]University of Belgrade, Faculty of Biology, Belgrade, Serbia
[2]Honey Bee Watch, Galway, Ireland
[3]Galway Honey Bee Research Centre, Galway, Ireland
[4]BeeSources, Bologna, Italy
[5]Universidad de Murcia, Murcia, Spain
[6]Edmund Mach Foundation, San Michele all'Adige (Trento), Italy
[7]Department of Livestock Population Genomics, University of Hohenheim, Stuttgart, Germany
[8]Aarhus University, Slagelse, Denmark
[9]University of Galway, Galway, Ireland
[10]Kazimierz Wielki University, Bydgoszcz, Poland
[11]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal
[12]Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, Gif-sur-Yvette, France
[13]Honey Bee Watch, Berlin, Germany
[14]Agroscope, Zürich, Switzerland
[15]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

Abstract

It was widely assumed that wild populations of the Western honey bee (*Apis mellifera*) had disappeared from Europe due to habitat loss and other stressors, such as the spread of *Varroa destructor*, whose severe effects on managed colonies led many to believe that wild cohorts had been even more detrimentally affected. However, over the past decade, several studies have documented the existence of free-living colonies (FLCs) throughout Europe and beyond.

During a three-year process, a consortium of experts coordinated through Honey Bee Watch reassessed the IUCN Red List status of wild *A. mellifera* in Europe, which had previously been classified as Data Deficient in 2014. Based on demographic evidence compiled from free-living colonies across multiple countries, wild *A. mellifera* met the criteria for the Endangered category within the European Union's 27 Member States. An important unresolved issue concerns the conservation significance of locally adapted subspecies, a dimension of diversity not directly captured by the current species-level IUCN assessment criteria.

At the broader, pan-European scale, significant knowledge gaps remain, particularly in underrepresented regions such as the Balkans, the Baltic region, Scandinavia, and Western Russia, where available data are scarce, fragmented, or completely lacking. These gaps highlight the need for coordinated data collection, further monitoring, and additional research resources and capacity.

This latest reassessment underscores several conceptual and practical challenges, including debates over the definition of "wild" and "managed" colonies, and how to account for the conservation significance of local genetic lineages. It also identifies priorities for future research and policy: improving the documentation and monitoring of FLCs beyond the EU-27, increasing awareness among stakeholders about the presence and importance of FLCs, and developing conservation strategies and policy frameworks that recognize wild honey bees as a native component of European biodiversity and that support the protection of their habitats and survival.

OC 37

STRATEGIC CHOICES WHEN THE RETURN TO RESILIENT HONEY BEES IS DIFFICULT: A CONCEPTUAL NOTE

Dirk C. de Graaf[1], Séverine D. Kotrschal[2], Martin Bencsik[3], Nuno Capela[4], Bjørn Dahle[5], Daniel S. Dezmirean[6], Giovanni Formato[7], Raquel Martín-Hernández[8], Robert J. Paxton[9], Maria Alice Pinto[10], Emma Bossuyt[1], Ellen Danneels[1]

[1]Ghent University, Ghent, Belgium
[2]Wageningen University and Research, Wageningen, Netherlands
[3]Nottingham Trent University, Nottingham, United Kingdom
[4]University of Coimbra, Coimbra, Portugal
[5]Norwegian Beekeepers Association, Kløfta, Norway
[6]Universitatea de Stiinte Agricole si Medicina Veterinara Cluj Napoca, Cluj Napoca, Romania
[7]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma, Italy
[8]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha, Marchamalo, Spain
[9]Martin-Luther-Universitaet Halle-Wittenberg, Halle, Germany
[10]Instituto Politécnico de Bragança, Bragança, Portugal

Abstract

Selection for resilient honey bees is a sustainable response to the damage caused by the varroa mite and the viruses it transmits. There are numerous examples where researchers and/or beekeepers have successfully implemented this approach. Often, it was possible to start with bee populations that had already underwent a rewilding process and thus were exposed to natural selection for some years. But what can be done if this approach is not feasible, e.g. because there are no rewilded honey bees and/or the available domesticated honey bees lack the intrinsic qualities to make the transition quickly? This presentation proposes a conceptual strategy that can offer a solution in such cases. It is based on the separation of the reproductive unit from the production unit in the beekeeping management. The reproductive unit is then subjected to low-stress beekeeping practices, thus giving breeding colonies with limited resilience every opportunity to thrive. On the contrary, no restrictions are imposed on the colonies of the production unit. The concept was rolled out for the first time in 2025 to the Belgian colonies of the Better-B project, which had been subjected to a Black Box Darwinian selection process. Compared with the BBD experiment in 2024, where all the 100 young experimental colonies died (0% survival), the 2025 version, under the given concept, resulted in a spectacular improvement in survival rates (80% in March). The changes to the DBB protocol included 1) fewer splits at the start, 2) a single oxalic acid treatment at the start, 3) better distribution of the colonies across the landscape and 4) removal of colonies with high varroa infestation (8 colonies out of 63). In the presentation we will shortly discuss how other outcomes from the Horizon Europe Better-B project can feed into this conceptual strategy.

"MANY CAN DO SOMETHING": LOCAL ENGAGEMENT AND BEEKEEPER STEWARDSHIP FOR SUSTAINABLE POLLINATOR LANDSCAPES

OC 38

Lotta Fabricius Kristiansen

Department of People and Society, Competence Centre for Advisory Services, Swedish University of Agricultural Sciences, Skara, Sweden

Abstract

Flora & Fauna Citizen Science is a Swedish project involving beekeepers, farmers, landowners, and local residents in recording and improving floral resources for honey bees and wild pollinators. Through coordinated floral inventories and beekeeper monitoring, including pollen sampling and hive scales, the project establishes ecological baselines and monitors changes in forage availability across seasons and years. This place-based data provides an accessible foundation for understanding the importance of floral continuity in supporting pollinator needs across the landscape at a local level.

Central to the project's approach is recognising beekeepers and local communities as stewards of sustainable pollinator landscapes. Their local observations, practical experience, and ongoing involvement enhance scientific monitoring and aid in interpreting ecological patterns in ways directly relevant to their local environments. This community-led stewardship shows that shifts towards pollinator-friendly environments are most successful when grounded in local knowledge and community participation.

The project also employs innovative communication and engagement tools. Its dedicated podcast, *Flora & Fauna medborgarforskning* (Flora & Fauna Citizen Science), acts as an intermediary object that supports participatory research, promotes knowledge sharing, and fosters dialogue among beekeepers, landowners, citizens, and community actors, including advisory services. Through storytelling and lived experiences, the podcast reduces barriers to engagement and encourages deeper involvement in monitoring and landscape stewardship.

Flora & Fauna Citizen Science acts as an intermediary object that supports participatory research, promotes knowledge sharing, and fosters dialogue among beekeepers, landowners, citizens, and community actors, including advisory services. Through storytelling and lived experiences, the podcast lowers barriers to engagement and encourages deeper involvement in monitoring and landscape stewardship.

Early findings indicate that merging community insights with systematic ecological data enhances understanding of forage continuity and helps in identifying locally relevant restoration measures. By integrating science, practice, and social learning, *Flora & Fauna Citizen Science* provides a scalable model for co-creating resilient, pollinator-friendly landscapes.

OC 39

HONEY BEES IN LAVA: A SELF-SUSTAINING POPULATION OF WILD HONEY BEE COLONIES ON THE ISLAND OF PANTELLERIA (SICILY, ITALY)

Paolo Fontana[1], Cecilia Costa[2], Giovanni Cilia[2], Livia Zanotelli[1], Patrick L. Kohl[3]

[1]Edmund Mach Foundation, San Michele all'Adige, Italy
[2]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[3]Department of Livestock Population Genomics, University of Hohenheim, Stuttgart, Germany

Abstract

A growing number of studies is demonstrating the occurrence of wild honey bee *(Apis mellifera)* colonies in Europe. Understanding whether these colonies form self-sustaining populations requires demographic data. Since 2021, wild colonies on the island of Pantelleria have been monitored, commissioned by its National Park. The study began with reports from local beekeepers, who noted an abundance of wild colonies on the island. Over the years, 57 nesting sites have been identified and recorded using the application "BeeWild". Of these, 58% are found within the walls of buildings, 16% in natural cavities within lava deposits, and 9% in olive trees. Wild and managed colonies present on the island were sampled, and genetic analyses (sequencing of the $tRNA^{leu}$-cox2 mitochondrial DNA region)) clearly show assignment of wild colonies to the African lineage, while results from the managed colonies provide evidence of introduction of different genetic origins from mainland Sicily and Italian peninsula. The monitoring data indicate that the annual colony survival rate of wild colonies is greater than 50%, meaning that surviving colonies would need to produce fewer than one swarm per year on average for the population to be self-sustaining. Both the wild and the managed colonies do not show signs of collapsing due to varroa, and the most important factor for the survival of these colonies appears to be extraordinary high temperatures and drought in the summer, when the highest mortality rates are observed. Another common cause of colony mortality is targeted removal by humans. All over the island, especially in the Northern part, swarms are abundant, and recolonization of suitable sites is frequent. Local beekeepers often capture swarms from wild colonies to enlarge their apiaries. The wild populations thus likely function as a demographic source, the opposite to what is observed in many other European regions.

JUVENILE HORMONE INCREASES *VARROA DESTRUCTOR* FERTILITY: EVIDENCE FROM ARTIFICIAL INFESTATION EXPERIMENTS

OC 40

Davide Frizzera, Elisa Seffin, Virginia Zanni, Desiderato Annoscia, Francesco Nazzi

University of Udine, Udine, Italy

Abstract

Understanding the biology of *Varroa destructor* is essential to develop effective control strategies against this major ectoparasite of honey bees. However, some crucial information is still missing, including the identity of the stimuli inducing the mite's oviposition. Early studies suggested that the juvenile hormone (JH) of the honeybee host could be involved in the synchronization of mite's reproduction with host development, by triggering *Varroa* oogenesis and oviposition; however, this hypothesis was later abandoned based on observational studies. We revisited this hypothesis using an artificial infestation method, allowing to observe *Varroa* reproduction on individual immature honeybees under controlled conditions. To this aim we injected L5 honeybee larvae with two doses of JH (2X and 10X the physiological level) and assessed mite fertility across seven independent biological replicates. We show that JH injection increases *Varroa* fertility, with a significant effect at the higher concentration, providing the first direct experimental evidence supporting the original hypothesis. Using a different experimental setup, we also show that, at the beginning of the reproductive phase, mites preferentially select larvae exhibiting lower expression of juvenile hormone esterase: an enzyme involved in JH degradation, suggesting that *Varroa* may actively target hosts with higher circulating JH levels. These findings reopen a long-dismissed hypothesis and highlight JH as a possible key factor of the host–parasite interaction, with potential implications for understanding Varroa reproduction and for the development of novel control strategies.

OC 41

GOOD VETERINARY REGULATIONS FOR GOOD BEEKEEPING PRACTICES

Claudia Garrido

BeeSafe, Bielefeld, Germany

Abstract

Honey bees, as food producing animals, are a subject of veterinary regulations. However, not enough attention is paid to their unique characteristics compared to other managed animals. This includes the complexity of the superorganism honey bee colony that challenges the concept of the individual usually considered in veterinary regulations.

These pecularities influence the practicability of implementing general regulation into beekeeping practice and the development of medicinal products for honey bees. On the other hand, health and welfare of managed honey bee colonies face increasing challenges from invasive pests and parasites and/or emerging diseases.

Current regulations do not give a good framework to address these challenges, for instance for developing veterinary medicinal products. In addition, a patchwork of practices throughout the EU or at regional level often hinders the implementation of the regulation and the collaboration between beekeepers and veterinary authorities.

Considering the challenges for bee health looming on the horizon in addition to current issues, an integrated approach is necessary to improve beekeeping practices, the collaboration with authorities, and, in consequence, bee health. This includes all levels involved, for instance:

- Guidelines open to new technologies for developing treatments against existing and future parasites and diseases.
- More knowledge in veterinary authorities about the pecularities of honey bees.
- Joint knowledge transfer for beekeepers and supervisory authorities and incentives for collaboration.
- Incentives for applied research for updating treatment schemes and management options for emerging issues.

Some starting points for creating a more integrated framework for the implementation of veterinary regulation into good beekeeping practice are presented.

THRIVING HIVES: INTEGRATING BEST PRACTICES AND ANIMAL WELFARE STANDARDS IN MODERN BEEKEEPING

OC 42

Claudia Garrido[1], Antonio Nanetti[2]

[1]BeeSafe, Bielefeld, Germany
[2]CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Honey bee colonies are highly organized functional entities with high resilience, operating as superorganisms capable of buffering a wide range of environmental and biological stressors. However, modern beekeeping is facing an increasingly complex set of challenges, driven primarily by the spread of invasive parasites such as *Varroa destructor*, the growing scarcity of floral resources due to land-use changes, and the direct and indirect effects of global warming on colony dynamics and forage availability. These pressures not only threaten colony health and productivity, but also jeopardize the critical ecosystem services that managed honey bee colonies provide.

Good beekeeping practices, in this context, become of crucial importance. Rather than offering rigid protocols, they should provide an adaptable and evidence-based framework that empowers beekeepers to proactively maintain the health and productivity of their colonies under varying conditions and regional circumstances. Importantly, sound beekeeping practices also carry broader implications for food safety, given the central role of honey bees in agricultural pollination and honey production.

Effective beekeeping practices must be firmly grounded in honey bee biology, acknowledging the complexity of colony-level organization and the intricate relationships between bees, pathogens, and their environment. Focusing on key areas such as nutrition, disease management, and environmental impact represents a first and essential step toward creating a flexible, science-based framework capable of addressing both current and emerging challenges. Particular attention should be given to the welfare of managed honey bee colonies — not merely as a moral consideration, but as a functional prerequisite for colony performance and longevity.

By integrating animal welfare standards into beekeeping best practices, the overall condition of managed colonies could be significantly improved, thereby strengthening their capacity to deliver ecosystem services. Ultimately, healthier colonies and more sustainable beekeeping systems translate into broader benefits for biodiversity, agricultural productivity, and human welfare.

OC 43

MIXED EFFECTS THROUGH MIXED MICROPLASTIC POLLUTION: EFFECTS OF MICROPLASTICS ON TWO WILD BEE SPECIES LARVAE (APIDAE, MEGACHILIDAE; APIFORMES)

Kenneth Kuba, Raquel Morell Kessler, Kim König, Carmen A. Nebauer, Sara D. Leonhardt

Plant-Insect Interactions, TUM School of Life Sciences, Technical University Munich, Freising, Germany

Abstract

Plastic production has been increasing for decades, resulting in a continuous release and accumulation of microplastics in soils and water bodies. Often dispersed via air, microplastic particles may also settle on plant surfaces, including flowers, potentially contaminating floral resources and exposing pollinators to microplastics. Experimentally, detrimental effects of such contamination in sugar water have been demonstrated in adult honeybees *(Apis mellifera)*, while corresponding experiments with larvae remain scarce.

In this study, the effects of pollen contamination with a microplastics mixture at two concentrations (Low: 0.5 mg/kg; High: 50 mg/kg) were tested on the development and survival of larvae of two wild bee species *(Osmia bicornis* and *Bombus terrestris)*. Results indicate that *Bombus terrestris* larvae fed low microplastics concentrations consumed up to 20% less pollen and produced significantly fewer pupae (up to 26% fewer) than the control group. *Osmia bicornis* larvae fed low microplastics concentrations developed more slowly, hatched later, and exhibited higher body mass as well as an increased conversion efficiency of pollen to body mass.

Overall, the effects of microplastics on pollinators were dose-dependent, non-linear, and species-specific, with the two bee species showing different responses to microplastic exposure. One possible explanation for the non-linear dose effect is that higher concentrations may trigger immune or protective responses, whereas lower concentrations remain below the activation threshold. The species-specific effects demonstrated here clearly highlight the need for more comprehensive research, as we are only beginning to understand the magnitude of the impacts these pollutants exert on insect diversity and, consequently, on our ecosystems.

PROJECT 10'000 COLONIES

OC 44

Peter Neumann[1,2], Giles E. Budge[3], Kirsten S. Traynor[4]

[1]Institute of Bee Health, University of Bern, Bern, Switzerland
[2]COLOSS (prevention of honeybee COlony LOSSes), Bern, Switzerland
[3]Newcastle University, Newcastle upon Tyne, United Kingdom
[4]State Institute of Bee Research, Stuttgart, Germany

Abstract

Managed honey bees are key pollinators globally, but beekeepers continue to struggle with unsustainable high colony losses that require dedicated research to resolve. However, high variability between colonies, and the influence of the apiary environment restrict inference from small-scale experiments. The impact of these local effects can only be compensated for by large multi-site experiments, which are expensive and difficult for individual researchers to manage. These local constraints have hampered our global understanding of bee health, preventing the identification of multiple subtle stressors that likely drive colony losses. We propose globally coordinated, large-scale studies using standardized experimental methods which feed data into an accessible platform that enables analyses. Such large scale experiments will provide a step-change in our understanding of bee health.

COLOSS (prevention of honeybee COlony LOSSes) has established these standard methods (BEEBOOK, https://coloss.org/activities/beebook/) and currently brings together 2,447 members from 127 countries, offering a unique base for global projects. We will create a cloud-based web interface and the infrastructure for large-scale, standardized experiments. The aim is to enable studies with 1,000+ colleagues, each collecting and uploading data from 10 to 20 bee colonies, strengthening global research collaborations, and enabling beekeepers to become citizen scientists. In this way, standardized data from > 10,000 colonies worldwide could be collected concurrently. This global infrastructure opens the opportunity to conduct simple but meaningful experiments to address key challenges for bee health (e.g. *Varroa destructor*, climate change, pesticides). We applied for funds to develop the interactive IT platform and cover administration costs. We will ensure the long-term further development and maintenance of the platform using COLOSS resources. In conclusion, we're convinced that this project constitutes a major transition in honeybee research, enabling collection of novel big scale data and better approaches to understanding the health and supporting the wellbeing of these precious pollinators.

OC 45

PESTITRACK: BUILDING A HARMONISED AND OPEN DATABASE ON PESTICIDE USE IN EUROPE

Lauréna Porcel[1], Marion Laurent[1], Marie-Pierre Rivière[1], Marie-Pierre Chauzat[1,2]

[1]Unit of Honey Bee Pathology, Sophia Antipolis Laboratory, ANSES, 06410 Biot, France
[2]Laboratory for Animal Health, ANSES, Paris-Est University, 94701 Maisons-Alfort, France

Abstract

Assessing pesticide exposure in agricultural landscapes remains a major challenge for accurately evaluating risks to pollinators. While bees and pollination are essential for agricultural production, agricultural intensification has led to increased chemical pressures on ecosystems. However, pesticide-use data remain scattered across heterogeneous sources and are difficult to compare across countries, highlighting the need for improved data accessibility and harmonisation.

Within the WildPosh project (Pan-European assessment, monitoring, and mitigation of chemical stressors on the health of wild pollinators), we developed a strategy to centralise and standardise pesticide-use data across European countries. As a proof of concept, the dataset included the four countries of the WildPosh site network (Estonia, Germany, the United Kingdom and Spain), focusing on oilseed rape (pollinator-attractive crop) and wheat (anemophilous crop), and on active quantified substances screened in different bee matrices.

We compiled heterogeneous data sources, including European and national databases, farmer pesticide usage surveys and regulatory information on authorised plant protection products. PestiTrack integrates data on pesticide application practices (commercial formulations, authorised application rates, timing, application methods, and crop-specific uses), as well as national-level pesticide-use data. Our approach relies on automated data extraction methods, including large-scale text mining and parsing of diverse formats. A major effort was dedicated to data harmonisation to enable comparisons across countries.

In 2025, the UK authorised the highest number of plant protection products for oilseed rape, followed by Germany. For wheat, Germany led, while Estonia authorised the fewest. Authorised fungicides dominated in Germany, whereas herbicides were the most represented group in other countries.

By centralising and making pesticide-use data openly accessible, this work provides a robust basis for defining realistic exposure scenarios, supporting environmental risk assessment, and informing on pesticide policies. Furthermore, ecotoxicological data on pollinators will be integrated into the database, with particular attention to source traceability and deduplication.

NUTRITIONAL MODULATION OF VITELLOGENIN AND VIRAL RESILIENCE IN *APIS MELLIFERA*

OC 46

Luca Rubbini[1], Alessandra Giacomelli[2], Daniele Alberoni[1], Chiara Braglia[1], Philippe Bulet [3,4], Michel Bocquet[5,4], Diana Di Gioia[1]

[1]Department of Agricultural Science, University of Bologna, Bologna, Italy
[2]Unione Nazionale Associazioni Apicoltori Italiani (UNA API), Florence, Italy
[3]Département Sciences Analytiques (DSA), Institut Pluridisciplinaire Hubert Curien (IPHC), Strasbourg, France
[4]Plateforme BioPark d'Archamps, Archamps, France
[5]Apimedia BP22-Prigny, Annecy, France

Abstract

The decline of the western honeybee, *Apis mellifera*, is a multifactorial phenomenon driven by interactions between the ectoparasitic mite *Varroa destructor* and a complex RNA virus pathobiome. Central to honeybee resilience is vitellogenin (Vg), a pleiotropic glycolipoprotein regulating longevity, behaviour, and immune priming. While pollen-derived amino acids support Vg synthesis, *Varroa* infestation imposes physiological stress by depleting host resources and vectoring viruses such as Chronic Bee Paralysis Virus (CBPV).

In this study, we investigated the relationship between nutrition, Vg expression, and viral abundance using RT-qPCR and MALDI-TOF mass spectrometry at an early time point (T1), complemented by temporal analyses under controlled treatments. Mixed-effects modelling (GLMM) and Tukey post-hoc comparisons revealed strong treatment-dependent effects on Vg expression, whereas CBPV exhibited a broader and less consistent response, with significant differences limited to specific treatment–time combinations.

Pairwise comparisons indicated that variation in Vg was more structured and treatment-driven, whereas CBPV responses were more heterogeneous, suggesting differential sensitivity to nutritional modulation. A negative association between Vg and CBPV was consistently observed, supporting a trade-off between host physiological investment and viral load.

MALDI-TOF analysis identified multiple spectral features significantly correlated with CBPV fold change (Spearman, FDR < 0.05), including peaks at m/z ≈ 9613.6, 12846.5, and 4512.3, indicating detectable virus-associated spectral signatures at early stages, even in untreated individuals. The partial overlap between Vg- and CBPV-associated spectral features suggests that viral infection reshapes host protein allocation. Overall, these results indicate that nutrition modulates host–virus interactions, while proteomic responses remain only partially coupled to transcriptional dynamics.

OC 47

BEYOND THE HONEY BEE: INTERSPECIFIC VARIABILITY IN SENSITIVITY TO INSECTICIDES AND IMPLICATIONS FOR RISK ASSESSMENT

Artur Sarmento[1], Lais Inoue[2], Mykola Rasko[1], Gabriela Garcia[3], Ivo Roessink[4], José P. Sousa[1], Nuno Capela[1]

[1]University of Coimbra, Coimbra, Portugal
[2]São Paulo State University, Rio Claro, Brazil
[3]University of São Paulo, São Carlos, Brazil
[4]Wageningen University & Research, Wageningen, Netherlands

Abstract

Plant Protection Products (PPPs) are widely recognized as one of the major stressors driving the global decline of bee populations, posing a serious threat to pollination services essential for ecosystem stability and agricultural productivity. Despite the diversity of this group, pesticide risk assessment has historically relied on an Apis-centric framework, in which the honey bee (*Apis mellifera*) is used as the primary indicator species.

Recognizing the limitations of this approach, the European Food Safety Authority (EFSA) has recently revised its guidance document to promote the inclusion of non-*Apis* bee species (e.g. *Bombus terrestris* and *Osmia* spp.). Nevertheless, regulatory implementation remains constrained by a lack of comparative toxicity data across bee taxa.

This study addresses this critical gap through a cross-continental comparative assessment designed to test the robustness of honey bees as surrogates. We evaluated the effects of three widely used insecticides with distinct modes of action - acetamiprid, cypermethrin, and cyantraniliprole - across six ecologically and geographically diverse bee species (*Apis mellifera, Bombus terrestris, Osmia bicornis, Megachile rotundata, Tetragonisca angustula* and *Scaptotrigona postica*).

Using harmonized laboratory methodologies, bees were exposed via oral and contact routes to capture compound-specific toxicity profiles and interspecific variability in sensitivity, enabling a robust comparison of the estimated endpoints across species under controlled conditions.

First results already reveal pronounced interspecific differences in sensitivity that depend on both the insecticide and exposure route, suggesting that honey bees do not consistently represent/protect the most sensitive species.

Overall, this work will provide comparative evidence to support the refinement of pesticide risk assessment frameworks. By promoting multi-species testing strategies, our study aims to support a more ecologically realistic and internationally relevant approach to pollinator protection.

SOLBEEPOP$_{ECOTOX}$ – A POPULATION MODEL FOR USE IN PESTICIDE RISK ASSESSMENTS FOR SOLITARY BEES

OC 48

Amelie Schmolke[1], Johannes Lückmann[1], Thomas G. Preuss[2], Mark Miles[3], Vanessa Roeben[2]

[1]RIFCON GmbH, Hirschberg, Germany
[2]Bayer AG, Monheim, Germany
[3]Bayer Crop Science, Cambridge, United Kingdom

Abstract

Potential risks to honey bees, bumble bees and solitary bees from pesticide exposures have to be assessed for their environmental impact in the EU. Thereby, data on organism-level toxicity, exposure estimates and potential population-level effects in the field need to be addressed. This is particularly challenging for for solitary bees, a diverse group of species with a wide range of ecological traits. Ecological effect models are identified as valuable tools to complement study data and extrapolate to untested conditions or scenarios. Models can integrate information about toxicity and exposure as well as field-relevant environmental conditions such as weather and landscape composition. We will provide an introduction to mechanistic effect models in pesticide risk assessments. As an example, we present the model SolBeePop$_{ecotox}$ that addresses populations of solitary bees in agricultural landscapes. The model can be used to simulate a range of solitary bee species based on their ecological traits, includes the explicit representation of exposures to pesticides from relevant routes and links the individual-level exposures to effects derived from laboratory toxicity study data. The model was used to simulate semi-field studies with exposures and effects from the reference insecticide dimethoate conducted with two bee species, *Osmia bicornis* and *Megachile rotundata* which differ considerably in multiple traits, including their phenology, body size and nesting ecology. The demonstration that the model can capture empirical data indicates that it is a useful tool in the context of pesticide risk assessments. We discuss how the model can be applied in risk assessments, including the simulations of untested scenarios such as different landscape compositions and exposure levels or patterns. Ecological effect models provide tools to enhance environmental risk assessments by integrating and extending available study data.

OC 49

POLLINATORS AND BEEKEEPERS IN THE EU COMMON AGRICULTURAL POLICY: CURRENT INTERVENTIONS, IMPLEMENTATION GAPS, AND OPPORTUNITIES FOR THE POST-2027 REFORM

Noa Simon Delso[1], Cindy Adolphe[1], Andrés Salazar Abello[1], Sebastian Spiewok[2]

[1]BeeLife European Beekeeping Coordination, Brussels, Belgium
[2]Deutscher Imkerbuny, Wachtberg, Germany

Abstract

The EU Common Agricultural Policy (CAP) contains a substantial, if underutilised, toolkit for supporting pollinators, beekeepers, and the agricultural habitats on which they depend. This contribution presents an analysis of the legal framework, budgetary allocation, and practical implementation of CAP measures relevant to bees and pollinators across the 2023–2027 programming period, and identifies strategic opportunities within the ongoing 2028–2034 CAP reform negotiations.

Our analysis identifies over 1,000 CAP interventions with positive potential for pollinators across Member States (MSs), aggregating a budget exceeding €100 billion, yet most are financially marginal, voluntary, or poorly targeted. All MSs include apiculture interventions in their National Strategic Plans, backed by €60 million in annual EU funding with a minimum 50% national co-financing. Seven intervention types are available, yet implementation is uneven: knowledge exchange and investments dominate, while restocking and quality enhancement remain marginal. While Agri-environment climate measures (AECMs) and eco-schemes provide significant benefits, such as enhancing pollinator habitats, they remain financially marginal (<1% of the CAP budget). Eco-schemes rarely address pollinators explicitly; benefits remain largely indirect and unmeasured. The greatest leverage for pollinator conservation lies not in beekeeping-specific instruments but in scaling biodiversity-oriented measures within mainstream agriculture, where farm management decisions determine forage availability, habitat connectivity, and pesticide exposure at the landscape scale.

Looking ahead, the Commission's proposal for the 2028–2034 CAP (2025/0241(COD)) introduces coupled income support for apiculture, alongside a unified agri-environmental framework and a farm stewardship system replacing conditionality, offering concrete entry points for embedding pollinator-friendly practices across the agricultural landscape.

The CAP already contains the instruments needed to support pollinators effectively. The challenge is not legislative invention but political prioritisation, adequate funding, and coherent implementation across governance levels. We provide recommendations for the ideal shaping and design of the CAP National Plans.

CAPTURING EXPERTS' OPINIONS ON SOIL-DEPENDENT POLLINATORS AS A PART OF THE PAN-EUROPEAN PROPOLLSOIL PROJECT

OC 50

Aleksandra Splitt[1], Jacek Jachuła[1], Claus Rasmussen[2], Jeff Ollerton[3,4], Maria Clara Castellanos[3], Zuzanna M. Filipiak[5], Michał Filipiak[5], Sara D. Leonhardt[6], Astrid E. Neumann[6], Carmen A. Nebauer[6], Jovana Bila Dubaić[7], Nigel Raine[8], Alexander Keller[9], Martin H. Thorsøe[10], Edward A. D. Mitchell[11], Dara A. Stanley[12], Markus A. K. Sydenham[13], Simon Hodge[12], Jorrit Poelen[14], Fabrice Requier[15]

[1]The National Institute of Horticultural Research, Skierniewice, Poland. [2]Aarhus University, Department of Agroecology, Aarhus, Denmark. [3]University of Sussex, School of Life Sciences, Brighton, United Kingdom. [4]University of Northampton, School of Life and Environmental Sciences, Northampton, United Kingdom. [5]Jagiellonian University, Faculty of Biology, Institute of Environmental Sciences, Krakow, Poland. [6]Technical University of Munich, TUM School of Life Sciences, Plant-Insect Interactions, Freising, Germany. [7]University of Belgrade, Faculty of Biology, Belgrade, Serbia. [8]University of Guelph, School of Environmental Sciences, Guelph, Canada. [9]Ludwig Maximilians University of Munich, Cellular and Organismic Networks, Munich, Germany. [10]Aarhus University, Department of Agroecology, Tjele, Denmark. [11]University of Neuchâtel, Laboratory of Soil Biodiversity, Neuchâtel, Switzerland. [12]University College Dublin, School of Agriculture and Food Science, Dublin, Ireland. [13]Norwegian Institute for Nature Research, Oslo, Norway. [14]University of California, Santa Barbara, Cheadle Center for Biodiversity and Ecological Restoration, Minneapolis, United States. [15]Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, Gif-sur-Yvette, France

Abstract

Many pollinator species rely on specific soil conditions for i.a. nesting and reproduction, making soil health a key factor in sustaining pollinator populations and the ecosystem services they provide. The pan-European project ProPollSoil (Horizon Europe: Mission Soil) aims to improve understanding of the interactions between soil health and soil-dependent pollinators. The first steps to be taken to realise this ambition are consolidating existing knowledge on the biology, ecology and threats to soil-dependent pollinators both below and above-ground. A key challenge is that much knowledge about the ecology of soil-dependent pollinators remains undocumented in the scientific literature and databases. Instead, it exists as tacit knowledge developed through years of field experience by professional researchers and experienced non-academic practitioners. This distributed expertise represents a valuable but underutilized source of information on soil-dependent pollinators. To capture and synthesize this knowledge, we will conduct a Delphi-style survey consisting of two rounds of structured questionnaires. The survey will be conducted from April to July 2026. Through this iterative process, the study will systematically compile and structure empirical knowledge on soil-dependent pollinators, making it more accessible for ecological research and conservation. During the presentation, we will share the preliminary results of this study.

This project receives funding from the European Union's Horizon Europe Framework Programme under Grant No.: 101219108 — ProPollSoil — HORIZON-MISS-2024-SOIL-01, the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract No. 25.00306, and the Hungarian NRDI Fund [2025-3.1.2-KÖA-2025-00014]

OC 51

LOCAL ECOLOGICAL NARRATIVES OF BIODIVERSITY LOSS AND ITS IMPLICATIONS TO SCIENTIFIC INQUIRY: INSIGHTS FROM GROUND NESTING STINGLESS BEE HONEY COLLECTORS

Yoseph Y. Zecharias[1,2], Yvonne Treis[1,2]

[1]Le Centre français des études éthiopiennes (CFEE), Addis Ababa, Ethiopia
[2]Langage, Langues et Cultures d'Afrique (Llacan)), Villejuif, France

Abstract

Because of their direct interactions with their environment, local communities generate and possess ecological knowledge, narratives, beliefs and perceptions that shape their ecosystem and livelihood. However, these local ecological narratives are insufficiently explored and poorly integrated into scientific inquiry. This study explores local ecological narratives of biodiversity loss, focusing on the decline of ground-nesting stingless bees (Meliponini). Meliponini are diverse yet understudied eusocial pollinators compared to the well-documented Apis mellifera, despite their fundamental roles in tropical ecosystems, including pollination services that support food security, biodiversity, agricultural productivity, and socioeconomic and cultural values. Based on results from in-depth interviews and field observations, we explore how traditional beekeepers and honey hunters in Kambaata, Ethiopia, understand and interpret the decline of ground-nesting stingless bees within broader biodiversity loss processes. The participants consistently associate decline in ground nesting stingless bees' abundance, distribution, and behavior to excessive pesticide use, agricultural expansion, drought, deforestation, and exploitative harvesting. Furthermore, nearly all participants reported that ground nesting stingless bees are neither manageable nor conservable, with domestication considered mostly "impossible" contributing for the decline. Some participants differentiated between lowlands and highlands, noting that ground nesting stingless have moved to mountainous or less disturbed areas, where chemicals are less applied and where there is no much farm activity. These findings contribute to emerging scholarship on biodiversity loss such as decline of ground nesting stingless bees by integrating local ecological perspectives, knowledge and narratives as a source of empirical insight that complements scientific evidence. Based on the study's results, we recommend an in-depth, context-specific research on the ecological biology, domestication, and conservation of ground-nesting stingless bees, alongside interdisciplinary approaches that integrate local knowledge narratives.

Microbiome and nutrition

OC 52

EFFECTS OF PROMOTOR L APIS SUPPLEMENTATION ON HONEYBEE COLONY PERFORMANCE AND VITELLOGENIN EXPRESSION UNDER VARIABLE CLIMATE

Diana Asciano[1], Alessandra Giacomelli[2], Chiara Braglia[1], Diana Di Gioia[1], Federico Casillas Santos[3], Daniele Alberoni[1]

[1]Department of Agricultural and Food Sciences, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy
[2]Unione Nazionale Associazioni Apicoltori Italiani (UNA API), Florence, Italy
[3]Calier Headquarters, Barcelona, Spain

Abstract

In the recent decade, climate change has deeply threatened the beekeeping sector by affecting the availability of trophic resources. Between 2017 and 2023, Italy experienced prolonged and abnormal droughts, which brought the beekeeping sector to its knees. Rainfall instability and nutritional stress significantly contributed to changes in honeybee survival and immunity.

This study evaluated the impact of the feed supplement Promotor L Apis on colony performance and physiological status of Apis mellifera under two distinct Italian climatic conditions (North and South). About 90 colonies were assigned to three treatments: Promotor L Apis(PLA), sugar syrup(SYR), or no supplementation(CTR) for eight weeks following queen caging. Brood area, adult honeybee population, pollen, and honey storage were evaluated as colony strength parameters with the ColEval method at 4 different sampling points post-nourishment. qPCR was employed to quantify vitellogenin (Vg) expression and viral loads of CBPV, DWV, and BQCV.

Although the anticipated drought condition did not occur in 2023 season, in the North, PLA colonies exhibited significantly enhanced brood development, with a 69.5% larger brood area at T2 compared to CTR (1,911 vs 1,128 cm^2; $p<0.05$) and showed the highest adult bee population (4,220 vs 3,739 cm^2 in CTR). In the South, PLA colonies accumulated significantly more honey across the overwintering period (~19% higher at T2–T4; $p<0.05$), indicating improved resource storage before winter.

At the molecular level, PLA supplementation was associated with a marked upregulation of Vg expression at T4, particularly in the North Italy, Vg reached 9.445 FC nearly fivefold higher than CTR, suggesting enhanced colony resilience. South Italy showed a 100% CBPV reduction by T4; notably, PLA reached zero by T2, while the CTR decline was more gradual.

This integrative approach aims to clarify potential correlations between nutritional supplementation, vitellogenin regulation, and improved immune status in honeybees.

FIRST METAGENOMIC CHARACTERIZATION OF BACTERIAL AND VIRAL COMMUNITIES IN ITALIAN HONEY BEE (*APIS MELLIFERA*) COLONIES

OC 53

Fulvio Bordin[1], Arianna Peruzzo[2], Gianpiero Zamperin[3], Michela Bertola[1], Elisa Palumbo[3], Adelaide Milani[3], Massimiliano Orsini[2], Alice Fusaro[3], Paola Mogliotti[4], Monica P. Cerioli[5], Giovanni Formato[6], Luciano Ricchiuti[7], Anna Cerrone[8], Pasquale Troiano[9], Antonio M. Salvaggio[10], Antonio Pintore[11], Franco Mutinelli[1], Anna Granato[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Laboratory For Honey Bee Health, Legnaro, Italy. [2]Istituto Zooprofilattico Sperimentale delle Venezie, Laboratory of Microbial Ecology and Genomics, Legnaro, Italy. [3]Istituto Zooprofilattico Sperimentale delle Venezie, Department of Virology, Research and Innovation Health, Legnaro, Italy. [4]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Asti, Italy. [5]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Brescia, Italy. [6]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Roma, Italy. [7]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Lanciano, Italy. [8]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy. [9]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy. [10]Istituto Zooprofilattico Sperimentale della Sicilia, Catania, Italy. [11]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy

Abstract

Honey bees are key pollinators, essential for crop production and biodiversity. Their health is influenced by environmental and anthropogenic factors that can alter their resident microbiota, composed of diverse microbial communities associated with the bee body. Due to the lack of information on the microbiota composition of Italian honey bees, this study investigated bacterial and viral communities in *Apis mellifera* colonies from 19 apiaries across different Italian regions using a metagenomic approach based on Next Generation Sequencing, aiming to expand current knowledge on honey bee microbiota and circulating pathogens. For each apiary, honey bee specimens were collected and pooled. DNA and RNA extracted from the pools were analysed to: *(a)* detect known pathogens (viruses, fungi, and trypanosomatids) using targeted molecular assays; *(b)* perform a metagenomic analysis of bacterial communities through amplification and Illumina sequencing of the V3–V4 hypervariable regions of the 16S rRNA gene; *(c)* characterize the virome by RNA sequencing (RNA-seq), using Illumina technology. Results showed the absence of *Vairimorpha apis*, Israeli acute bee paralysis virus and Kashmir bee virus in any sampled apiary, whereas Sacbrood bee virus and Black Queen Cell virus were consistently present. Bacterial community composition appeared homogeneous among apiaries and dominated by core taxa including *Lactobacillus*, *Gilliamella*, *Snodgrassella*, and *Bifidobacterium*. Conversely, RNA-seq analysis revealed both known and previously unreported viral species in Italian honey bees, such as Lake Sinai virus, Apis rhabdovirus, and Bee macula-like virus. These findings were further confirmed by targeted RT-PCR assays and Sanger sequencing. Conducted within project RC IZSVE 09/20, funded by the Italian Ministry of Health, this study provides the first large-scale metagenomic characterization of the Italian honey bee microbiome. The identification of novel viral species expands current epidemiological knowledge and offers valuable reference data to enhance molecular diagnostic tools.

OC 54

GUT MICROBIOTA INFLUENCE ON LOCOMOTION AND SOCIAL BEHAVIOUR OF HONEYBEES (*APIS MELLIFERA*)

Maria Bortot, Davide Liga, Irmak Pismisoglu, Albrecht Haase, Elisa Frasnelli

Center for Mind/Brain Sciences, University of Trento, Rovereto, Italy

Abstract

The gut microbiota of honeybees (*Apis mellifera*) is critical for their survival, affecting weight, longevity, homeostasis, behaviour, and cognition. Factors like diet, development, and environmental stressors can disrupt their microbiota, potentially causing dysbiosis. Recent studies have demonstrated that *Lactobacillus* (i.e., one of the most popular bee bacteria strains) enhances learning and memory of bees. The microbiota can also influence host locomotion, as fruit flies without normal microbiota showed signs of hyperactivity. However, links between microbiota and honeybee locomotion and studies on its cognitive effects remain limited.

This study explores the effects of gut bacteria (e.g., *Lactobacillus*) on honeybee locomotion and social behaviour. Pupae were extracted from a sterilized comb and raised under sterile conditions. Individuals were divided into two main groups: germ-reduced (GR) and conventional (Conv) bees, retaining the full microbiota. GR individuals were subdivided and mono-colonized with specific bacteria, enabling identification of microbial taxa involved in normal behavioural expression. After a 4-day acclimation, we assessed fine motor movements (legs and antennae), overall activity, and social behaviours (e.g., trophallaxis). This allowed us to examine how microbiota reduction affects behaviour and to identify the bacteria capable of restoring conventional activity levels.

Preliminary results will be discussed, presenting behavioural pattern analysis of locomotion abilities of Conv and GR bees, providing insights into microbiota's role in their motor and social behaviour.

ENHANCING HEALTH AND LONGEVITY OF *APIS CERANA* USING ARTIFICIAL POLLEN DIETS WITH SELECTED MARINE PROTEIN-BASED INGREDIENTS

OC 55

Bajaree Chuttong[1], Pornprapa Saenluang[2], Pichet Praphawilai[3], Chuleui Jung[4,5], Khanchai Danmek[2]

[1]Meliponini and Apini Research Laboratory, Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand
[2]School of agriculture and Natural Resources, University of Phayao, Phayao, Thailand
[3]Office of Research Administration, Chiang Mai University, Chiang Mai, Thailand
[4]Department of Plant Medicals, Gyeongkuk National University, Andong, Korea, Republic of Korea
[5]Agriculture Research Institute, Gyeongkuk National University, Andong, Korea, Republic of Korea

Abstract

Apis cerana beekeeping in Thailand relies on capturing wild colonies for domestication. During initial establishment, supplemental feeding is essential to prevent absconding and stimulate population growth before the primary honey flow. Given the periodic scarcity of natural pollen, marine-derived proteins rich in omega-3 fatty acids offer a promising nutritional alternative to enhance physiological health. The objective of this study was to assess the nutritional composition of a marine-derived protein artificial pollen diet (utilizing seabass and eastern little tuna) compared to controls (mixed pollen and sucrose syrup) and its impact on the health of *A. cerana*. The experimental diet was formulated with hydrolyzed marine-derived protein (53.9%), mixed pollen powder (10%), sugar (35%), vitamins and minerals (0.1%), xanthan gum (1%), and rum (2% of 40% alcohol). This formulation contained 14-16% crude protein and 0.5-1.0% ether extract, providing a gross energy of 2,500-3,000 kcal/kg. The use of marine-derived sources produced an artificial pollen diet (e.g., seabass-based diet) with a more complete amino acid profile and omega-3 fatty acids included alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) compared with pollen-based diets, which primarily contain ALA. Laboratory trials were conducted to measure hypopharyngeal gland development, lifespan, and gut microbiome composition. Laboratory evaluations demonstrated that these dietary treatments significantly enhanced hypopharyngeal gland development, maintained high survivability (21 days), and showed no significant differences in gut microbiome composition compared to the control (pollen-based diet). Utilizing alternative protein sources that meet the nutritional requirements of honey bees offers a practical and effective strategy for beekeepers. These marine-derived diets provide a solution for supporting the health and development of managed *A. cerana* colonies during periods of natural pollen scarcity.

OC 56 MOLYBDENUM: A TRACE ELEMENT THAT HELPS IMPROVE HONEY BEE HEALTH

Loïc Colin-Duchevet[1], Jean-Christophe Sandoz[1], Sébastien Floquet[2]

[1]Evolution Genomes Behaviour & Ecology, University Paris-Saclay, CNRS, IRD, Gif-sur-Yvette, France
[2]Institut Lavoisier of Versailles, UVS/CNRS, Université Paris-Saclay, Versailles, France

Abstract

The nutrition of honeybees is key to improving their health and resilience in the face of the many sources of stress to which they are exposed. Whilst there are numerous studies on the benefits of protein, lipids and vitamin intake, the impact of trace elements on their health remains largely unexplored. In this contribution, we will first examine the levels of certain trace elements found naturally in bees as well as in pollen, an important source of minerals. Particular attention will be paid to molybdenum, an essential trace element in biology whose role in bees remains unexplored. The second part of this presentation will then focus on a molybdenum-based coordination compound, which we have demonstrated bees can bioassimilate both under laboratory conditions and within the hive in the field. Through X-ray fluorescence studies carried out at the SOLEIL Synchrotron, we will demonstrate that feeding bees with this complex leads to a significant increase in molybdenum levels in the hypopharyngeal glands, the brain and the neurolemma surrounding the brain. A final part will examine the impact of feeding this complex on the cognitive abilities of bees, which will be exposed to sublethal doses of fipronil in order to demonstrate its protective effect against this pesticide.

BEYOND CORRELATION: A FRAMEWORK FOR UNDERSTANDING GUT MICROBIOME VARIATION IN SOCIAL BEES (GEM–P)

OC 57

Simone Cutajar[1,2], Daniele Alberoni[2], Diana Di Gioia[2], David Mifsud[1]

[1]Institute of Earth Systems, Università di Malta, Msida, Malta
[2]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy

Abstract

The gut microbiome of social bees is increasingly recognised as a key determinant of nutrition, detoxification, immunity, and behaviour. However, microbiome variation is still often interpreted primarily through correlations with host traits or environmental exposures, making it difficult to determine whether microbial shifts drive bee health outcomes or instead reflect upstream genomic or environmental processes.

Here, we present the Genome Exposome Microbiome Phenome (**GEM–P**) framework, a conceptual model that integrates host genome, exposome (lifetime exposures), microbiome and phenome (individual- and colony-level traits), collectively **G**, **E**, **M** and **P**, within a single dynamic system. GEM–P treats the microbiome as an active mediator linking host genome, lifetime exposures, and measurable traits, while also recognising feedbacks whereby phenotypes such as foraging, trophallaxis, and social organisation reshape subsequent exposures and microbial transmission. The framework organises current evidence across nested biological scales, from species and population to colony and individual bee, and across temporal dimensions including ontogeny, task transitions and seasonal change. It highlights recurrent causal pathways such as **G→M**, **E→M** and **M→P**, together with indirect and feedback routes that help explain why microbiome–phenotype associations are so context dependent. By structuring evidence in this way, GEM–P supports pathway-specific hypothesis generation and more informative study designs. In particular, it emphasises the value of temporally and spatially stratified sampling, explicit measurement of exposure histories, and attention to colony identity and transmission structure. GEM–P provides a practical conceptual scaffold for interpreting microbiome variation in social pollinators and for guiding future mechanistic, applied and evolutionary research in apidology.

OC 58 YEAR-LONG DYNAMICS OF THE GUT MICROBIOME IN MALTESE AND ITALIAN HONEY BEES

Simone Cutajar[1,2], Daniele Alberoni[2], Chiara Braglia[2], Loredana Baffoni[2], David Mifsud[1], Diana Di Gioia[2]

[1]Institute of Earth Systems, Università ta' Malta, Msida, Malta
[2]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy

Abstract

The gut microbiota of honey bees is central to nutrition, immunity and resilience, yet little is known about how it varies across subspecies and through seasons. This study investigates the year-long dynamics of the gut microbiome in *Apis mellifera ruttneri*, the endemic Maltese honey bee, and *Apis mellifera ligustica*, the Italian honey bee. Using synchronised test apiaries in Malta and Italy, worker bees were sampled monthly over a full annual cycle, with forager bees targeted to capture both internal and external environmental exposures. Particular attention is given to environmental drivers of microbiome variation, including seasonal changes in forage availability and pollen-derived nutrition. DNA has been extracted from the gut of over 1,200 individuals and analysed downstream using Oxford Nanopore full-length 16S rRNA sequencing to determine microbiome structure, along with qPCR-based quantitative analyses of specific targets.

By combining a year-long comparative design across the two subspecies with parallel environmental observations, this work reveals substantial shifts in beta diversity indices linked to host background and local conditions. Stable core taxa and microbiome signatures associated with host background and local conditions were identified in *Commensalibacter, Bartonella* and *Bombilactobacillus*, with specific core microbial taxa emerging as markers of the Maltese environment. Seasonal variation in environmental, non-core taxa was also detected, particularly in honey bees reared in Malta. Overall, the findings of this study reveal whether the two subspecies maintain consistent differences in gut microbial composition, how seasons shape microbiome structure, and whether temporal patterns are conserved or context-dependent across environments. This work contributes to understanding how host genetic background and local environment interact to shape gut microbiome structure in honey bees.

CORE GUT MICROBIOME DIVERSITY IN SOUTH AMERICAN WILD BEES ACROSS SPECIES AND ENVIRONMENTS

OC 59

Gregorio Fernandez de Landa[1], Daniele Alberoni[1], Loredana Baffoni[1], Mateo Fernandez de Landa[2], Chiara Braglia[1], Matias D. Maggi[2], Diana Di Gioia[1]

[1]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy
[2]Instituto de Investigaciones en Producción Sanidad y Ambiente (IIPROSAM), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Centro Científico Tecnológico Mar del Plata, CONICET. Centro de Asociación Simple CIC PBA, Mar del Plata, Argentina

Abstract

The gut microbiome of bees plays a fundamental role in host physiology, nutrition, and ecological adaptation. While most studies have focused on managed or social species, information on the core microbiome of wild bees in South America remains limited. In this study, we characterized the gut bacterial communities of multiple native bee species, including solitary and social taxa, sampled across contrasting environments in the Pampean region of Argentina.

Using high-throughput 16S rRNA gene sequencing, we identified consistent patterns in microbiome composition across species, highlighting the existence of a shared yet flexible core microbiome. Across all analyzed bees, *Pseudomonas* emerged as a dominant and recurrent genus, suggesting a strong environmental component in microbiome acquisition. In addition to this shared core, species-specific signatures were observed: *Sodalis* was characteristic of *Lasioglossum*, *Spiroplasma* of *Eucera fervens*, and Lactobacillus showed higher prevalence in *Xylocopa augusti* and bumble bees. Social species were additionally enriched in canonical core taxa such as *Snodgrassella*, *Gilliamella*, and *Bifidobacterium*, consistent with previously described stable microbiomes in corbiculate bees. Despite differences in land use, microbiome composition remained relatively stable at higher taxonomic levels, with variation primarily driven by host species rather than environmental conditions. These findings suggest that wild bee microbiomes combine a conserved bacterial core with flexible, host-associated components, reflecting both ecological interactions and evolutionary history.

Overall, this work provides a comprehensive overview of gut microbiome diversity in South American bees and contributes to the understanding of core microbial assemblages across phylogenetically and ecologically diverse pollinators.

OC 60 WIDESPREAD RNA TRANSFER FROM THE MICROBIOTA TO HONEYBEES DRIVES HOST GENE SILENCING

Anastasios Galanis, Nikolaos Vergoulidis, Eyal Maori

Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

Abstract

Transmissible RNA has emerged as a means of communication between organisms, both within and across different kingdoms of life. Donor organisms transmit long base-paired RNA, tRNA-fragments and other small RNAs to elicit RNAi responses in recipient individuals, affecting their gene expression and phenotypes. We previously reported that honeybees possess a transmissible RNA pathway through which they share RNAs between individuals and across generations via the secretion and ingestion of worker and royal jelly. However, the role of the microbiota in this RNA transmission route has not been investigated. Here, we show that endogenous RNAs originating from a gut-restricted symbiotic bacterium, *Snodgrassella alvi (S. alvi)*, are exported from the adult honeybee gut, circulate systemically via the hemolymph, and are secreted into royal jelly, where they are subsequently ingested and systemically disseminated in larvae in the absence of the bacterium. Further, we demonstrate that transmissible bacterial small RNAs associate with the honeybee RNA-induced silencing complex (RISC), and host target genes are down-regulated in *S. alvi*-colonised bees. Expanding beyond a single symbiont, we reveal a complex 'transmissible RNAome' landscape comprising RNAs transmitted from diverse microbiota taxa, many of which are recruited into host RISC assemblies. Finally, analysis shows that transmitted microbiota-derived RNAs could potentially trigger RNAi also against honeybee pathogens such as Nosema and viruses. A naturally occurring microbiota-to-host RNA communication pathway and its potential roles in cooperative transgenerational honeybee-microbiota interactions will be discussed.

POLLEN COMPOSITION OF LARVAL FOOD PROVISIONS AFFECTS DEVELOPMENT AND ADULT INSECTICIDE SENSITIVITY IN A POLYLECTIC SOLITARY BEE

OC 61

Alejandro Garcia-Gomez[1], Laura Ciot[1], Sergio Albacete[2], Marta Barnadas[1], Anselm Rodrigo[1], Jordi Bosch[1]

[1]Centre for Ecological Research andForestry Applications (CREAF), Barcelona, Spain
[2]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy

Abstract

Bees have been experiencing changes in pollen diet composition over the last decades. These changes can be attributed to alterations in floral resource availability caused by multiple factors, such as landscape simplification, agricultural intensification and climate change. The potential consequences of these diet shifts on development, adult performance and pesticide sensitivity are still poorly understood, especially for solitary bees. *Osmia cornuta* is a polylectic solitary bee that collects pollen from many plant families but shows a strong preference for *Prunus* pollen. Although *O. cornuta* larvae develop successfully on monofloral *Brassica* or *Sinapis* provisions, *Brassicaceae* pollen is usually a secondary pollen source. We used *O. cornuta* provisions to prepare three larval diets with different ratios of *Prunus:Brassicaceae* pollen (43:57, 20:80 and 0:100). We reared larvae on individual provisions of these three diets, monitored development and measured adult body mass and winter mortality. Upon emergence the following spring, we exposed adults to an insecticide sublethal dose and then assessed response to a phototaxis test and monitored longevity. Bees reared on the 0% *Prunus* diet exhibited lower developmental rates and higher mortality both during development and wintering. Adults reared on this diet also had lower body mass and reduced post-winter longevity. Bees reared on the 20% *Prunus* diet developed similarly to the 43% *Prunus* diet but had higher winter mortality. Following insecticide exposure, both bees reared on the 0% and 20% *Prunus* diets showed reduced survival. Our results demonstrate that larval provision pollen composition can negatively affect bee development and produce carry-over consequences on adult longevity and pesticide sensitivity independently of body mass. Our study shows synergistic effects between two major drivers of bee declines.

OC 62

ENVIRONMENTAL DRIVERS OF THE HONEY BEE (*APIS MELLIFERA*) GUT MICROBIOME AND ITS ASSOCIATIONS WITH VIRUSES AND PESTICIDES ACROSS CANADIAN AGROECOSYSTEMS

Aleksandra Kozlova[1], Huan Zhong [2], Leonard J. Foster[2]

[1]Center for Bio- and Medical Technologies, Moscow, Russia
[2]Department of Biochemistry & Molecular Biology, Michael Smith Laboratories, Life Sciences Institute, University of British Columbia, Vancouver, BC, Canada

Abstract

The goal of this study is to provide a comprehensive analysis of the gut microbiota of Canadian *Apis mellifera* colonies. We aim to (1) characterize the overall composition of the core microbiome, (2) assess the variability of microbial communities across samples collected from different ecosystems, and (3) investigate the abundance of known immunostimulatory symbionts in relation to environmental parameters, like pesticides and pathogens.

Samples for metagenomic sequencing were collected from sites across Canada that represented the country's diverse climate and landscape as described in Zhong et al. (2025). Sequencing was performed on an Illumina NovaSeq 6000 instrument. Reads were taxonomically classified as bacterial using Kraken 2 (v2.1.2) with the BeeRoLaMa v1 Honey Bee Microbiota Database. In total 55 microbial species were identified across all samples.

Major honey bee viruses assessed via real-time qPCR, primers and detailed methods are available in a previous report in the Supplementary data 2 of McAfee et al's paper. Separately agrochemical residue analysis using LC-MS/MS at the Agriculture and Food Laboratory, University of Guelph, an ISO/IEC 17025 accredited laboratory was performed. All virus pathogens and pesticide variables were normalized with log(x+1).

Analysis of microbial community composition was performed using R (version 4.4.2). By integrating three complementary analytical frameworks – ANCOM-BC2 differential abundance testing, centered-log-ratio (CLR) correlations, and SelBal balance modeling we identified bacterial taxa that respond consistently to specific viral infections.

We identify consistent associations between specific bacterial taxa and major RNA viruses, including enrichment of *Serratia marcescens* with SBV and depletion of *Bombella intestini* with BQCV. Pesticide exposure is likewise linked to reproducible shifts in key microbial taxa. Together, these findings reveal that interacting environmental stressors jointly shape the bee gut microbiome and enable prediction of microbiome responses in agroecosystems.

A REFERENCE ATLAS OF THE GUT MICROBIOME IN THE EUROPEAN HONEYBEE (*APIS MELLIFERA*)

OC 63

Jorge Langa[1], Melanie Parejo[1], June Gorrochategui-Ortega[1], Sofia Marcos[1], Marin Kovačić[2], Gennaro Di Prisco[3], WP[1] Partners BEE-GUARDS[4], Cecilia Costa[4], Iratxe Zarraonaindia[1,5]

[1]University of the Basque Country UPV/EHU, Leioa, Spain
[2]University of Osijek, Faculty of Agrobiotechnical Sciences, Osijek, Croatia
[3]IPSP-CNR Institute for Sustainable Plant Protection, Naples, Italy
[4]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[5]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Abstract

A balanced microbiota enhances bees' resilience to infections and environmental stressors. To fully characterize the honey bee gut microbiome beyond the species level and capture the functional capacities of its key bacteria, a comprehensive atlas of the microbial community is needed. To address this gap, within the Horizon Europe-funded BEE-GUARDS project, we aim to produce a comprehensive microbial reference through shotgun deep sequencing and bioinformatic analysis of hindgut samples collected across Europe. We thoroughly surveyed worker honeybee guts from 77 colonies collected in summer and autumn of 2024, across the whole European continent including 12 countries, six subspecies, and multiple climatic regions. Adopting a host-depletion molecular protocol to enrich microbiota, we retrieved more than 75% of shotgun sequenced reads belonging to bacteria, and 15% to viruses. The assemblies and coassemblies conducted (a total of 555) produced a MAG catalogue of 96 high-quality bacterial species and a total of 411 strains. Among them, *Snodgrassella*, *Bombilactobacillus*, *Bifidobacterium*, and *Gilliamella*, commonly identified species, accounted for the highest levels of intraspecies variability with 108, 48, 43 and 35 strains respectively. The presence, absence and abundances of several of these identified strains differed between regions and honeybee subspecies, highlighting host-specificity within the holobiont. In addition 6 new potential bacterial species were discovered. On top of that, we also have insights of the DNA-based gut virome, a fragment of the microbiome usually overlooked in honeybees.
In summary, this work describes, to our knowledge, the most comprehensive metagenome-assembled genome catalogue of the European honey bee to date, providing a high-quality reference resource for future research aimed at conducting comparative metagenomics in honeybee studies.

OC 64

DUAL EFFECTS OF THE BRASSICACEAE PHYTOCHEMICAL S-METHYL-L-CYSTEINE SULFOXIDE ON HONEY BEE PHYSIOLOGY AND METABOLISM

Saetbyeol Lee[1], Pavel Dobeš[2], Jacek Marciniak[2], Anna Mascellani Bergo[1], Martin Kamler[3], Petr Marsik[1], Radek Pohl[4], Dalibor Titera[3], Pavel Hyršl[2], Jaroslav Havlík[1]

[1]Czech University of Life Sciences Prague, Prague, Czechia
[2]Masaryk University, Brno, Czechia
[3]Bee Research Institute, Maslovice, Czechia
[4]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Science, Prague, Czechia

Abstract

Phytochemicals present in nectar and pollen can influence pollinator physiology, yet their functional roles under natural exposure remain poorly understood. Here, we investigated the distribution, seasonal dynamics, and physiological effects of S-methyl-L-cysteine sulfoxide (SMCSO), a sulfur-containing phytochemical abundant in Brassicaceae, in honey bees (*Apis mellifera*).

SMCSO and its metabolites were identified by ^{1}H NMR and quantified across seasons and castes. SMCSO concentrations showed strong seasonal variation, peaking in summer (1.36 µmol g^{-1}), approximately 5–6-fold higher than in spring, autumn, and winter ($q < 0.0002$). Higher accumulation was observed in foragers compared to nurse bees, particularly in the abdomen (2.8 vs. 1.28 µmol g^{-1}; $q = 0.0286$), consistent with increased dietary exposure during foraging.

Controlled feeding experiments were conducted to assess physiological effects. Acute exposure (1 mg mL^{-1}) did not affect survival but increased antioxidant capacity in multiple body parts (up to 2-fold; $p \leq 0.0411$). In contrast, long-term exposure (10 days) reduced head and thorax mass ($p = 0.0086$ and $p = 0.0028$, respectively), suggesting potential effects on glandular and flight-related tissues.

1H NMR-based metabolomic analysis further indicated that prolonged SMCSO exposure altered amino acid metabolism, including changes in key metabolites such as lysine and γ-aminobutyric acid, pointing to potential effects on nutritional physiology and behaviour.

Overall, SMCSO exhibited context-dependent effects, enhancing antioxidant capacity under short-term exposure while inducing metabolic and physiological trade-offs during prolonged intake. These findings highlight the complex role of plant-derived compounds in shaping honey bee health in agricultural landscapes dominated by Brassicaceae crops.

This research was supported by the Ministry of Agriculture of the Czech Republic (QL26010035 and QK21010088).

CONTEXT-DEPENDENT EFFECTS OF BACTERIAL SUPPLEMENTATION ON THE HONEY BEE GUT MICROBIOME AND PHYSIOLOGICAL RESPONSES

OC 65

Saetbyeol Lee[1], Pavel Dobeš[2], Anna Mascellani Bergo[1], Jacek Marciniak[2], Jana Hurychova[2], Sara Sreibr[2], Martin Kamler[3], Vojtech Purnoch[3], Jiri Killer[1,4], Lucie Hlinakova[5], Dalibor Titera[3], Ondrej Cinek[5], James C. Carolan[6], Pavel Hyršl[2], Jaroslav Havlík[1]

[1]Czech University of Life Sciences Prague, Prague, Czechia
[2]Masaryk University, Brno, Czechia
[3]Bee Research Institute, Maslovice, Czechia
[4]Institute of Animal Physiology and Genetics v.v.i, the Czech Academy of Sciences, Prague, Czechia.
[5]Charles University and University Hospital Motol, Prague, Czechia
[6]Maynooth University, Maynooth, Ireland

Abstract

Improving the honey bee gut microbiome has been the focus of increasing research interest, with several approaches, including probiotic supplementation, proposed to enhance core gut symbionts. However, these approaches have largely been evaluated in isolated settings, limiting understanding of how microbiome-based interventions translate across laboratory and field conditions. Here, we applied several omics techniques to assess the effects of gut-derived bacterial supplementation under controlled, semi-controlled (with nestmate interaction), and field conditions. Newly emerged bees received a defined mixture of *Lactobacillus helsingborgensis*, *Lactobacillus apis*, *Bifidobacterium choladohabitans*, and *Bifidobacterium polysaccharolyticum* for treatment groups, or a sucrose for control groups. Gut samples collected at day 10 were analyzed using 16S rRNA sequencing, ^{1}H NMR metabolomics, and label-free quantitative proteomics (controlled conditions only).

Bacterial supplementation altered the gut microbiota, with the strongest effects observed under controlled condition, where supplemented bees were enriched in *Lactobacillus* and *Bifidobacterium*. Under field condition, overall genus-level restructuring was limited, but *Lactobacillus helsingborgensis* remained significantly enriched in supplemented bees. Proteomic analysis showed supplemented bees displayed increased abundance of major royal jelly protein precursors and mitochondrial-associated proteins, whereas controls showed higher abundance of ribosomal and translation-related proteins in the controlled conditions. In the semi-controlled condition, supplemented bees exhibited a proteomic profile characterized by proteins associated with energy metabolism, while controls were enriched in proteins related to translation pathways. Metabolomic profiling revealed reproducible treatment-associated trends across all conditions, including increased microbial fermentation products such as acetate and succinate, as well as neuromodulatory metabolites including ornithine and γ-aminobutyrate. In contrast, sucrose, N-acetylglucosamine, and uridine were consistently lower in supplemented groups.

Together, these findings highlight reproducible, context-dependent effects of bacterial supplementation on honey bee gut physiology and support the interpretation of microbiome-based interventions under different environmental conditions.

This research was supported by the Ministry of Agriculture of the Czech Republic (QL26010035 and QK21010088).

OC 66 THE IMPORTANCE OF GUT FLORA IN THE OVERWINTERING PROCESS FOR SWEDISH HONEY BEES (*APIS MELLIFERA*)

Sonja Leidenberger, Erik Gustafsson, Niclas Norrström

University of Skövde, School of Bioscience, Skövde, Sweden

Abstract

Overwintering is one of the most crucial and risky periods in Swedish beekeeping. During this time, bee colonies are completely dependent on the food stores available to them. Winter losses can have major economic and ecological consequences. Additionally, there is a significant lack of scientific knowledge about how different types of winter feed affect bee health under Swedish conditions. This project compared three feeding strategies and their effects on the gut microbiome: honey, sugar and a combination of both. We analyzed (1) the gut microbiome of 10 Swedish honeybee (*Apis mellifera*) colonies using Illumina sequencing (shotgun metagenomics), (2) compared the bacterial composition and relative abundance across the three different diets (honey, sugar, honey-sugar mixture) over one winter season (October 2024 to March 2025) and (3) examined whether the overwintering diets affect the bees' health condition at the beginning of the new season.

The results show that the choice of winter feed is related to both changes in the bees' gut microbiome and the strength of colony development during the following spring. Colonies that overwintered on honey generally showed the fastest and strongest spring development with higher brood production and greater pollen collection. Colonies that overwintered on sugar generally developed more slowly and had weaker growth, while colonies that received a combination of honey and sugar in most cases fell between these extremes. At the same time, changes in the composition of core gut bacteria genus (core microbiome) were observed depending on diet, suggesting that winter feed affects the microbial environment that bees bring into the new season. For Swedish beekeepers, this means that the choice of winter feed may be important for how quickly a colony gets started in the spring. This could in turn affect pollination, honey production and the general strength of the bee colony.

ASSESSING MICROPLASTIC-INDUCED TOXICITY IN HONEY BEES (*APIS MELLIFERA LIGUSTICA*) AND THEIR ASSOCIATED MICROBES

OC 67

Maria Ludovica Saccà[1], Carmen Fajardo[2], Giovanni Cilia[1]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]Department of Biomedicine and Biotechnology, Faculty of Pharmacy, University of Alcala, Alcala de Henares, Spain

Abstract

Microplastics (MPs) represent one of the most significant environmental threats of the present century. Honey bees are recognised as good candidates for biomonitoring environmental MP pollution due to their exposure to complex mixtures of MPs under field conditions. However, our understanding of the risks posed to these pollinators by chronic exposure to MPs is still limited. This study investigated the effects of three types of MPs (polystyrene, polyethylene and polymethyl methacrylate) on *Apis mellifera ligustica* mortality and key bacterial taxa, using qPCR. Laboratory assays involved feeding the bees MPs either individually or in binary and ternary combinations. Exposure to all of the tested MPs reduced the bees' survival rates significantly, with the combined treatments having the most severe impact. However, the detrimental effect of the MP ternary mixture on bee survival (Median Lethal Time of 9 days compared to 19 days in the control) was not reflected in its effect on bee microbiota, which did not differ from the untreated control. This suggests that the mortality of bees fed with the ternary mixture was related to factors other than changes in the abundance of the analysed bacterial taxa. More than half of the analysed bacterial taxa increased over the course of the experiment, with a stronger acute increase in bees fed MPs, which may be due to an initial compensatory response to maintain microbial functional stability. Furthermore, bacterial strains were isolated from MP-treated bees to investigate their potential for plastic biodegradation. Understanding the relationship between gut bacteria and host health following bee exposure to MPs may be key to comprehending their environmental impact.

OC 68 PROBIOTIC POTENTIAL OF GUT BACTERIA FROM THAI HONEY BEES IN ENHANCING QUEEN HEALTH DURING LARVAL REARING

Sanchai Naree[1], Worachote Boonsriwong [1], Guntima Suwannapong[2]

[1]Pharmacology and Biopharmaceutical Department, Faculty of Pharmaceutical Sciences, Burapha University, Chon Buri, Thailand
[2]Department of Biology, Faculty of Science, Burapha University, Chon Buri, Thailand

Abstract

Honey bees play an important role as pollinators in both natural and agricultural environments. The health and quality of the queen are essential for the productivity and survival of the colony. Recently, using beneficial gut bacteria has emerged as a promising way to improve honey bee health. This study investigated the probiotic potential of gut bacteria isolated from Thai honey bees, including *Apilactobacillus kunkeei* and *Bifidobacterium* sp., in improving queen health during larval rearing. One day old larvae were grafted and fed with basic larval diet (BLD) supplemented with 2×10^5 colony-forming units (CFU)/ mL of gut bacterial isolates. Then, all larvae were kept in desiccator at 34 ± 2°C with 95 ± 2% relative humidity. We evaluated key developmental and physiological parameters of queens and compared them to untreated controls. The results showed that probiotic supplementation considerably improved larval survival rates, successful queen emergence, and overall queen quality. Queens from the treated groups had a higher body weight at emergence. These findings suggest that using honey bee gut bacteria as probiotic supplements is a promising and sustainable way to enhance queen rearing results and overall colony performance.

EFFECTS OF DIETARY SUPPLEMENTATION ON IMMUNE GENE EXPRESSION IN HONEY BEES INFECTED WITH *NOSEMA CERANAE*

OC 69

José Sánchez-Sánchez[1], Antonio Martínez Mateo[2], Pedro Díaz Molins[2], Pilar De la Rúa[1], Álvaro Urueña[1]

[1]Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain
[2]ZUKAN Pol. Ind. La Estrella, Molina de Segura, Spain

Abstract

Honey bee colony losses remain a major concern worldwide, and pathogen infections are recognized as one of the key drivers behind this decline. Among these pathogens, *Nosema (Vairimorpha) ceranae*, an obligate intracellular microsporidian parasite, has become the predominant species infecting honey bees, causing nosemosis type C. This disease negatively affects worker survival, behaviour, and various physiological functions, ultimately compromising colony performance. Recent studies suggest that certain dietary supplements may alleviate the effects of N. ceranae infection by improving the gut microbiota or limiting parasite proliferation, highlighting nutrition as a promising avenue for mitigation.

In this study, we evaluated the influence of dietary supplementation on the western honey bee subspecies *Apis mellifera iberiensis* in the context of *N. ceranae* infection. Worker bees were experimentally infected with *N. ceranae* spores and subsequently fed a sugar syrup–based diet enriched with compounds with potential immunomodulatory properties. The tested supplements included fructooligosaccharides (FOS), postbiotics, and the phyto-pharmacological preparation Nozevit. These additives represent different modes of action, ranging from prebiotic enhancement of the gut environment to plant-derived compounds reported to reduce microsporidian development.

To assess the physiological impact of these treatments, we carried out gene expression analyses targeting immune-related pathways. This approach allowed us to quantify the honey bees' immune response under each dietary regimen and evaluate whether supplementation contributes to improved resilience against infection. The results provide valuable insights into how nutritional strategies may modulate honey bee immunity during *N. ceranae* infection. Our findings support the growing evidence that diet plays a crucial role in shaping host–pathogen interactions and may contribute to the development of alternative, sustainable methods to mitigate the impact of this pervasive pathogen on honey bee health.

OC 70

MAPPING MICROBIOTA-DERIVED METABOLITES FROM THE GUT TO THE BRAIN IN HONEY BEES

Laurie Thouvenel, Julie Pacull, Damien Justrobe, Simon Duthen, Amélie Cabirol

Center for Research on Animal Cognition - CNRS - University of Toulouse, Toulouse, France

Abstract

Honey bee behaviour is under the influence of the micro-organisms located in their gut and the metabolite they produce. While gut bacteria have evolved unique metabolic functions to degrade the nutrients and non-nutritive compounds ingested by the bee, few microbiota-produced metabolites have been identified for their effect on the neural functions supporting behaviour. Here, we applied an integrative metabolomics framework to investigate gut–brain metabolic communication in microbiota-deprived bees and in bees colonized with either a native microbiota or a defined synthetic community of native gut bacteria.

Untargeted LC–MS profiling of gut, hemolymph, and brain tissues identified metabolites differentially abundant between conditions across compartments, revealing candidate molecules potentially involved in systemic signaling. To characterize microbiota-dependent shifts in both gut and brain metabolism, we performed 13C-glucose labeling, enabling the tracing of carbon incorporation into metabolites. Finally, we used desorption electrospray ionization (DESI) spatial metabolomics to map the localization of differentially regulated metabolites within the brain. By integrating these complementary approaches, we reconstruct the trajectory of microbiota-associated metabolites from their production in the gut to their detection and spatial distribution in the brain. This strategy allows us to distinguish candidate metabolites potentially transferred across compartments from those arising through local metabolic reprogramming.

Together, our results provide a systems-level view of microbiota-driven metabolic changes along the gut–brain axis and establish a framework for linking microbial metabolism to brain biochemistry in honey bees. This work advances our understanding of host–microbiota interactions and their potential impact on neural function in this key pollinator species.

Plasticity and genomic variation to overcome threats

OC 71

ORGANIC FARMING AND LANDSCAPES SHAPES BEE COMMUNICATION, PLANT-POLLINATOR INTERACTIONS AND DIVERSITY OF CAVITY NESTING SOLITARY BEES

Samuel Boff[1,2], Sara Olberz[3], Sophie Hecht[4], Josué Raizer[5], Tina Schultz[1], Ola Olsson[4], Hannah Burger[1], Manfred Ayasse[1]

[1]Ulm University, Ulm, Germany
[2]Federal University of Maranhão, São Luis, Brazil
[3]Technical University Munich, Munich, Germany
[4]Lund University, Lund, Sweden
[5]Federal University of Grande Dourados, Dourados, Brazil

Abstract

Agricultural intensification is a major driver of pollinator decline, yet the mechanisms by which farming systems influence wild bees remain poorly understood. We present an integrative assessment of how organic versus conventional farming affects the ecology, behavior, and population dynamics of cavity-nesting solitary bees, focusing on *Osmia bicornis*, across agricultural landscapes in Germany. Using standardized trap nests ("bee hotels") across 17 sites, we evaluated reproductive output, population structure, species diversity, floral resources, and chemical communication. Organic farming consistently promoted higher brood cell production, nesting frequency, and the abundance of both female and male bees at local and landscape scales. Landscapes with a greater proportion of organic farming supported increased species richness and diversity, while forest cover showed no strong effects. Organic management also enhanced meadow plant diversity, which translated into a more diverse pollen diet in bee nests, indicating improved foraging conditions. Despite these benefits, organic farming influenced bee phenotype and behavior. Individuals from organic sites were smaller and exhibited distinct cuticular hydrocarbon profiles compared to those from conventional systems. Behavioral bioassays revealed stronger male attraction to females from conventional farms; however, this difference was driven by chemical cues, as applying synthetic semiochemicals to females from organic sites restored their attractiveness. Overall, our findings demonstrate that organic farming enhances key ecological parameters of solitary bees, including reproduction, diversity, and resource use, while also shaping phenotypic traits and mating-related chemical communication. These results highlight the importance of integrating ecological and behavioral perspectives when designing conservation strategies and support the expansion of organic farming as a means to promote pollinator biodiversity in agricultural landscapes.

MONITORING THE DISTRIBUTION OF *APIS MELLIFERA* MITOCHONDRIAL DNA LINEAGES OVER EIGHT YEARS IN THE EMILIA-ROMAGNA REGION USING HONEY EDNA

OC 72

Maria Letizia Calabri, Valeria Taurisano, Anisa Ribani, Luca Fontanesi

Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

Abstract

Honey contains DNA derived from all organisms involved in its production, including honey bees (*Apis mellifera*), making it a non-invasive and valuable source of genetic information for honey bee population genetic analysis. In this study, we used DNA assays we previously developed to identify the mitochondrial DNA lineages of the honey bees that produced honey. These assays differentiate three mitochondrial DNA (mtDNA) lineages (A, C, and M) of *Apis mellifera* and distinguish two mitotypes within the C lineage: C1 and C2, predominantly associated with *A. m. ligustica* and *A. m. carnica*, respectively. This study included a total of approximately 1200 honey samples produced from 2018 to 2025 in the Emilia-Romagna region (in the North of Italy). The C1 mitotype was the most frequent, indicating the ongoing prevalence of *A. m. ligustica* in regional beekeeping. Some samples carried the C2 mitotype, and a few contained both C1 and C2, suggesting genetic heterogeneity. Additionally, a small number of honey samples included A and/or M lineages in addition to the C lineage. Geographical data from the honey samples allowed us to map mitotype distribution across the Emilia-Romagna region and evaluate the overtime modifications in local honey bee populations. These findings offer valuable information for developing conservation strategies for honey bee genetic resources and preserve the genetic integrity of local *A. mellifera* populations.

OC 73

INTERFERENCE OF NUCLEAR MITOCHONDRIAL INSERTIONS (NUMTS) IN THE MITOGENOME ANALYSIS OF ALGERIAN HONEYBEES *APIS MELLIFERA INTERMISSA* AND *SAHARIENSIS*

Amira Chibani Bahi Amar[1,2], Alain Vignal[3], Pierre Faux[3], Nacera Tabet Aoul[2,4], Thibault Leroy[3], Emmanuelle Labarthe[3], Kamila Canale Tabet[3]

[1]Department of Biology, Faculty of Natural and Life Sciences, University of Saida Dr. Moulay Tahar, 20000, Saida, Algeria
[2]Laboratory of Molecular and Cellular Genetics (LGMC), Department of Applied Molecular Genetics, University of Sciences and Technology of Oran Mohamed Boudiaf (USTOMB), Oran, Algeria
[3]GenPhySE, Université de Toulouse, INRAE, INPT, INP-ENVT, France
[4]Department of Biotechnology, Faculty SNV, University of Oran1 Ahmed Ben Bella, Saida, Algeria

Abstract

The honeybee *Apis mellifera* is a key pollinator insect that plays a major role in maintaining biodiversity and ecosystem stability, yet faces multiple threats affecting its survival worldwide. In light of these concerns, characterizing and understanding the genetic makeup of *Apis mellifera* subspecies is essential to assess their genetic diversity, to understand their evolutionary history and to highlight their adaptative potential. In Algeria, studies have shown that two morphologically distinct subspecies exist and occupy different geographic ranges. *Apis mellifera intermissa* is found in the north of the country, while *Apis mellifera sahariensis* is rather found in the south. In this study, we aimed to genetically characterize the Algerian honeybees by analysing the mitochondrial DNA (mtDNA) of 151 Algerian haploid drones obtained from whole genome Illumina sequencing. Integrated analyses were performed on a dataset containing also African and European reference populations. The results showed no substantial admixture in the Algerian honeybee populations studied and confirmed the absence of genetic differentiation between the two local subspecies, as reported in our previous study based on whole genome sequencing analyses. The genetic proximity between the Algerian subspecies and the Iberian Peninsula subspecies, *A. m. iberiensis* was confirmed. Alignment biases were detected during the mitogenomes analyses, probably caused by the presence of Nuclear Mitochondrial DNA (NUMTs) insertions, which may lead to variant calling errors, and potentially cause misinterpretations of genetic variations. We indeed found that the reference genome used for read mapping can influence the results, potentially creating coverage gaps or misalignments of certain genes. Overall, our study provided a comprehensive view of genetic diversity of Algerian honeybees. Also, these findings highlight the importance of careful bioinformatics analyses for mitochondrial sequences analyses and suggests using pangenome approaches to solve the problems appearing when using a single reference genome.

GENETIC PARAMETERS FOR HONEY PRODUCTION, GENTLENESS AND HYGIENIC BEHAVIOR IN THE "ABEILLE LIGÉRIENNE" SELECTIVE BREEDING PROGRAM IN FRANCE

OC 74

Jonanthan d'Ambrosio[1], Robin Azemar[2], Benjamin Poirot[3], Michel Sourdioux[4], Benjamin Basso[5], Florence Phocas [6], Pierrick Haffray[1]

[1]SYSAAF, Rennes, France
[2]Abeille Ligérienne, Lagord, France
[3]Abeille Ligérienne, Lagord, France
[4]SYSAAF, Nouzilly, France
[5]INRAE, Avignon, France
[6]INRAE, Jouy-en-Josas, France

Abstract

We report estimations of genetic parameters and breeding values (EBVs) for honey production traits in a small-sized breeding program initiated in 2016 by 17 professional beekeepers (L'Abeille Ligérienne, Lagord, France) in a multi-subspecies "Buckfast" type line.

Virgin queens were mated in island mating station or inseminated with selected drone-producing colonies. 1 727 queens were phenotyped through 9 year-classes (29 apiaries). The pedigree relationship matrix was calculated using an R program (Brascamp and Bijma, 2019). Genetic parameters and EBVs were estimated using AIREMLF90 of BLUPf90 software package, applying a linear mixed model with a single direct genetic effect considered, either the queen effect (Queen Model: QM) or workers' one (Workers Model: WM).

Honey production heritability (standard error) for Workers (WM) and Queen (QM) Models were 0.43 (0.09) and 0.28 (0.08). The colony ranking for honey production did not change according to the model used (Pearson correlations 0.88). The colony rankings between honey production EBVs and colony honey performances corrected for year and apiary were consistent (correlations of 0.71 with WM and 0.78 with QM). Selection the 10% best queens on pedigree-BLUP EBVs by Queen model led to increases in selection response of 16% when compared to mass selection on phenotypes corrected for environmental effects. Heritability with Queen Model were 0.54 (0.08) for gentleness and 0.27 (0.12) for hygienic behavior (pint test). Pearson correlations between honey production and gentleness or hygiene or between gentleness and hygiene were limited to null. Heritabilities in this limited size, but optimized, selective breeding program were in the higher range previously reported in generally less genetically optimized breeding schemes. The correlations between EBVs are favorable to introduce multi-trait breeding index to balance genetic selection.

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OC 75 ALARM PHEROMONE AND STRESS RESPONSE: UNDERLYING MECHANISMS AND FACTORS OF PLASTICITY IN HONEY BEES

Jean-Marc Devaud[1,2], Alice M. Durand[1], Simon Marty[1]

[1]Research Centre on Animal Cognition (CRCA), Centre for Integrative Biology (CBI); CNRS, Toulouse University, Toulouse, France
[2]Institut Universitaire de France, Paris, France

Abstract

In honeybees, the sting alarm pheromone is emitted upon direct threat and promotes recruitment of nestmates for colony defense. Exposure to this pheromone, or to its main component IPA (isopentylacetate), triggers stereotyped responses reminiscent of some effects of stress in vertebrates. At the behavioural level, these include aggressiveness, decreased motivation for food and decreased sensitivity to aversive stimuli, as well as reduced learning performances.

Using learning as a behavioural readout, we have identified some allatostatins and their receptors as mediators of a stress response triggered by IPA. Allatostatins are a family of neuropeptides which were primarily identified for their role in pacing development through inhibition of juvenile hormone (JH) release, but their functions in the adult brain had remained elusive. We showed that signaling through the A-type allatostatin (ASTA) is necessary and sufficient to induce some effects of IPA exposure, and we propose that ASTA plays a major role in the neuro-hormonal coordination of the stress response in adult bees, and in particular in regulating a behavioural switch between foraging and defense activities.

Our results also indicate that this stress response is plastic since, among the colony, different categories of individuals display variable levels of sensitivity to IPA, and because some effects of IPA exposure can be experimentally modulated by manipulating the bee's experience. Our current research aims at characterizing the extent of such plasticity, and at exploring the neural and/or hormonal components of the stress response as possible targets to improve resilience in bees.

SELECTBEES 2.0: IMPLEMENTATION OF ASSORTATIVE MATING, VARROA MITE POPULATION DYNAMICS AND SINGLE DRONE INSEMINATION IN AN AUSTRIAN RESISTANCE-BREEDING RESEARCH PROGRAM

OC 76

Thomas Druml[1,2], Stefan Mandl[3], Berthold Heinze[1]

[1]BFW-Austrian Research Centre for Forests, Department for forest growth, silviculture and genetics, Vienna, Austria
[2]BBS, Nötsch, Austria
[3]Bienenhof Mandl GmbH, Traiskirchen, Austria

Abstract

Varroa resistance in honeybees represents a complex trait caused by multiple environmental effects, polygenic background, and/or gene regulatory networks. However, measuring reliable and cost-effective resistance traits in the framework of breeding programs still remain an open question.

In this paper we present an approach that combines population genetic measures as founder gene contributions with repeated measures of phoretic mite population growth, SMR/brood infestation levels, honey yield in order to design a breeding plan applying assortative mating strategies at phenotypic and genetic level.

The designed mating plan aimed to increase resistance related founder gene contributions (derived from pedigree analysis) from parental to offspring generations. From the phenotypic side we selected from 1.945 testing colonies (resistance related founder contributions = 26.2%) 69 potential parents (resistance related founder contributions = 56.2%). By assortative mating the resistance related founder contributions could be increased to 62.3% in the offspring generation, that finally descended from 37 dams (breeding queens) and 10 sires (drone producing queens). Because of genetic diversity matters we aimed to keep the gene pool as wide as possible including a maximum number of 15 potential allele segregation axes (i.e. maternal dam lines) in the mating plan, which represents 52% of total segregation axes within the total population. Among performance testing colonies we could show, that the phenotypic selection approach was able to increase the amount of SMR>40% from 11% in the years 2022/23 to 59% in 2025. The first generation of SDI colonies (130 colonies in total) resulted in 46 colonies (35.4%) with a SMR level > 40%. Data for the second SDI generation will be available in August this year.

Finally, we aim in this research project to gather high-density and high-quality phenotypic data to set up a genome-wide association study at the final step of the research program.

OC 77 THE IMPACT OF HYGIENIC BEHAVIOR AND INDIVIDUAL IMMUNITY ON THE SPREAD OF VIRAL INFECTION WITHIN THE COLONY

Tal Erez[1,2], Angelina F. Osabutey[1], Elad Bonda[1], Assaf Otmy[1], Sofia Levin-Nikulin[3], Poppy J. Hesketh-Best[3], Clarissa Pellegrini Ferreira[4], Nor Chejanovsky[1], Marla Spivak[4], Declan C. Schroeder[5], Victoria Soroker[1]

[1]ARO, Rishon LeTzion, Israel
[2]The Hebrew University of Jerusalem, Rehovot, Israel
[3]University of Minnesota, Saint Paul, Israel
[4]University of Minnesosota, Saint Paul, Israel
[5]Minnesosota, Minneapolis, Israel

Abstract

The combination of *Varroa destructor* and the viruses the mite vectors is a major driver of honey bee (*Apis mellifera*) colony losses. Individual immunity, and social immunity through hygienic behavior enable bees to defend against some pests and parasites. However , the efficacy of hygienic behavior against virus-infected pupae, independent of Varroa infestation, has been questioned. In field studies, in USA and Israel we inoculated mite-free pupae with DWV local B-A recombinant collected from high and low hygienic colonies and quantified the rates of uncapping and removal of sham and virus-inoculated pupae by the bees in the colony as well as expression of individual immunity in worker bees. Our results demonstrate that both virus injected and PBS injected pupae experienced significantly higher removal by hygienic colonies. Moreover, the brood treatment resulted in dynamic changes in both viral loads and immune genes in nurse bees within the colony. The viral loads of both the DWV B-A recombinant and IAPV changed significantly with time. Surprisingly, despite the direct inoculation of pupae with DWV B-A, the DWV loads as measured by the DWV-A RdRp primers in sampled adult worker bees decreased 24 hours post inoculation, mainly in workers from hygienic colonies, whereas the DWV-B loads (DWV-B RdRp primers) did not change with time in both phenotypes, but IAPV loads increased in both phenotypes. The impact of hygienic behavior on viral transmission and colony health will be discussed.

BUMBLEMATE – AGENT-BASED MODELLING OF BUMBLEBEE MATING FLIGHTS TO PREDICT GENE FLOW AND DELIMIT POPULATIONS

OC 78

Lilian Gornall[1], Frank Sommerlandt[2], Anne-Kathrin Schneider-Hohenbrink[2], Jens Dauber[1,2], Wiebke Sickel[2]

[1]TU Braunschweig, Braunschweig, Germany
[2]Thünen Institute of Biodiversity, Braunschweig, Germany

Abstract

Monitoring genetic diversity in bumblebees is crucial, as its loss poses a significant threat to populations and compromises their resilience and adaptability to changing environmental conditions. Due to their high agility and widespread distribution, the delimitation of bumblebee populations remains a major challenge for genetic diversity assessments and has so far only been possible by means of complex and expensive genetic analyses. Alternative strategies for delimiting bumblebee populations that do not rely on genetic data are required.

To remedy this issue, we have developed BumbleMATE, an agent-based model. BumbleMATE depicts dispersal capabilities of bumblebees by modelling sex-specific behavioural patterns such as flight and dispersal trajectories during mating season and outputs mating networks, which cannot be produced through DNA-based methods at a sufficient scale and resolution. Based on a generic bumblebee ecotype, we ran nine scenarios with ten repeats each, that differed in nest densities and distributions.

Overall, all mating networks exhibited a dominant gene flow cluster that encompassed the majority of colonies, indicating sufficient gene flow. Reducing nest densities increased network modularity and the number of unmated agents. Differences in spatial distribution had a smaller effect on mating networks, but local nest clusters led to shorter distances between colonies of mating partners, indicating an increased probability of population sub-structuring. As these patterns align well with literature, BumbleMATE has the potential to become a powerful tool to model gene flow and establish a basis for *in silico* genetic diversity monitoring of bumblebees.

OC 79

GENETIC DIVERSITY IN HONEYBEE SUBSPECIES AND MATING STATIONS IN AUSTRIA – MICROSATELLITE DNA TESTS

Berthold Heinze

Austrian Research Centre of Forests BFW, Vienna, Austria

Abstract

The microsatellite DNA method was established and applied to accompanying and counselling breeders of the dark bee (*A.m. mellifera*) in Austria over several years, to investigate mating patterns in various Carnolian (*A.m. carnica*) mating stations, and to investigate the local gene pool in southern Austria. Breeding lines of the Austrian Mellifera Breeders (AMZ organisation, 2018-2024) showed, in most cases, acceptable low levels of admixture and introgression from other species (though a bit higher than in Carnolian bees), and no marked inbreeding. Genetic lineages could be distinguished within the material. Four breeding stations for the Carnolian bee were analysed, including one in the city of Vienna with no marked geographically isolating landscape features. Still, correct mating rates were mostly at 80 % and above, up to almost 100 % in mountainous areas. Individual queen behaviour seemed to be responsible for variability in correct matings – some queens seemed to 'catch' different drone genetics, compared to the rest. An initial exploration of Carnolian genetic diversity in Southern Austria showed that genetic integrity of these honeybees remains intact and follows a clinal pattern, with some queen imports (of other Carnolian lines) occasionally disturbing such an interpretation. Distinction from neighbouring regions and other breeds, especially from Italian *A.m. ligustica*, were not straightforward with the microsatellite markers, but can be enhanced with mitochondrial DNA sequence variants. – While these "patchy" results do not give a complete status of genetic diversity of honeybees present in Austria, a picture of considerable levels of diversity in both subspecies, more marked in Carnolian bees, emerges, which calls for efforts in conservation and careful planning of breeding efforts, especially when choosing drone producing colonies for mating stations.

DE NOVO ASSEMBLY OF *CSD* ALLELES FROM MULTIPLE HONEY BEE SUBSPECIES

OC 80

Guilhem Huau, Kamila Canale Tabet, Alain Vignal

GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet Tolosan, France

Abstract

In honey bees, sex is determined by a haplodiploid system, typical of hymenopterans. Heterozygosity is required at the hypervariable region of the protein coded by the csd gene so that embryos develop as females, whereas hemizygous unfertilised eggs or eggs homozygous at csd, will develop as males. However, these diploid males are killed by workers in the colony and as a consequence, to avoid homozygosity, a very high diversity is found at the locus and studying this diversity is essential to the management of closed populations. Over 370 csd alleles have been described to date in the hymenoptera genome database, constructed by collecting data across several studies, each of which target a specific population and detect around 100 alleles for a few hundred workers or drones sequenced. Although some studies were performed by NGS sequencing, most of the alleles described were analysed by Sanger sequencing a PCR product. However, in our experience, some samples fail to amplify, probably due to sequence divergence in the primer sequences and preventing the discovery of some of the alleles. Here, we take advantage of over 1200 haploid male genomes sequenced by Illumina in the SeqApiPop project, to reconstruct csd alleles by de novo assembly. Cases in which drones were sampled from the same colony are used to check the accuracy of the method, as two alleles are expected at most. The rest of the data is used to detect alleles in samples from various subspecies including *A.m. mellifera*, *ligustica*, *carnica*, *caucasia*, *intermissa* or *adansonii*, whose genetic background has been defined by admixture analyses in previous projects. Analysis of the sequences flanking the locus will also allow to improve primer design for PCR and lower-cost analyses.

OC 81

MANAGEMENT OF GENETIC DIVERSITY IN HONEYBEES

Jana V. Huml[1,2,3], Richard Bernstein[4], Andreas Hoppe[4], Mairi E. Knight[3], Jonathan S. Ellis[3], Victoria Buswell[5], Gabriele Soland-Reckeweg[1]

[1]Apigenix, Twann, Switzerland
[2]mellifera.ch, Urdorf, Switzerland
[3]University of Plymouth, Plymouth, United Kingdom
[4]Länderinstitut für Bienenkunde, Hohen Neuendorf, Germany
[5]James Hutton Institute, Dundee, United Kingdom

Abstract

The loss of genetic diversity and the increase of inbreeding in honeybees are issues of concern both in a conservation as well as in a breeding context, due to small population sizes and selective breeding processes. An increase in inbreeding can show negative effects in the short term, such as diploid drone production, and impact colony performance. In the long term, the loss of genetic diversity reduces the evolutionary potential to adapt to changing environmental conditions and emerging threats. Here we evaluate methods to measure inbreeding, genetic diversity and effective population size in honeybees in a routine monitoring scheme to assist management decisions with the aim to reduce the loss of genetic diversity. Whilst whole genome sequencing (WGS) offers the most detailed results on genetic diversity patterns, it is generally less suitable than SNP arrays for routine monitoring, where a quick turnaround is required at a reasonable cost. The Illumina Honeybee_2021 SNP-array, for which methods for subspecies classification and quantification of introgression have recently been developed (Huml et al., in Review) is a promising tool for the assessment of genetic diversity because of its high SNP density (~70K markers). Therefore, we compare estimates of genetic diversity between 94 WGS and Honeybee_2021 SNP-array replicate samples to optimize and validate the performance of the SNP-array. We discuss the utility of different diversity matrices to inform management decisions.

SEASON SHAPES THE MOLECULAR RESPONSE OF HONEY BEES TO VARROA PARASITISM

OC 82

Jana Hurychová[1], Jacek Marciniak[1], Rachel Ward[2], Saetbyeol Lee[3], Sara Šreibr[1], Romana Moutelíková[4], Pavel Hyršl[1], Jana Prodělalová[4], Martin Kamler[5], Jaroslav Havlík[3], Kevin Kavanagh[2], Pavel Dobeš[1]

[1]Masaryk University, Brno, Czechia
[2]Maynooth University, Maynooth, Ireland
[3]Czech University of Life Sciences Prague, Prague, Czechia
[4]Veterinary Research Institute, Brno, Czechia
[5]Bee Research Institute in Dol, Máslovice, Czechia

Abstract

The impact of the ectoparasitic mite *Varroa destructor* on honey bee physiology is well documented, but its effects across different seasonal worker phenotypes remain poorly understood. We applied combined metabolomic and proteomic approaches to investigate how parasitism alters the molecular physiology of short-lived summer and long-lived winter workers. In all tissues analysed, seasonal phenotype was the dominant source of variation. In summer bees, parasitism caused high post-emergence mortality and pronounced metabolic and proteomic shifts, including altered amino acid metabolism, protein maintenance, cuticle remodelling, and redox homeostasis. Parasitised winter bees showed limited metabolic plasticity but exhibited disrupted neural function, and selective depletion of antioxidant enzymes and simple carbohydrates. Despite these seasonal differences, *Varroa* parasitism also triggered universal responses, with parasitised bees investing more in fatty acid metabolism and membrane remodelling, while reduced activity of TCA cycle and depletion of their immune and nutritional proteins indicated impaired longevity maintenance. Overall, our results show that *V. destructor* does not trigger a uniform stress response, instead it exploits seasonally distinct physiological states to produce divergent molecular stress patterns with consequences for worker survival and colony resilience.

This research was supported by the Ministry of Agriculture of the Czech Republic (QK21010088 and QL24010241).

OC 83 HARNESSING NATURAL SELECTION TO DEVELOP *VARROA*-RESISTANT HONEYBEES IN AN ISOLATED POPULATION

Benoît Jobart, Jean-Marie Chatel

GDS Réunion, La Plaine des Cafres, France

Abstract

The rapid global spread of the parasitic mite *Varroa destructor* has not spared La Réunion, an island in the south-western Indian Ocean. First detected in 2017, this parasitic mite has caused an unprecedented health crisis in the local beekeeping sector and continues to result in colony losses. The island is home to a single subspecies of honeybee, *Apis mellifera unicolor*, which is endemic to this part of the Indian Ocean. Strict regulations prohibit the importation of honeybees, creating a closed population and limiting selection and breeding to local genetic resources.

Current control strategies rely primarily on chemical treatments; however, the emergence of acaricide resistance in *V. destructor* represents a major concern for the sustainability of these approaches. In this context, selective breeding for natural resistance appears as a promising alternative yet remains challenging in isolated systems.

This study aims to evaluate the effectiveness of targeted natural selection in enhancing resistance to *V. destructor* in *A. m. unicolor*. An experimental apiary was set up in accordance with the principles of the 'Darwinian Black Bee Box'. Around 30 colonies were monitored over three 10-month periods without the use of acaricides. During these periods, monthly monitoring of phoretic mite infestation levels, colony strength and health status was carried out. Colonies that survived while maintaining a low phoretic mite infestation level were selected and multiplied using controlled breeding techniques (instrumental insemination, guided mating).

To measure the progress made, a group of descendant colonies of resistant phenotypes was compared with a group of control colonies not selected for resistance. We expect the selected colonies to show improved survival, reduced mite infestation and better health status. This work would contribute to highlighting the potential of directed natural selection as a sustainable strategy for managing *V. destructor* in isolated honeybee populations.

COMPARATIVE POPULATION GENOMICS OF WILD AND MANAGED HONEY BEES

OC 84

Patrick L. Kohl[1], Gabriel Ahlenstorf[2], Nadine Chapman[3], Josh D'Ambrosio[4,5], Myra J. Dickey[6], Fernando Fleites-Ayil[7], Paolo Fontana[8], Sonja Kersten[1], Margarita M. López-Uribe[9], Delphine Panziera[10], Javier Quezada-Euan[11], Juliana Rangel[12], Francis Ratnieks[13], Fabrice Requier[14], Benjamin Rutschmann[15,16], Oliver Visick[13], Martin Hasselmann[1]

[1]Department of Livestock Population Genomics, University of Hohenheim, Stuttgart, Germany. [2]University of Hohenheim, Stuttgart, Germany. [3]NSW Department of Primary Industries and Regional Development, Queanbeyan, Australia. [4]The Urban Bee, Adelaide, Australia. [5]Department of Primary Industries and Regions SA, Adelaide, Australia. [6]Department of Entomology, University of Illinois Urbana-Champaign, Champaign, United States. [7]Department of Entomology, University of California Riverside, Riverside, United States. [8]Edmund Mach Foundation, San Michele All'Adige, Italy. [9]Department of Entomology, The Pennsylvania State University, University Park, United States. [10]Biointeractions and Plant Health, Wageningen University & Research, Wageningen, Netherlands. [11]Departamento de Apicultura Tropical, Universidad Autónoma de Yucatan, Mérida, Mexico. [12]Department of Entomology, Texas A&M University, College Station, United States. [13]Laboratory of Apiculture and Social Insects (LASI), Department of Ecology and Evolution, School of Life Sciences, University of Sussex, Brighton, United Kingdom. [14]Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, Gif-sur-Yvette, France. [15]Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Würzburg, Germany. [16]Agroecology and Environment, Zurich, Switzerland.

Abstract

The Western honey bee (*Apis mellifera*) has been kept in hives for millennia and is therefore generally regarded as a managed bee. However, the species has a dual nature, with wild (including feral) colonies being more than twice as abundant as managed ones globally. Since honey bee queens naturally mate with multiple drones drawn from a vast area, and beekeepers have limited breeding control, it is generally unclear whether, or to what degree, managed honey bee populations have been functionally changed by humans. This study aims to quantify genomic signatures of modern beekeeping at a global scale by jointly comparing the genomes of pairs of self-sustaining wild and managed honey bee populations from 15 regions in Europe, Africa, Australia, and North America. We analysed whether wild and managed honey bee populations differ in genome-wide levels of genetic diversity, how the species' original geographic lineages are represented in each group, and whether there are any signatures of adaptation to managed conditions. This study will reveal whether wild honey bee populations harbour additional genetic diversity compared to managed populations and provide insights into whether beekeeping per se leads to honey bee domestication.

OC 85

REDUCED BODY SIZE OF *VARROA DESTRUCTOR* ASSOCIATED WITH VARROA-RESISTANT HONEY BEE COLONIES ACROSS EUROPE

Adéla Krajdlová[1], Václav Krištůfek[2], Alena Krejčí[1,3]

[1]University of South Bohemia, Faculty of Science, České Budějovice, Czechia
[2]Biology Centre, Institute of Soil Biology and Biochemistry, Czech Academy of Sciences, České Budějovice, Czechia
[3]Biology Centre, Institute of Entomology, Czech Academy of Sciences, České Budějovice, Czechia

Abstract

The ectoparasitic mite *Varroa destructor* is the most significant parasite of the Western honey bee (*Apis mellifera*) and a major driver of colony losses worldwide. Although extensive research has focused on behavioral and physiological mechanisms of host resistance, comparatively little attention has been paid to potential phenotypic responses of the parasite itself. Here we investigated body size variation in *Varroa destructor* associated with varroa-resistant and non-resistant honey bee colonies across four European countries. We quantified the dorsal shield area of adult female mites from multiple colonies differing in the honey bee colonies resistance status, using standardized digital image analysis. Across geographically distant non-resistant populations, mite body size was remarkably consistent, with a median dorsal shield area of 1.47 mm². In contrast, mites originating from varroa-resistant colonies were consistently smaller, with a median dorsal shield area of 1.37 mm², representing an approximately 6.8% reduction in body size. This pattern was reproducible across different geographical areas, honey bee genetic origin and beekeeping practices. The striking stability of mite body size in non-resistant populations contrasted with the consistent reduction observed in mites associated with resistant hosts, suggesting a host associated shift in parasite phenotype. Because body size in arthropods integrates developmental conditions, nutritional availability and resource allocation, the observed pattern may reflect altered developmental environments and selective pressures imposed by resistant hosts. Our results show a consistent morphological shift in this globally important parasite associated with resistant hosts and suggest that dorsal shield size in *Varroa* could serve as a new selection marker for varroa-resistant honey bee colonies.

RETHINKING BEE GENOMICS: HOW CAN WE BETTER INTEGRATE ENVIRONMENTAL IMPACTS AND ETHICAL CONSIDERATIONS INTO FUTURE RESEARCH?

OC 86

Thibault Leroy

GenPhySE, Université de Toulouse, INRAE, INPT, INP-ENVT, Toulouse, France

Abstract

Our BeeGEES team at INRAE Toulouse contributes to large-scale genomic studies by sampling bees from different continents. Over the last decade, this work allowed us to participate in several projects, especially on honey bees, studying their population structure, evolutionary history, genetic resilience to different stressors, and their microbiota.

However, one major challenge in obtaining genetic information arises from sampling. Collecting bees often requires long international field trips, building collaborations with local partners, and dealing with many administrative procedures. When field trips are finally possible, they also represent a significant carbon footprint for our research activities.

Another concern relates to the number of bees used for research. Over the last decade, we estimate that nearly one million honey bees (mostly workers) were sacrificed for our projects. Although these projects are contributing to major advances in bee conservation and health, they raise ethical questions, especially in light of growing societal demands for more responsible animal experimentation. They lead us to consider whether genomic research can be carried out in ways that reduce, or even eliminate, the need to sacrifice bees.

I will present our recent efforts to explore environmental DNA approaches. More specifically, we investigate the possibility of performing whole-genome sequencing of honey bees using DNA directly extracted from different beehive products. Our results show that several products can be used and provide different levels of genomic resolution.

This approach can also simplify sampling. Indeed, some hive products can be obtained from local markets due to the global trade of bee products. In addition, partners from other countries can more easily collect and send samples without requiring our presence. We will present some first results of this project and discuss how these approaches could provide additional genetic insights into bee-associated ecosystems, while reducing the environmental impacts of genomic research on bees.

OC 87

LANDSCAPE GENOMICS OF PESTICIDE EXPOSURE IN *APIS MELLIFERA*

Fernanda Li[1], Daniela Barbosa[1,2], Sana Bashir[1], Carlos A. Yadró García[1], Ana R. Lopes[1], Demetris Taliadoros[3], Maria Murcia-Morales[4], Amadeo Rodríguez Fernández-Alba[4], Matthew T. Webster[3], José A. Rufino[5], Dirk C. de Graaf[6], Ivo Roessink[7], Bas Buddendorf[7], Jozef van der Steen[8], Maria Alice Pinto[1], Dora Henriques[1]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal. [2]Pontificia Universidade de Minas Gerais, Minas Gerais, Brazil. [3]Department of Medical Microbiology and Biochemistry, SciLifeLab, Uppsala University, Uppsala, Sweden. [4]Chemistry and Physics Department, University of Almeria, Agrifood Campus of International Excellence (ceiA[3]), Almería, Spain. [5]CeDRI, SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal. [6]Laboratory of Molecular Entomology and Bee Pathology (LMEB), Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium. [7]Wageningen Environmental Research (WENR), Wageningen, Netherlands. [8]AlveusAB Consultancy, Kerkstraat 96, NL-5061 EL, Oisterwijk, Netherlands.

Abstract

Genome-environment association (GEA) analysis has been applied to investigate local adaptation to ecological and climatic gradients, such as temperature, precipitation, pollution and human density. By linking genomic variation to environmental drivers, GEA frameworks reveal how natural selection shapes patterns of local adaptation. In this study, we extend this approach to anthropogenic environmental gradients. Specifically, we use GEA to explore how human-driven stressors, such as pesticide exposure, may contribute to genomic variation in *Apis mellifera*.

We combined whole-genome data from 1096 *A. mellifera* individuals sequenced within the BETTER-B project, covering more than 30 countries and multiple European subspecies, with detailed pesticide-exposure data obtained through the INSIGNIA-EU monitoring scheme. For each colony with genomic data, pesticide residues were quantified using APIStrips, providing a direct, colony-level measure of in-hive chemical exposure. These empirical pesticide measurements were then compared with publicly available environmental datasets, namely PEST-CHEMGRIDS (global estimates of pesticide application rates) and CORINE Land Cover (land-use and agricultural intensification), to assess the concordance between local chemical residues and broader spatial pesticide and land-use gradients. Integration of genomic and environmental layers was performed using three complementary GEA approaches, SAMBADA (spatial logistic regression), LFMM (latent factor mixed models), and RDA (redundancy analysis), each capturing distinct aspects of genome-environment covariance.

Concordance among datasets was limited, reflecting the different dimensions of environmental pressure captured by each source, from direct in-hive chemical exposure measured by INSIGNIA-EU to broader landscape-level indicators of pesticide use and land-use. Although further studies are required to strengthen these associations, several loci linked to stress, apoptosis, ion homeostasis and transmembrane transport were simultaneously detected by all GEA methods.

By identifying candidate loci associated with specific stressors, we gain a deeper understanding of the genetic mechanisms that enable *A. mellifera* to persist in increasingly contaminated and intensified agricultural landscapes.

GEOMETRIC MORPHOMETRICS REVEAL THE EROSION OF THE M LINEAGE WITHIN THE HONEY BEE (*APIS MELLIFERA*)

OC 88

Julita Machlowska[1], Dariusz Gerula[2], Andrzej Oleksa[3], Henk Kok[4], Grace P. McCormack[5], Alexandra Valentine[5], Lars Kirkerud[6], Per Kryger[7], Laima Blažytė-Čereškienė[8], Martin Hasselmann[9], Teweldemedhn G. Hailu[9], Benjamin Rutschmann[10], Adam Tofilski[1]

[1]University of Agriculture in Krakow, Kraków, Poland
[2]National Institute of Horticultural Research, Puławy, Poland
[3]Kazimierz Wielki University in Kazimierz Wielki University in Bydgoszcz, Bydgoszcz, Poland
[4]Nederlandse Bijenhoudersvereniging, Ede, Netherlands
[5]University of Galway, Galway, Ireland
[6]Norsk Brunbielag, Lillestrøm, Norway
[7]Aarhus University, Aarhus, Denmark
[8]Nature Research Centre, Vilnius, Lithuania
[9]University of Hohenheim, Hohenheim, Germany
[10]University of Würzburg, Würzburg, Germany

Abstract

Evolutionary lineage M of the Western honey bee (*Apis mellifera*) occurs naturally in western, central, and northern Europe. Several populations belonging to this lineage are increasingly threatened by genetic erosion associated with the widespread introduction of commercially bred queens, which may lead to introgression from non-native lineages. To assess the extent to which the native phenotype persists across Europe, we analysed a large dataset of worker wing images using geometric morphometrics.

We newly collected 29,043 images of worker forewings representing 1,342 colony samples from ten European countries. In addition, publicly available wing images from two further countries were incorporated to broaden the geographic coverage. Each wing was digitized using 19 homologous landmarks describing venation geometry. Based on these landmarks, specimens were assigned to one of four major evolutionary lineages (A, C, M, or O) through comparison with reference samples from the Morphometric Bee Database in Oberursel.

Across the analysed material, 63.7% of colonies displayed a wing phenotype characteristic of lineage M, whereas 27.5% were classified as lineage A, 7.9% as lineage C, and 0.8% as lineage O. The lineage M phenotype remains prevalent in several unprotected populations, particularly in Portugal, Spain, and Ireland, as well as in protected populations elsewhere in Europe. In contrast, a pronounced decline of the lineage M phenotype was detected in unprotected populations from northeastern Poland.

These results reveal substantial regional differences in the persistence of lineage M phenotypes across Europe. The findings highlight areas where native populations remain relatively intact, as well as regions where introgression appears more advanced. Together, they emphasize the importance of coordinated monitoring and conservation strategies aimed at preserving the genetic and phenotypic diversity of European honey bees.

OC 89

INTEGRATED BREEDING STRATEGY FOR APIS MELLIFERA: TOWARDS SUSTAINABLE BEEKEEPING

Ségolène Maucourt[1,2], Andrée Rousseau[2], Pierre Giovenazzo[1]

[1]Université Laval, Québec, Canada
[2]Centre de Recherche en Sciences Animales de Deschambault, Deschambault, Canada

Abstract

The Canadian beekeeping industry has faced significant challenges in recent years, with average colony losses reaching 26% since 2007 and peaking at 46% in 2022. This situation has increased reliance on imported honeybees and queens, which presents several risks, including the introduction of new diseases, the spread of treatment-resistant pathogens, and reduced queen reproductive quality. Moreover, imported bees are generally less adapted to Canada's cold climate, resulting in lower winter survival compared to locally bred bees, which are better suited to these conditions. In response, the CRSAD/Université Laval honeybee genetic selection program has achieved substantial progress over the past fifteen years by improving key traits such as hygienic behavior, honey production, and spring development. Using the BLUP-animal model, breeding values have been estimated for all colonies, enabling the selection of the best individuals for reproduction and leading to measurable genetic gains.

The current goal of the program is to develop a methodology that makes these improved genetics available to the entire Canadian beekeeping industry. To achieve this, a pyramidal selection strategy has been implemented at the Deschambault Animal Sciences Research Center. A selection core maintains pure lines, from which 180 improved queens are distributed annually to six partner breeders for multiplication. These breeders collect phenotypic data on honey production under field conditions, comparable to those of the research center. This combined dataset is then used to refine breeding value estimates and guide future selection decisions.

Results indicate strong genetic potential in several distributed queens. Through multiplication by breeders, who collectively produce approximately 28,000 queens per year, these improved genetics can be disseminated widely across Canada. This pyramidal approach is designed to enhance the sustainability and autonomy of the Canadian beekeeping industry while also allowing evaluation of its effectiveness, particularly regarding improvements in honey production.

COMBINING STANDARDISED PROTOCOLS, CITIZEN SCIENCE AND GENOMICS TO MONITOR WILD *APIS MELLIFERA MELLIFERA* COLONIES IN IRELAND

OC 90

Grace P. McCormack[1], Alexandra Valentine[2], Mick Verspuij[2], Stephen Smith[2], Markus Neuditschko[3], Sonya E. Eynard[4], Keith A. Browne[2], Arrigo Moro[5]

[1]Galway, Galway, Ireland
[2]University of Galway, Galway, Ireland
[3]Agroscope, Posieux, Switzerland
[4]Université de Toulouse, Tolosan, France
[5]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

Abstract

The wild honey bee population in Ireland is an important genetic resource of *Apis mellifera mellifera*, a honey bee subspecies that is threatened, endangered, or extinct across much of its original range (Western Europe). Due to intensive apicultural practices in Europe, and the global trade in a few highly bred honey bee lines, honey bee populations across Europe are at risk of becoming genetically homogenized reducing their ability to adapt to new threats (e.g. environment, pathogens). Despite the reporting of honey bee colonies in wild habitats in recent years in Europe most do not become established, and self-sustaining populations are unconfirmed. Here we present the monitoring of hundreds of wild colonies by citizen scientists and the research team. To avoid the risk of reporting continued activity when a colony has in fact died out, we applied a standardised monitoring protocol, recording activity four times each year at key points in the honey bee life cycle. We supported citizen scientists with a web-based monitoring platform and a social media community. Using a genomic approach we confirmed the persistence of honey bee lineages at nest sites over a multi-year period. The longest confirmed to date is >5years with the great granddaughter of the original queen present in the nest. We have also identified where new lineages have taken up residence at nest sites. Both genomic and monitoring data is applied to identify adaptive traits which can be used to advocate for the importance of protecting this unique genetic resource for its own sake and for sustainable beekeeping practices.

OC 91 AGRICULTURE AND URBAN LANDCOVER CLASSES CRITICAL FOR WILD HONEY BEE CONSERVATION IN EUROPE; LESSONS FROM IRELAND

Grace P. McCormack, Mick Verspuij

University of Galway, Galway, Ireland

Abstract

Deforestation and intense agriculture has reduced natural habitat availability for honey bees in Ireland. Furthermore, while distribution and density data from managed and free-living honeybee colonies is essential for understanding population dynamics and future conservation efforts of the Western European honeybee (*Apis mellifera*), such data remain limited. We carried out a detailed census of honey bees, managed and wild, within a 229 km² study area in Ireland, linking this with landcover information relating to nest site and forage resources, thus providing new insights into colony density, spatial relationships and landcover associations. An overall density of 0.87 colony/km² was found in the study area where approximately 80% of all colonies were associated with urban and agricultural landcover classes, highlighting their importance. Native forest supported the highest colony density, though making up a small proportion of the study area. Free-living colonies were found in close proximity to managed apiaries, indicating substantial overlap in foraging grounds and potential ecological and genetic interactions. These findings demonstrate that free-living honeybee colonies persist within a human-modified landscape and form part of a shared metapopulation with managed colonies. As a result, free-living colonies were further classified according to the degree of human influence, distinguishing wild, free-living, human-assisted and managed colonies. The study highlights the importance of agriculture to the persistence and health of honey bees both managed and wild and the necessity of having a gradated colony classification for accurately assessing honeybee ecology in this altered environment.

THE ROLE OF OLFACTORY ADAPTATION TO BROOD ESTER PHEROMONE IN *VARROA*-RESISTANCE

OC 92

Amélie Noël, Barbara Locke

Swedish University of Agricultural Sciences, Uppsala, Sweden

Abstract

The ectoparasite, *Varroa destructor*, remains the leading cause of honey bee colony loss worldwide. Selective breeding for mite-resistant honey bee populations is considered the most promising long-term solution to fight against the mite, yet the biological basis of resistance is not yet fully understood, hindering efforts to identify and propagate resistant traits.

A naturally resistant population on Gotland Sweden expresses resistance by disrupting mite reproduction. The reproductive success of *Varroa* is intricately linked to the brood development cycle of honey bees, particularly through their reliance on Brood Ester Pheromone (BEP), a complex blend of fatty acid esters emitted by the brood. BEP emissions are reduced in the Gotland resistant population, which may play a key role in lowering mite reproductive success by disrupting the mite's ability to detect reproductive signals.

BEP serves primarily as an essential signal for intra colonial communication influencing worker behaviour, including brood feeding, cell capping, foraging activity, and ovary development. A reduction in BEP could thus disrupt critical processes, potentially compromising brood care or foraging efficiency. The question remains: how do resistant colonies maintain colony functional stability despite lowered BEP levels?

We propose that resistant bees possess heightened olfactory sensitivity, allowing them to distinguish between subtle variations in BEP composition more effectively than non-resistant bees. To test this, we employed the proboscis extension reflex bioassay to assess the responsiveness of nurse bees to different pheromone blends. Our findings indicate that resistant bees initially respond broadly to BEP bouquets but gradually develop the ability to discriminate between them, exhibiting improved memory retention. This suggests that resistant colonies may adapt their olfactory processing to preserve colony function while simultaneously reducing mite reproductive success.

OC 93

CONTRIBUTIONS OF THE WILD BEE FAUNA OF CYPRUS

Özge Özden[1], Fatih Dikmen[2], Salih Gücel[1], Ece Kamalak[3], Çiğdem Özenirler[3], Gonca Yeşilyurt[2], Denis Michez[4]

[1]Near East University, Nicosia, Cyprus
[2]Istanbul University, Istanbul, Turkey
[3]Hacettepe University, Ankara, Turkey
[4]University of Mons, Mons, Belgium

Abstract

Cyprus, the third largest island in the Mediterranean Basin, represents a biodiversity hotspot characterized by high levels of plant endemism. The island's wild bee fauna has been extensively documented, notably by the eminent taxonomist George Mavromoustakis, whose collections (1916–1957) and subsequent publications provided a foundational framework for regional melittological research. According to the most recent checklist published by Varnava et al. (2020), a total of 369 verified wild bee species have been recorded from the island.The present study was conducted during 2024–2025 in northern Cyprus. Field surveys were carried out using entomological sweep nets, and both wild bee specimens and their foraging plants were systematically recorded. In addition, habitat characteristics were documented for each sampling locality. As a result of these surveys, *Hylaeus punctatus* (Brullé, 1832) is reported as a new record for the island. Furthermore, updated distributional data were obtained for 101 species, alongside the identification of their associated floral resources. Among the recorded taxa, two species are classified as Near Threatened (NT), seven as Data Deficient (DD), and the remaining species as Least Concern (LC) according to IUCN criteria. Notably, 12 of the 21 bee species endemic to Cyprus were documented within the scope of this study. Overall, these findings contribute to a more comprehensive understanding of the island's wild bee diversity and provide a valuable baseline for future ecological and molecular studies, particularly in the context of climate change and pollinator conservation.

ACCELERATING SELECTION FOR *VARROA* RESISTANCE IN IBERIAN HONEYBEES USING A PHEROMONE-BASED HYGIENIC BEHAVIOUR ASSAY

OC 94

Melanie Parejo[1], Luis Javier Chueca[2], Egoitz Galartza[3], Pablo Revillas[1], Kaira Wagoner[4], Iratxe Zarraonaindia[1,5]

[1]University of the Basque Country, Leioa-Bilbao, Spain
[2]BC3 - Basque Centre for Climate Change - Klima Aldaketa Ikergai, Leioa, Spain
[3]ERBEL, Iberian Bee Breeding Association, Zaldibia, Spain
[4]University of North Carolina, Greensboro, Spain
[5]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Abstract

Breeding for hygienic behaviour is used to improve honeybee health, yet standard assays such as the pin-test mainly measure dead brood removal and may not specifically capture responses to varroa-infested brood. Here, we evaluated the applicability of the UBeeO-test (OpteraLLC), a pheromone-based assay that mimics the odour of unhealthy and varroa-infested brood, to select *Apis mellifera iberiensis* colonies with enhanced hygienic behaviour. The method consists of spraying the pheromone solution onto capped brood and quantifying the proportion of cells uncapped by workers within two hours.

In 2023 and 2024, 77 colonies from various apiaries in the North of Spain were screened. UBeeO scores in this baseline Iberian population ranged from 0 to 95.9%, with a low overall expression of the trait (45% of colonies had zero response).

In spring 2025, two colonies with high scores (21.4%, 67.3%) were selected as breeder queens from which 10 queens each (F1) were reared and naturally mated at a mating station. Mated nucs were evaluated with UBeeO after 40 days, and again 10 queens were crafted and mated from the two highest-scoring F1-queens (58.3%, 95,6%), producing selected F2-queens in the same year. On average, the F1-generation (24.5%) showed higher average scores than the parental generation (16.3%), indicating a positive response to selection. Preliminary analysis estimated high heritability of the trait (0.42), suggesting a substantial additive genetic component, making it suitable for implementation in breeding programs. Moreover, the possibility to phenotype and breed two generations per year, enables an accelerated breeding scheme.

In 2026, breeding continues by adding targeted crossing of high-scoring colonies via artificial insemination. At the conference, we will present updated results from F3 (and F4) generations. Together, our results support the UBeeO assay as an efficient phenotyping tool for breeding varroa-resistant Iberian honeybees.

OC 95

A PERSISTENT LEGACY: DYNAMICS OF ACARICIDE RESISTANCE MUTATIONS IN *VARROA DESTRUCTOR* IN THE ABSENCE OF SELECTION PRESSURE

Antonio Pérez-Pérez[1], Maíra Costa[2], Dora Henriques[2], Carolina Bartolomé[3], Julio M. Maside[4], Delphine Panziera[5], Séverine D. Kotrschal[5], Bjørn Dahle[6], Robert J. Paxton[7], Martin Bencsik[8], Alexandru-Ioan Giurgiu[9], Maria Alice Pinto[2], Nuno Capela[10], Soledad Sagastume[1], Matthew T. Webster[11], Emma Bossuyt[12], Lina De Smet[12], Dirk C. de Graaf[12], Raquel Martín-Hernández[1], Mariano Higes[1]

[1]Laboratorio de Patología Apicola, IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Consejería de Agricultura de La Junta de Comunidades de Castilla, Marchamalo, Spain. [2]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal. [3]Fundación Pública Galega de Medicina Xenómica (FPGMX), Hospital Clínico Universitario de Santiago, Edificio de Consultas, Santiago de Compostela, Spain. [4]Departamento de Zoologia, Genética e Antropologia Física, Faculdad de Biología Universidad de Santiago de Compostela, España. [5]Stichting Wageningen Research (WR) Droevendaalsesteeg, Wageningen, Netherlands. [6]Norwegian Beekeepers Association (NB) Dyrskuev, Kløfta, Norway. [7]Martin-Luther-Universität Halle-Wittenberg (MLU) Institute for Biology/General Zoology Hoher Weg, Halle, Germany. [8]The Nottingham Trent University, Nottingham, United Kingdom. [9]Universitatea de Științe Agricole și Medicină Veterinară din Cluj-Napoca (USAMV), Cluj-Napoca, Romania. [10]Universidade de Coimbra (UCOI), Coimbra, Portugal. [11]Uppsala University (UU) IMBIM, Uppsala, Sweden. [12]Ghent University (UGENT) Laboratory of Molecular Entomology and Bee Pathology (L-MEB) & Honeybee Valley Department of Biochemistry and Microbiology (WE10), Ghent, Belgium.

Abstract

Varroosis, caused by the parasitic mite *Varroa destructor*, represents one of the major health challenges in global beekeeping. The intensive use of synthetic acaricides, as well as the residues they leave within the hive, has favored the selection of mite populations with genomic mutations associated with resistance, primarily through alterations in their pharmacological targets.

In this context, mutations in the gene encoding voltage-gated sodium channels (VGSC), specifically M918L and L925V, have been described in association with pyrethroid resistance. In addition, several mutations in the β2-adrenergic octopamine receptor gene (N87S, Y215H, F290L, Y337F, and T115N) have been identified as potentially related to amitraz resistance, although their functional role remains under debate.

This study hypothesizes that, in the absence of acaricide selection pressure, the frequency of these mutations should decrease in mite population. To evaluate this, adult female *Varroa destructor* were analyzed from colonies included in the Darwinian Black Bee Box selection program, under the framework of the European Better-B project, where colonies receive no acaricide treatments. For each generation, up to 30 adult female mites were randomly sampled from different European regions. DNA was extracted individually, and the genes of interest were amplified using an optimized PCR protocol, followed by Sanger sequencing.

The results show that the overall frequency of the analyzed mutations is approximately 50%, with some individuals from diferente regions carrying with mutations in both genes. Furthermore, similar patterns were observed in successive generations, suggesting that acaricide selection pressure may not be the sole driving factor. This work details the prevalence of each mutation by country, assesses their trends, and evaluates the emergence of new mutations. These findings suggest the potential involvement of other unidentified factors and underscore the need for continued research into resistant *Varroa destructor* populations.

ARE HONEYBEE SUBSPECIES DISAPPEARING? GENOME-WIDE EVIDENCE OF WIDESPREAD HUMAN-MEDIATED ADMIXTURE

OC 96

Maria Alice Pinto[1], Carlos A. Yadró García[2], Ana R. Lopes[1], Demetris Taliadoros[3], Dora Henriques[1], Fernanda Li[1], Raquel Martín-Hernández[4], Dirk C. de Graaf[5], Matthew T. Webster[3]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal
[2]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal
[3]Uppsala University, Uppsala, Sweden
[4]Centro de Investigación Apicola y Agroambiental, Marchamalo, Spain
[5]Ghent University, Ghent, Belgium

Abstract

Many papers open with a statement referring to the extensive diversification of *Apis mellifera* into ~30 subspecies, including the 24 originally described by Ruttner (1988). However, since Ruttner's pioneering work on honeybee taxonomy, based on specimens collected in the mid-20th century, beekeeping has undergone dramatic intensification, reflected in a substantial increase in the global number of managed colonies. This intensification has driven a growing demand for queens and intensified competition for floral resources, thereby promoting both large-scale queen trade and migratory beekeeping, facilitating increased gene flow between historically isolated subspecies. This raises a fundamental question: To what extent has this intensification reshaped the honeybee gene pool to the point that its subspecies can no longer be considered distinct entities? Here, we address this question at an unprecedented scale by integrating whole-genome data (N=2,240) from the Medibees and Better-B projects, encompassing 17 continental and island subspecies sampled across 46 countries in Europe, the Middle East, and North Africa. After analysing admixture patterns, the answer is clear: nearly all subspecies show signatures of recent human-mediated gene flow, albeit at varying levels. Gene exchange occurs both within the same lineage (e.g., in Türkiye, Italy) and between divergent lineages, with *A. m. ligustica* and, in particular, *A. m. carnica* (e.g., in Iran, Egypt) acting as major sources of introgression across the range, while *A.m. lamarckii* also emerges as an important source in the Levant and Arabian Peninsula. Several subspecies (e.g., *A. m. syriaca*) and regional populations (e.g., UAE) exhibit alarmingly high levels of introgression, raising concerns about their persistence as distinct genetic entities. Our findings suggest that, across large portions of the species' native range, patterns shaped by thousands of years of evolution have been largely erased in less than a century of human-mediated change, calling into question the long-standing paradigm of discrete subspecies.

OC 97

NOT ALL DIFFERENCES MATTER EQUALLY: QUANTITATIVE ANALYSIS OF PSD DOMAIN INTERACTIONS IN HONEY BEE COMPLEMENTARY SEX DETERMINER PROTEIN

Natalia Romek[1], Agnieszka Laszkiewicz[1], Aleksandra Ertman[1], Joanna Niedbalska-Tarnowska[1], Patryk Skinderowicz[1], Kristina Jancelowska[1], Ajda Moskric[2], Malgorzata Cebrat[1]

[1]Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wroclaw, Poland
[2]Agricultural Institute of Slovenia, Ljubljana, Slovenia

Abstract

The complementary sex determiner (*csd*) gene is responsible for sex determination in *Apis mellifera*, with its function depending on interactions between allelic variants of the CSD protein. A key role is played by the PSD domain: identical CSD variants form an inactive complex mediated by PSD–PSD interactions, whereas different variants form an active complex driven by interactions between their N-terminal regions. However, the minimal sequence differences within the PSD domain required to disrupt homozygous interactions and enable functional heterozygosity remain unclear.

To address this, we developed an *in vitro* assay to quantitatively assess PSD–PSD interactions. Using the luciferase complementation system, PSD domains from selected CSD alleles were cloned into vectors encoding either the small or large NanoLuc subunit and expressed in HEK293T cells. Protein–protein interactions were quantified based on restored luminescence.

We first tested pairs of alleles occurring naturally, including homozygous combinations of several alleles and their known functional heterozygous pairs. As expected, all homozygous combinations exhibited strong luminescence signals, while functional heterozygous pairs showed little to no interaction. Interaction strength was quantified as the ratio of luminescence in homozygous versus heterozygous configurations for each allele pair.

Subsequently, the same alleles were analyzed in non-natural combinations, designed to provide further insight into the basis of functional heterozygosity. Results from this extended analysis revealed that even small differences in allele length within the hypervariable region (HVR) led to a noticeable reduction in interaction strength, whereas amino acid substitutions alone did not produce a comparable effect.

Our results demonstrate that the NanoLuc-based assay is a robust and reproducible tool for studying PSD domain interactions *in vitro*. Future work will focus on testing naturally occurring allele combinations with reduced co-occurrence frequencies and engineered mutations targeting predicted protein–protein interaction sites within the PSD domain.

EXPRESSION OF KEY INNATE IMMUNE GENES IN VIRUS-TOLERANT HONEY BEES FOLLOWING AN ACUTE BEE PARALYSIS VIRUS INFECTION

OC 98

Xinyan Ruan[1], Amélie Noël[1], Srinivas Thaduri[2], Joachim R. de Miranda[1], Barbara Locke[1]

[1]Swedish University of Agricultural Science, Uppsala, Sweden
[2]Surabhi Institute of Medical Science, Telananga, India

Abstract

Honey bees (*Apis mellifera*) have a complex immune system to cope with pathogens, with both innate immunity at the individual level and social immunity at the colony level. The honey bee population from the island of Gotland, Sweden, is well-known for their independent resistance to Varroa mite infestation and tolerance to virus infections. However, the immune mechanisms underlying the virus tolerance characteristic in this population are still unclear. We investigated the innate immunity of Gotland honey bees towards virus infections to determine possible genetic variation of the expression of different immune genes. In this study, the Gotland honey bees and mite-susceptible honey bees were collected at different time points (12h, 24h, 48h, 72h, 120h) over the course of a controlled oral inoculation of Acute Bee Paralysis Virus (ABPV) to detect the function of innate immunity in the competition between honey bees and viruses. The Gotland honey bees survived better than non-selected mite-susceptible honeybees. Several genes involved in humoral response, cellular response and RNA interference pathways were tested using qRT-PCR. Six internal housekeeping genes were selected as the candidates of reference genes in this study, but one gene from JAK/STAT pathway showed the highest stability. Immune-related gene expression exhibited distinct temporal changes with a distinct differentiation between Gotland and susceptible honey bees following virus infection. The results of this study, and their potential contribution to the population's virus tolerance, will be discussed. As the innate immunity is a primary defense against viruses, these findings shed new light on pathogen–host interactions and will help mitigate escalating colony losses worldwide. Comprehensively investigating innate immune strategies, their relative importance for bee health and their costs and benefits, will benefit breeding efforts to safeguard honey bees and the important pollination services they provide for human well-being.

OC 99

THE ROLE OF SOCIAL IMMUNITY IN RESPONSE TO ACUTE BEE PARALYSIS VIRUS INFECTION IN THE GOTLAND VIRUS-TOLERANT HONEY BEES

Xinyan Ruan, Amélie Noël, Barbara Locke

Swedish University of Agricultural Science, Uppsala, Sweden

Abstract

Social immunity, encompassing the coordinated behavioral, physiological, and organizational defenses of group members, plays a critical role in protecting social animal colonies from pathogen pressure and ultimately determines colony fitness and survival. In honey bees (*Apis mellifera*), colony-level defenses complement innate immunity, with behaviors such as *Varroa* sensitive hygiene (VSH) behavior, strongly associated with resistance to *Varroa destructor* mite. The honey bee population from the island of Gotland, Sweden, are well-known for their independent resistance to *Varroa destructor* mite infestation and tolerance to virus infections, in particular to Deformed Wing Virus (DWV) and Acute Bee Paralysis Virus (ABPV). Yet, the contribution of social immunity mechanisms in this population, that support virus tolerance, remains insufficiently understood. Here, we compared the behavioral responses of Gotland and susceptible honey bee colonies following a controlled oral infection of ABPV. In total, 12 behaviors were detected including brood care, grooming, antenna contact, and aggressive behavior. The results of this study will be presented and discussed. By integrating behavioral profiling with a pathogen challenge, we provide novel insights into how social immunity contributes to colony-level resilience. These results advance our understanding of host–parasite dynamics and may inform future breeding strategies for disease-resistant honey bee populations.

TRANSITION OF *TROPILAELAPS MERCEDESAE* FROM *APIS DORSATA* TO *APIS MELLIFERA* RESULTS IN ALTERED TRANSCRIPTOMES AND VIROMES IN PARASITE AND HOST

OC 100

Olav Rueppell[1], Orawan Duangphakdee[2], Ajay Poudel[1], Carina Lai[1], Robert X. Lu[3], Jacob J. Herman[1], Prabashi M. Wickramasinghe[1]

[1]University of Alberta, Edmonton, Canada
[2]King Mongkut's University of Technology Thonburi - KMUTT, Ratchaburi, Thailand
[3]Oxford University, Oxford, United Kingdom

Abstract

The ectoparasitic mite *Tropilaelaps mercedesae* poses an emerging threat to global apiculture because it is expanding its range after its host shift from *Apis dorsata* to *Apis mellifera*. Similar to *Varroa* in many regards, it has the potential to be more harmful to bee colonies and more difficult to control. However, little information exists on its direct impact on host physiology and its capacity to vector bee viruses. Thus, we compared the host transcriptome response to *Tropilaelaps* infestation between the novel host, *Apis mellifera*, and its original host, *Apis dorsata*. We also compared the transcriptome profiles of *Tropilaelaps* infesting either the original or the new host species. In addition, we quantified known viruses and searched for novel viruses in the mites and bees of both species. Corresponding to our prediction, we found significantly different physiological responses to *Tropilaelaps* infestation between *Apis* species with a stronger response in the original host. We also identified differences in the mites' gene expression, indicating differences in host-parasite interactions. Finally, we detected numerous viruses, including several uncharacterized ones, some of which were abundant. Differences in virome composition of hosts and *Tropilaelaps* on different hosts were pronounced despite all samples coming from the same area in Thailand. Combined, these results may explain how *Tropilaelaps* is more damaging to the novel *Apis mellifera* than its original *Apis dorsata* host and inform risk assessment for *Tropilaelaps'* potential to introduce novel viruses into the apicultural populations it will invade.

OC 101 A SUPERORGANISM FRAMEWORK FOR SOCIAL BEE HEALTH

Daniel Schläppi, Peter Neumann

Institute of Bee Health, University of Bern, Bern, Switzerland

Abstract

Social bees play crucial roles in terrestrial ecosystems and are key to global food security. Yet, despite decades of research to mitigate ongoing declines of wild populations and unsustainably high losses of managed colonies, what exactly constitutes health in such decentralised systems remains poorly defined, limiting our ability to develop effective diagnostic and intervention strategies. Here, we establish a conceptual framework for defining health in bee colonies grounded in the superorganism concept and medical principles. We define superorganism health as the capacity of interacting individuals to maintain colony-level homeostasis through collective behavioural processes under varying environmental conditions. Central to this definition is the balance between functional demands arising from internal and external conditions and the colony's ability to meet them. The framework explicitly captures resilience at the superorganism level as the colony's capacity to buffer against and recover from perturbations through flexible task allocation and the mobilisation of reserve worker cohorts. This shifts the focus from commonly used static indicators, such as population size or pathogen presence, toward dynamic measures that capture how effectively colonies maintain functional integrity. By linking individual-level impairments to disruptions in colony-level processes, functional metrics enable early detection of declining health and provide a foundation for improving ecological risk assessment, guiding targeted management strategies, and supporting the long-term sustainability of social bees in changing environments.

GENOMIC SPOTLIGHT ON A DIVERSITY HOTSPOT OF HONEY BEE–*VARROA* SPP. ASSOCIATIONS

OC 102

Anaïs K. Tallon[1], Jessica Baroga-Barbecho[2], Cleofas Cervancia[2], Vincent Dietemann[3], Peter Neumann[4], Alexis L. Beaurepaire[4,2]

[1]Animal GenoPhenomics Group, Agroscope, Posieux, Switzerland
[2]UPLB Bee Program, University of the Philippines Los Baños, College, Laguna, Philippines
[3]Swiss Bee Research Centre, Agroscope, Liebefeld, Switzerland
[4]Institute of Bee Health, University of Bern, Liebefeld, Switzerland

Abstract

Allopatric speciation is a major driver of biodiversity, particularly in island systems where geographic isolation, environmental heterogeneity, and limited dispersal promote genetic divergence. Islands can thus be used as natural laboratories for studying evolutionary processes, including host–parasite coevolution. The Philippine archipelago represents an ideal region for studying the interactions between honey bees and Varroa spp. mites due to the considerable taxonomic diversity of these organisms occurring there. In addition to native Eastern honey bees (*Apis cerana*), Western honey bees (*Apis mellifera*) have been widely introduced for commercial beekeeping. Previous studies indicate that Philippine *A. cerana* and associated *Varroa* spp. populations are genetically distinct from mainland Asian populations, with substantial differentiation also occurring among islands. However, the extent and determinants of this variation remain poorly understood. Here, we use population genomic approaches to characterise genetic variation in *A. cerana*, *A. mellifera*, and their associated *Varroa* spp. mites sampled across multiple islands. Specifically, we investigate how island geography, host ecology, and human-mediated bee movement are associated with patterns of dispersal, gene flow, and adaptation in hosts and parasites. Analyses are ongoing, and results will be presented at the conference. We expect to find (i) ongoing gene flow between allopatric *A. mellifera* populations due to transportation by humans, but not in *A. cerana* populations relying on swarming for dispersal, (ii) host-associated genetic differentiation of *Varroa* spp. mites, with homogenous populations of *V. destructor* of the Korean haplotype primarily linked to introduced *A. mellifera* and more diverse local *Varroa* spp. lineages associated with *A. cerana*, and (iii) candidate genomic regions potentially involved in host-parasite interactions. By integrating genomic data from both hosts and parasites, this study will provide new insights into the drivers of diversification, adaptation, and coevolution of *Apis-Varroa* systems in a biodiversity hotspot.

OC 103 THE EFFECT OF EARLY LIFE SOCIAL ISOLATION ON SOCIAL BEHAVIOR IN HONEY BEE WORKERS

Itamar B. Treister, Kira Vyshevsky, Hagai Y. Shpigler

Department of Entomology, Agricultural Research Organization, The Volcani Institute, Rishon LeZion, Israel

Abstract

Early social interactions profoundly shape adult behavior across many taxa, yet the degree to which specific eusocial behaviors in honey bees (*Apis mellifera*) rely on early social experience remains underexplored. This study investigates whether early social isolation affects the responsiveness of worker honey bees to social signals by comparing bees isolated for seven days post-emergence to those reared in social cohorts. We evaluated three key social interactions: affiliative nursing behavior toward a queen larva, agonistic behavior toward an unfamiliar intruder, and nestmate recognition toward a sister bee. Our results indicate that early social isolation does not significantly alter fundamental affiliative or agonistic capacities. Both isolated and socially reared bees demonstrated similar frequencies, durations, and intensities of larval nursing, as well as comparable aggressive responses toward non-nestmate intruders. However, isolated bees exhibited a profound deficit in nestmate recognition, responding to a sister bee with severe aggression comparable to their reaction to an unknown intruder. In contrast, socially reared bees displayed normal tolerance and significantly lower aggression toward sisters. These findings suggest that unlike in mammals, where early isolation broadly abolishes social capabilities, fundamental task-based behaviors in honey bees are innate and highly robust to early social deprivation. Conversely, the ability to recognize and tolerate nestmates is strictly experience-dependent, highlighting a distinct evolutionary uncoupling of innate social tasks from acquired social recognition in eusocial insects.

Relationship between managed and wild bees

OC 104 WHEN MANAGEMENT MEETS WELFARE: INSIGHTS FROM MELIPONISTS KEEPING *MELIPONA QUADRIFASCIATA* IN BRAZIL

Celso Barbiéri[1], Maria Luiza P. Melantonio[2], Fabiana E. Casarin[2], Tiago M. Francoy[1]

[1]Universidade of São Paulo, São Paulo, Brazil
[2]Federal University of São Paulo, São Paulo, Brazil

Abstract

Bees play a crucial role in pollination, biodiversity maintenance, and agriculture. However, the indiscriminate use of agrochemicals and deforestation, among other factors, threaten their populations. In this context, meliponiculture, when properly managed, can contribute to the conservation of these pollinators. This study aims to evaluate the management practices adopted by meliponists to promote the well-being of **Melipona quadrifasciata**, the second most common species of stingless bee in Brazil. A structured questionnaire using Likert scales (0 to 5) was administered virtually to 50 meliponists, assessing seven dimensions of well-being. The mean scores for each dimension were: Product Collection (4.60), Resource Availability and Location (4.38), Comfort (4.22), Sanitary Conditions (3.20), Feeding (2.92), Maintenance and Inspection (2.75), and External Factors (1.94). Except for the External Factors dimension, higher scores indicate greater application of techniques aimed at promoting well-being. The dimensions Product Collection and Resource Availability and Location were the most significant determinants of well-being, highlighting the importance of proper harvesting methods and resource availability in the surrounding area. Conversely, the dimensions Maintenance and Inspection, Feeding, and External Factors revealed opportunities for improvement. The findings demonstrate that rational meliponiculture is compatible with bee well-being, positioning skilled meliponists as key contributors to the conservation of **M. quadrifasciata**. Our data indicate that meliponists can play a valuable role not only in conserving stingless bees but also in ensuring their well-being, even when harvesting products. This study also offers a methodological contribution to the assessment of stingless bee well-being and the development of good management practices.

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FIGHT CLUB: THE FIRST RULE IS YOU DON'T TALK ABOUT COMPETITION

OC 105

Gherardo Bogo[1], Nora T. Boraso[2], Laura Bortolotti[1], Cecilia Costa[1], Raffaele Dall'Olio[3], Marta Galloni[2], Angela Pepe[2], Davide Vignola[2], Marta Barberis[2]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy
[3]BeeSources, Bologna, Italy

Abstract

In recent years, the potential competition between managed bees and wild pollinators has attracted increasing attention. However, existing evidence remains conflicting, often influenced by environmental and ecological factors. Considering that some European countries have adopted precautionary measures, banning apiaries in certain areas, the need for further study to define sustainable coexistence strategies becomes essential. We present a case study conducted within the BEE-GUARDS project (www.beeguards.eu) at the Botanic Garden of the University of Bologna, aimed at analysing interactions between honey bees and wild pollinators. The site, a green island in an urban centre with no nearby apiaries, provides an ideal setting to assess the local effects of introducing managed *Apis mellifera* colonies. From mid-May to early August, wild pollinator abundance, diversity, and foraging behaviour were monitored before, during, and after the presence of a honey bee mating station, composed by 6 colonies and 30 mating nucs. Floral resource availability was also assessed throughout the study period. Network analyses performed for three comparisons (pre- versus post-introduction, pre- versus post-removal, and over the entire period of hive presence) showed that honey bees had only a marginal, if any, effect on wild pollinator diversity and abundance. On the other hand, some effects on foraging behaviour were observed, likely resulting from nectar depletion by the honey bees. In contrast, monthly vegetation mowing, which strongly reduced floral resources, consistently had a pronounced negative impact on pollinator communities. Overall, our findings highlight the prominent role of vegetation management in shaping pollinator communities, with floral resource depletion emerging as the dominant driver of change, while the impact of honey bee presence on wild pollinator diversity and abundance remained comparatively marginal under the studied conditions.

OC 106 REDUCING HONEYBEE DENSITY LED TO SHIFTS IN PLANT-POLLINATOR INTERACTIONS

Mariana Castro[1], Nuno Capela[1], Helena Castro[1], Hugo Gaspar[1], Sérgio Timóteo[1], João Loureiro[1], Dirk C. de Graaf[2], José P. Sousa[1], Sílvia Castro[1]

[1]Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[2]Laboratory of Molecular Entomology and Bee Pathology (LMEB), Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium

Abstract

Understanding how the presence of honeybees (*Apis mellifera*) influences wild pollinator communities is essential for designing sustainable pollination conservation strategies and avoiding unintended ecological impacts. Within the BETTER-B project, we evaluated how reducing honeybee foraging pressure alters plant–pollinator dynamics across seasons. We conducted a repeated experiment across three distinct periods (early spring, late spring, and summer) by temporarily restricting honeybee density through hive activity. Pollinator visitation rates, community composition, and plant–pollinator interaction networks were quantified under two conditions: normal honeybee presence and reduced honeybee presence.

Our results show that, although pollinator community composition remained largely stable after reducing honeybee activity, the structure of plant–pollinator interaction networks swiflty changed. Under lower floral resource availability, reducing honey bee density increased visitation by wild pollinators (Apoidea and Syrphidae), supporting the existence of a competitive effect associated with resource scarcity. When honeybee activity was reduced, wild pollinators may compensate for the decline in pollination services provided by honeybees, but network rearrangements indicated shifts in plant visitation patterns, with some plant species receiving fewer visits. These findings highlight that even when the pollinator community remains unchanged, interaction dynamics can shift markedly with honeybee density and seasonal fluctuations in resource availability.This work improves our understanding of how managed honeybees interact with wild pollinators across environmental contexts, offering evidence-based insights to help balance beekeeping productivity with ecosystem resilience. The outcomes contribute to the development of management recommendations that safeguard pollination services while mitigating ecological pressures on wild pollinator communities.

ECOLOGICAL AND HUMAN-DRIVEN DETERMINANTS OF PATHOGEN PREVALENCE IN ISLAND BUMBLEBEE POPULATIONS

OC 107

Daniel Doblas, Pilar De la Rúa, Vicente Martínez-López

Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, Murcia, Spain

Abstract

Understanding the mechanisms that shape pathogen dynamics in insular pollinator communities is essential for assessing disease risks and informing conservation planning. In this study, we examined the prevalence of both genus-specific pathogens and those associated with spillover processes in the endemic Canary Islands bumblebee *Bombus terrestris canariensis* and the introduced *B. ruderatus*. Our objective was to evaluate how environmental conditions and anthropogenic pressures jointly influence pathogen prevalence and transmission dynamics within insular bumblebee populations.

Bumblebee specimens were collected across a broad range of habitats representing different altitudinal zones, vegetation types and levels of human activity on the island of La Palma. Molecular screening techniques were applied to detect four key pathogens relevant to wild and managed pollinators: *Nosema bombi*, *Nosema ceranae*, *Crithidia bombi* and *Lotmaria passim*. This approach allowed us to compare pathogen occurrence across ecological gradients and to assess differences between the native *B. t. canariensis* and the introduced *B. ruderatus*.

Our results indicate that pathogen prevalence is not driven by a single dominant factor but instead emerges from a complex combination of environmental, geographic and biological variables. Although beekeeping intensity measured through *Apis mellifera* hive density, showed some association with pathogen distribution, it explained only a limited proportion of the observed variability. The native *B. t. canariensis* exhibited markedly higher susceptibility to *N. bombi* compared with *B. ruderatus*, suggesting species-specific vulnerability within insular systems. Additionally, *L. passim* prevalence increased significantly with altitude, underscoring the role of environmental gradients in shaping pathogen dynamics. Multivariate statistical models confirmed that spatial structure and habitat-related factors were more influential than direct anthropogenic pressure.

Overall, our findings highlight the need for integrated management strategies that address habitat conservation, environmental change and the regulation of beekeeping activities to effectively protect insular bumblebee populations and maintain healthy pollinator communities.

OC 108 APIARY PROXIMITY RE-SHAPES PATHOGEN DYNAMICS IN BUMBLEBEES

Liam Kendall[1], Joachim R. de Miranda[2], Olivia Bernhardsson[3], Julia Carlsson[2], Henrik G. Smith[1]

[1]Lund University, Lund, Sweden
[2]Swedish University of Agricultural Sciences, Uppsala, Sweden
[3]KU Leuven, Leuven, Belgium

Abstract

Managed pollinators form a ubiquitous part of agricultural landscapes, yet their effects on disease dynamics within wild pollinator communities remain poorly understood. Here, we used a replicated field experiment across eight agricultural landscapes in southern Sweden to assess how proximity to managed honeybee apiaries and landscape-level floral resource availability influence pathogen prevalence and infection load in managed and wild bumblebees. We screened a broad panel of viral and parasitic pathogens across pollinator taxa and analysed pathogen prevalence and loads in relation to apiary distance and floral resource availability. Pathogen communities differed markedly among managed honeybees, managed bumblebees, and wild bumblebees, indicating strong host structuring of disease assemblages. Apiary proximity altered pathogen infection patterns in bumblebees, but responses were pathogen-specific: several pathogens showed higher prevalence and infection loads near apiaries, consistent with increased exposure in shared environments, whereas others exhibited elevated infection farther from apiaries, suggesting transmission structured by interactions within wild pollinator networks modified by competition with honeybees. Floral resource availability further modified these patterns, with contrasting effects consistent with resource-mediated influences on both transmission and host susceptibility. These results demonstrate that pollinator disease dynamics in agricultural landscapes emerge from interacting effects of host identity, pathogen ecology, and landscape context, rather than apiary proximity alone.

BEELINING IN THE BUSH: WILD HONEY BEE NESTING ECOLOGY IN SOUTH AFRICA

OC 109

Kaylin G. Kleckner[1], Wandisile Mdiza[2], Martin Hill[2], Lindsay Campbell[1], James D. Ellis[1]

[1]University of Florida, Gainesville, United States
[2]Rhodes University, Makhanda, South Africa

Abstract

While the western honey bee (*Apis mellifera*) is well studied as managed livestock, relatively little is known about unmanaged populations of this important pollinator. Across Africa, the ecology of wild honey bees remains understudied. We worked in the Eastern Cape Province, South Africa to explore natural history, turnover, and environmental drivers of wild honey bee nests. We surveyed 1 km_2 grid cells for wild honey bee nests using beelining techniques, with which we followed foragers from stands with sugar water to their nest entrances. We monitored nests at three time points over 12 months to assess seasonal changes in nest vacancy and damage. We located 127 nests, 91 of which were located via beelining. Nests in the ground were most prevalent (69.0%), followed by nests in plants (23.8%), manmade structures (5.6%), and rock faces (1.6%). By the end of the study, over half of all nests were vacated. Nests in the ground were vacated and damaged more frequently than were nests in plants after 4-6 months of monitoring ($p < 0.05$). We then used model selection to explore impacts of 11 land management, vegetation, and terrain covariates on patterns of nest density (nests/0.25 km_2). Greater nest densities were associated with rangeland, low percent land cover and high edge density of low vegetation, and southward facing downhill slopes. While the western honey bee is often described as tree-dwelling, we demonstrate that nest ecology is regionally specific. Within our study area, wild colonies likely rely on other animals, such as aardvarks, to create nest sites in the ground that may be more prone to disturbances compared to plant-based nests. Our exploration of environmental features influencing honey bee nesting outlines future directions for hypothesis-driven experiments to inform land management strategies for the conservation of honey bees in South Africa.

OC 110

INVASIVE *BOMBUS TERRESTRIS* AS PATHOGEN RESERVOIRS IN PATAGONIA: INFECTION PHENOLOGY AND THE CHALLENGE OF NON-DESTRUCTIVE SCREENING

Josefina Lohrmann, Eduardo E. Zattara, Marina P. Arbetman

Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), Universidad Nacional del Comahue - CONICET, San Carlos de Bariloche, Argentina

Abstract

Arrival and spread of the invasive bumblebee *Bombus terrestris* to southern South America has reshaped plant-pollinator networks and host-pathogen interactions, with native *Bombus dahlbomii* populations declining coincident with the invasion. We investigated temporal patterns and spatial variation of pathogen occurrence in bumblebees of Northern Patagonia to identify the intrinsic and environmental drivers of infection dynamics. Periodic PCR screenings were conducted throughout the season across three sites to detect trypanosomatids, neogregarines, and microsporidians, alongside characterizations of *Bombus* and floral abundance. We analyzed internal tissues from 314 *B. terrestris* individuals and non-destructive fecal samples from 33 native B. dahlbomii. Additionally, paired samples were used to validate fecal screening accuracy against internal tissues. In *B. terrestris*, total pathogen prevalence reached 79.6%, with distinct dynamics observed across pathogen groups. Neogregarines showed low temporal persistence but a positive association with *Bombus* density. In contrast, trypanosomatids and microsporidians exhibited higher temporal persistence. These patterns suggest that intra-colony transmission and potential external reservoirs may buffer pathogens against environmental fluctuations. Significant spatial heterogeneity was observed in caste phenology and composition; notably, one site exhibited unusually abundant and early male emergence associated with high microsporidian levels that potentially reflects life-history plasticity in response to pathogen pressure. While recorded prevalence in *B. dahlbomii* fecal samples was low, our validation revealed that fecal screening—while effective for trypanosomatids (87.5%)—significantly underestimates microsporidians (33.3%) and neogregarines (27.3%) compared to internal tissues. Therefore, results from non-destructive sampling require careful interpretation, as group-specific biases may lead to underestimate total infection prevalence. Our findings highlight *B. terrestris* as a widespread pathogen reservoir whose numerical dominance likely imposes a high infection pressure on both *B. dahlbomii* and the broader native bee community.

COMPOUNDING EFFECTS OF CLIMATE CHANGE AND BIOLOGICAL INVASIONS DRIVE ICONIC NATIVE BUMBLEBEE DECLINE IN SOUTH AMERICA

OC 111

Carolina L. Morales[1], Marina P. Arbetman[1], Eduardo E. Zattara[1], Maureen Murua[2], Licán Martinez[1], Santiago Hurtado[3], Reto Schmucki[4], Ben A. Woodcock[4], Marcelo A. Aizen[1]

[1]Grupo Ecología de la Polinización (EcoPol). Instituto de Investigaciones en Biodiversidad y Medio Ambiente, INIBIOMA, Universidad Nacional del Comahue, CONICET, San Carlos de Bariloche, Argentina
[2]Centro de Genómica, Ecología y Medio Ambiente (GEMA), Universidad Mayor, Santiago, Chile
[3]Fundación Bariloche, San Carlos de Bariloche, Argentina
[4]UK Centre for Ecology & Hydrology, Crowmarsh Gifford, United Kingdom

Abstract

Understanding how drivers of global change interact to compound pollinator declines is crucial for native bee conservation and mitigation, yet these interactions remain largely untested for most species. Using data from a large-scale monitoring program (2011–2021) tracking the South American (Argentina and Chile) invasion of *Bombus terrestris* (introduced for crop pollinator), and the concurrent decline of native *Bombus dahlbomii*, we tested interactions among climate change (CLIM-CH), land-use change (LAND-CH), and plant biological invasions (PL-BIOINV). We surveyed both species and floral resources across their distributional range in 2020–2021 and calculated proxies for CLIM-CH (temperature and precipitation change between 1960–1990 and 2007–2024), LAND-CH (percentage of anthropogenic land cover), and PL-BIOINV (abundance of alien flowers), alongside current climate, land cover, and floral composition. We used these proxies in multi-drivers' models to predict the abundances of both species, including *B. terrestris* abundance as an additional predictor of B. dahlbomii. Abundances of both species were largely influenced by floral composition, but not by land cover. *B. terrestris* abundance increased with temperature change and alien flower abundance, while *B. dahlbomii* abundance was driven by *B. terrestris* abundance and the interactive effects of temperature and precipitation change. These patterns also predicted abundances in a smaller area (Andean Patagonia) surveyed earlier (2011). Between 2011 and 2021, *B. terrestris* presence increased from 88% to 94%, while *B. dahlbomii* presence declined from 48% to 36%. In less than a decade, the *B. terrestris:B. dahlbomii* abundance ratio rose from ~8:1 to ~28:1, suggesting large-scale species turnover. These results reveal that climate change and biological invasions of plants and pollinators interact to produce compounding negative effects on native bee populations, highlighting the need to account for interactive drivers when planning pollinator's introductions and conservation strategies for vulnerable pollinators under concurrent biological invasions, climate change and land-use change.

OC 112

LARGE SCALE OBSERVATIONS IN FLY ACTIVITIES WITH RADIO FREQUENCY TECHNOLOGY TO STUDY DIFFERENT SUBSPECIES OF HONEYBEES IN SOUTHWESTERN SWEDEN

Niclas Norrström, Sonja Leidenberger

University of Skövde, School of Bioscience, Skövde, Sweden

Abstract

Variation in honeybee flight activity has important implications for pollination dynamics and broader ecological processes. The foraging range, flight behavior and flower choices of honeybees influence their ability to access floral resources, which are essential for the survival, growth, and development of the colony. Radio Frequency Identification is a modern and practical technique to study flight patterns. In this study, we investigated the flight activities of three different *Apis mellifera* subspecies, including *A. m. carnica*, *A. m. ligustica*, the native *A. m. mellifer*a, and the hybrid Buckfast (*A. mellifera X*), in southwestern Sweden. In two apiaries (16 colonies each), we tagged 30 freshly hatched honeybee individuals per colony, once in spring and once in summer. Flight activities were recorded at the entrance from May-August each year. Generalized Linear Mixed-Effects Models (GLMMs) with zero-inflation were developed to analyze the hourly counts of nearly 220,000 bee registrations. The time of day at which the recording occurred was categorized as night or daytime. Environmental variables like temperature, light intensity (PAR), and wind speed, were included both as main effects and in interaction with subspecies, using Poisson and negative binomial distributions.

Our results reveal significant differences in flight activities among subspecies, underscoring the nuanced interplay between environmental factors and bee behavior. Key findings indicate a significant effect of bee age, temperature, light intensity and wind speed on bee activities, with distinct responses observed across the analyzed subspecies. Interestingly, the native *A. m. mellifera* showed a distinct response profile. The study enhances our understanding of bee ecology and behaviors, providing insights into how subspecies-specific adaptations to environmental variables could contribute to their foraging patterns and life history characteristics. Our results underscore the importance of considering subspecies diversity and environmental factors in honeybee conservation.

HONEYBEES' EFFECTS ON SPECIES DIVERSITY AND PLANT-POLLINATOR INTERACTIONS: INSIGHTS FOR IMPLEMENTING SUSTAINABLE BEEKEEPING

OC 113

Hedda Ørbæk[1], Erik Stange[2], Markus A. K. Sydenham[3], Bjørn Dahle[4], Linn F. Groeneveld[4]

[1]Norwegian Institute for Nature Research, Bergen, Norway
[2]Norwegian Institute for Nature Research, Lillehammer, Norway
[3]Norwegian Institute for Nature Research, Oslo, Norway
[4]Norwegian Beekeepers Association, Lillestrøm, Norway

Abstract

Beekeeping is frequently criticized because managed honeybees' high abundances and foraging behavior have the potential to reduce wild pollinator species' access to floral resources and thereby lead to changes in the wild bee community composition (e.g., species turnover and loss). Honeybees may also cause disruptions to plant–pollinator interaction networks (i.e., "rewiring"). The likelihood and magnitude of these effects are expected to depend on local resource availability and the degree of specialization within wild pollinator communities.

We present results from a manipulative field study conducted in Norway within a region where honeybee colonies are normally absent through restrictions to protect the genetic lineage of queen bees reared at the Norwegian Beekeepers' Association's facilities. This area provides a unique opportunity to experimentally assess how variation in honeybee density can affect native bee species assemblies along gradients of both floral resources and nesting site availability.

We sampled pollinators at 40 sites, using netting and pan traps at regular intervals over three consecutive years (2022-2024), to measure both the area's pollinator abundance/diversity and document plant-pollinator interactions before, during and after introduction of honeybees at varying densities. The data from 2022 describe baseline conditions, data from 2023 describe same-year effects of honeybee presence, and data from 2024 give insight into potential lasting effects following hive removal.

Preliminary results indicate wild bee community abundance and species richness decreased between 2022 and 2024 with increasing honeybee density, but that these effects are most pronounced in areas with limited floral resources, where niche overlap between honeybees and wild pollinators is high.

We demonstrate how these findings can inform a predictive framework for prescribing appropriate honeybee colony stocking densities. By accounting for local variation in floral resources and nesting site availability, this approach aims to reduce potential negative impacts on wild pollinators while supporting beekeeping practices.

OC 114 RISK OF VIRUS TRANSMISSION TO WILD BEES DUE TO POOR *VARROA DESTRUCTOR* CONTROL

Anja Probst[1], Orlando Yañez[1], Rebecca Denes[1], Vincent Dietemann[2,3], Peter Neumann[1], Alexis L. Beaurepaire[1,2]

[1]Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland.
[2]Swiss Bee Research Centre, Agroscope, Bern, Switzerland
[3]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

Abstract

Wild bee populations are declining globally, and virus host shifts from managed pollinators may be involved. In particular, inadequate management of ectoparasitic mites *Varroa destructor*, being potent honey bee virus vectors, may foster virus transmission from managed honey bees to other hosts. However, the actual role of *V. destructor* infestation levels in *Apis mellifera* colonies for virus transmission to local wild bees remains unclear. Here, we quantified infection rates of managed and wild bees with two viruses associated with *V. destructor* – Deformed wing virus (DWV) and Acute bee paralysis virus (ABPV) – and one virus which is not thought to be transmitted via *V. destructor*, Black queen cell virus (BQCV). By comparing infection rates, virus prevalence, and viral loads in managed and wild bees collected near highly (above 50 mites per day on the bottom board) and low (control; below 5 mites per day on the bottom board) infested apiaries, we assessed whether *V. destructor* infestations influence virus spillover to sympatric wild bees. We hypothesise that wild bees close to apiaries with high mite infestation levels have higher loads of DWV and ABPV than wild bees close to apiaries with low infestation levels. Further, we hypothesise that there is no difference in the load of BQCV when comparing wild bees collected close to highly infested apiaries to wild bees collected close to low infested apiaries. Altogether, this study will enable us to evaluate the risks associated with beekeeping practices while highlighting the benefits of effective mite control for both managed and wild bee health. The results will be presented and discussed.

Technological applications for the bee science & Bee-based monitoring networks

OC 115 WHAT SHAPES THE BUZZ? MORPHOLOGICAL AND KINEMATIC FACTORS INFLUENCING THE FLIGHT SOUND OF POLLINATORS

Simona Alberti[1,2], Abhishek Ray Mohapatra[2], Phu T. Nguyen[2], Ivan Sili[2], Sebastian Oberst[2], Luca P. Casacci[1], Francesca Barbero[1]

[1]University of Torino, Torino, Italy
[2]University of Technology Sydney, Sydney, Australia

Abstract

Passive acoustic monitoring is emerging as a promising non-invasive tool for pollinator biodiversity assessment. Automated species recognition from flight-generated buzz could enable large-scale, real-time monitoring without capturing or handling animals. However, developing reliable acoustic classifiers requires understanding how species-specific morphological and kinematic traits relate to the spectral structure of flight sound.

Here we present two complementary components of an ongoing project aimed at developing an automatic species recognition algorithm for bees and hoverflies based on flight buzz. The goal is to identify which morphological and kinematic variables co-vary with acoustic features.

In a first component, we report a pilot study on wing kinematics and flight sound in *Apis mellifera* (n=4 individuals), using synchronised high-speed videography (4,000 fps) and calibrated hemi-anechoic acoustic recordings. Wing landmarks were tracked with DeepLabCut, and four kinematic variables (pitch angle, wing flexion, stroke amplitude, wingbeat frequency) were correlated with acoustic features (F0, RMS, H2/H1, H3/H1). Pitch angle showed a near-perfect negative correlation with the relative energy of the third harmonic ($r = -0.999$, $p = 0.001$), suggesting direct empirical evidence that wing geometry shapes the harmonic structure of flight sound in a bee.

In a second component, we present a morphological analysis of 12 pollinator species (96 specimens; bees and hoverflies), examining which body measurements best discriminate species and which species cluster by morphological similarity. Individual morphological profiles were subsequently correlated with their corresponding acoustic recordings to evaluate whether interspecific morphological divergence is reflected in distinct acoustic signatures, and conversely, whether morphologically similar species can instead be reliably discriminated based on their acoustic characteristics.

Together, these analyses constitute the biological foundation for a data-driven acoustic classifier of wild pollinators.

AN INTEGRATED MONITORING AND TRACEABILITY SYSTEM FOR HONEY QUALITY USING CCP-BASED DIGITAL FRAMEWORK

OC 116

Sahin Aydin[1], Elif D. Yelmeoglu[1], Gulsum C. Cavdaroglu[1], Mehmet N. Aydin[2], Samet Okuyan[3], Serhat Solmaz[3], Gökhan Akdeniz[4], Gönül S. Tuzlu[5], Huseyin Deniz[1], Muhammet E. Ozkan[1], Deniz Altintas[1], Hasan Kivrakdal[1], Simge S. Iskaloglu[1]

[1]Isik University, Istanbul, Turkey
[2]Bogazici University, Istanbul, Turkey
[3]Apiculture Research Institute, Ordu, Turkey
[4]Aegean Agricultural Research Institute, Izmir, Turkey
[5]Kadir Has University, Istanbul, Turkey

Abstract

Ensuring the quality, safety, and traceability of honey across the supply chain remains a significant challenge due to fragmented monitoring practices and limited transparency between production and consumption stages. Traditional approaches often lack real-time data integration and fail to provide consumers with verifiable information regarding product origin and processing conditions.

This study proposes an integrated digital framework for honey quality monitoring and traceability based on Critical Control Point (CCP) principles. The system is designed to support end-to-end tracking of honey production, from apiary-level data collection to final batch processing and consumer-level information access. The framework incorporates structured CCP monitoring, where key quality parameters are recorded, evaluated, and managed through a centralized platform.

A key component of the proposed system is the dynamic linkage between production data and consumer-facing interfaces through QR code technology. Each honey batch is associated with a unique digital identity, enabling users to access detailed information about quality parameters, production conditions, and control processes through an interactive "honey story" interface. The platform also supports parameter-based educational content, enhancing consumer awareness and trust.

The system is implemented as a modular web-based application, integrating database-driven parameter management, monitoring logs, and batch composition tracking. By combining traceability, quality control, and user-oriented transparency, the proposed framework contributes to improved food safety, strengthened consumer confidence, and more efficient supply chain management.

The proposed approach aligns with emerging trends in digital agriculture and smart beekeeping, offering a scalable and adaptable solution for integrating monitoring technologies, decision support systems, and communication tools within the apiculture domain.

OC 117

TROPI ALARM: AI-POWERED NON-INVASIVE MONITORING OF *TROPILAELAPS* MITES IN HONEY BEE COLONIES

Davide Bassignana[1], Irakli Janashia[2], Gianluca Francini[1], Maurizio Ghirardi[1], Andrea Varesio[1], Chao Chen[3], Marin Kovačić[4], Aleksandar Uzunov[3,5,6], Cecilia Costa[7]

[1]Apisfero A.P.S., Torino, Italy
[2]Agricultural University of Georgia, Tbilisi, Georgia
[3]State Key Laboratory of Resource Insects, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences., Beijing, China
[4]J.J.Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences, Osijek, Croatia
[5]Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Skopje, North Macedonia
[6]Macedonian Academy of Sciences and Arts, Research Center for Environment and Materials, Skopje, North Macedonia
[7]CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Western apiculture is currently facing a new threat, posed by the westward spread of the parasitic mite *Tropilaelaps mercedesae*. The rapid reproductive cycle of this mite and the severe damage it causes to developing brood make early detection and monitoring essential for effective management and biosecurity. Current diagnostic methods rely largely on colony inspection and expert identification, which are not always possible. To address these limitations, we are developing Tropi Alarm, an artificial intelligence–based image recognition system designed to automatically detect and quantify *T. mercedesae* mites fallen onto sticky monitoring sheets placed on hive bottom trays.

Tropi Alarm builds upon the computer vision technology used in the Bee Varroa Scanner (BVS) developed by Apisfero A.P.S. for automated detection of *Varroa destructor*. For *Tropilaelaps*, a training dataset is being built using images collected from honey bee colonies infested with *Tropilaelaps* mites in Georgia, where the parasite is currently present. Sticky sheets placed on hive trays collect naturally fallen mites and debris. A tailored scanner is used to obtain high resolution images which are used to train the detection algorithm to recognize the morphology of *Tropilaelaps* mites and distinguish them from other debris commonly present on hive bottom boards, including *Varroa* mites and hive particles. The images are systematically annotated to generate a labeled dataset for supervised model training, validation and testing. The dataset captures variability on the sheets, including *Tropilaelaps* appearance and orientation, debris composition and illumination conditions representative of field monitoring.

A key advantage of Tropi Alarm is that it enables continuous monitoring of mite infestation levels without opening the hive. By combining non-invasive sampling with automated image analysis, the system can thereby support rapid, standardized, and scalable surveillance of *Tropilaelaps* infestations, improving early detection and strengthening biosecurity preparedness in beekeeping systems.

API-BASE CAGE: A NOVEL MODULAR AND STANDARDIZED CAGE SYSTEM FOR LABORATORY ASSAYS ON HONEY BEES

OC 118

Michela Bertola[1], Arrigo Moro[1], Edoardo Mazzucco[2], Davide Gamba[3], Franco Mutinelli[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, NRL for honey bee health, Legnaro, Italy.
[2]Freelance engineer, Legnaro, Italy
[3]Freelance, Padova, Italy

Abstract

Laboratory experiments using adult or newly emerged honey bees are essential to obtain reliable results that are not influenced by external environmental factors when testing stressors, contaminants, and the efficacy or toxicity of acaricides. In addition, resistance to acaricides in *Varroa destructor* is an increasingly relevant global issue, and several research groups are investigating its extent and underlying genetic mechanisms. In the absence of well-characterized resistance markers, field efficacy trials followed by laboratory confirmation through phenotypic assays remain the most reliable approach to detect resistance. Among the latter, the most common are toxicity tests, topical microapplication, and spray methods. Testing mites while they are infesting adult bees, thus mimicking natural field conditions, may provide more realistic results compared to testing mites alone, as it reduces stress factors such as starvation and dehydration.

Laboratory assays are typically conducted using containment systems such as glass vials, mason jars, or homemade cages. Although these devices share common features (i.e., ventilation openings, ports for substance application, feeding systems, and adhesive bottoms for mite collection) they often present limitations such as lack of standardization, fragility, non-reusability, and safety risks due to accidental opening or breakage.

Here, we present API-BASE cage designed for laboratory testing with honey bees. The system consists of a fixed base coupled with a detachable and customizable container, allowing flexibility in handling and cleaning. The device is 3D-printed, PFAS-free, reusable, and easily sanitizable. The absence of glass components reduces the risk of breakage, while the material minimizes electrostatic effects, improving handling and experimental consistency. A bayonet-secure closing enables safe collection of bees in the field, transport to the laboratory, and controlled exposure during testing.

This prototype represents a safer, standardized, and more sustainable tool for different typologies of experimental assays on honey bees.

OC 119 FROM NEURAL CODING TO BEHAVIOUR: PROBING ENVIRONMENTAL STRESS EFFECTS IN HONEYBEES

Amélie Cabirol[1], Mara Andrione[2], Davide Liga[3], Elisa Frasnelli[3], Albrecht Haase[3]

[1]Research Centre on Animal Cognition, Toulouse, France
[2]University of Vienna, Vienna, Austria
[3]University of Trento, Rovereto, Italy

Abstract

Environmental stressors such as pesticides, temperature extremes, and anthropogenic pollution are increasingly recognised as major drivers of pollinator decline. To link environmental change to ecological consequences, it is essential to understand how these factors affect neural processing and behaviour. In previous work, we showed that sublethal exposure to neonicotinoids disrupts olfactory processing in the honeybee brain, impairing odour discrimination. At the synaptic level, chronic exposure interferes with experience-dependent structural plasticity by suppressing synaptic pruning, which is associated with deficits in long-term memory formation.

Building on these findings, we will now investigate how a broader spectrum of environmental stressors, including temperature variation, light pollution, pathogens, chemical exposure, and electromagnetic fields, affects neural coding, behaviour, and cognitive performance in honeybees. Using *in vivo* two-photon calcium imaging under controlled environmental conditions, we quantify how neural representations of sensory stimuli change in peripheral and higher brain centers. In parallel, we investigate corresponding changes in behaviour, decision-making, as well as learning and memory.

By linking environmental stressors to neural encoding and ecologically relevant behaviour, this work aims to provide a mechanistic understanding of how changing environments influence the behaviour of pollinators and, ultimately, the ecosystem.

A POLLINATOR BIOSECURITY PLAN FOR THE ISLES OF SCILLY

OC 120

Norman L. Carreck

Carreck Consultancy Ltd, Shipley, United Kingdom
University of Sussex, Falmer, United Kingdom

Abstract

The Isles of Scilly are a small group of islands off the coast of south west England. Their pollinator fauna is not large, but includes some unique island variants. As part of a larger landscape recovery programme for the islands, a Pollinator Biosecurity Plan has recently been developed to assess the threats to all pollinators from the possible arrival of exotic pests and diseases. The limited information on the presence of bee pests and pathogens that is currently available indicates that some important species such as varroa and European and American foulbrood are not present. The presence of *Nosema ceranae* and deformed wing virus B suggest, however, their arrival with honey bees brought to the islands in the last 30 years. A honey bee sampling programme is being carried out in 2025-26 using Whole Genome Sequencing to provide greater clarity on pathogen presence. The beekeepers on the five inhabited islands do not wish to import further queen bees, and the possibility of a legal ban is being explored. The Biosecurity Plan explores all possible pathways of entry for exotic pests and pathogens. The islands are served by helicopters and fixed wing aircraft, and by regular ferry boat services from several locations on mainland UK, and by many types of smaller vessels. The islands are also visited by cruise ships, which may have visited a range of ports en route, including countries where yellow legged Asian hornets (YLAH) are present. The discovery of a YLAH nest in Tresco Abbey Gardens in September 2025 has emphasised the vulnerability of the island populations. Options for raising awareness of biosecurity among the resident population and visitors are discussed.

OC 121

HONEY BEE MATING CONTROL BY CIRCADIAN RHYTHM MODULATION

Chao Chen[1,2], Aleksandar Uzunov[1,2,3], Zhenxing Liu[1]

[1]State Key Laboratory of Resource Insects, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, China
[2]Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Skopje, North Macedonia
[3]Macedonian Academy of Sciences and Arts, Research Center for Environment and Materials, Skopje, North Macedonia

Abstract

Mating control is a crucial component of honey bee breeding programs for genetic improvement and conservation, and it often remains the limiting step. Among the methods for mating control, temporal isolation is gaining attention because it does not require geographic isolation and is therefore widely applicable regardless of the apiary's location. Nevertheless, the current method for temporal isolation (delayed or postponed flight time) suffers from drawbacks such as a narrow time window for mating, incomplete mating isolation due to the presence of feral drones, and the risk of queen losses. Our group has shown that the nuptial flights of queens are controlled by the circadian rhythm and further developed a protocol that modulates the circadian clock of queens and drones to its biological limits. Applying this protocol results in a nuptial flight window that ensures efficient mating of the selected queens and drones and isolation from unwanted (feral) drones. Details will be presented on field and lab studies, as well as the potential for utilization of the method in small-scale and commercial conservation and breeding programs will be discussed.

OC 122

FIRST EVIDENCE OF SOLITARY BEES (*OSMIA CORNUTA* AND *O. BICORNIS*) AS SENTINELS FOR ENVIRONMENTAL ANTIMICROBIAL RESISTANCE GENES (ARGs) SURVEILLANCE

Laura Ciot[1], Alejandro García Gómez[1], Marta Barnadas Serinanell[1], Rossella Tiritelli[2], Jordi Bosch[1], Anselm Rodrigo[1], Giovanni Cilia[3]

[1]CREAF, Bellaterra, Spain
[2]Unit of Biostatistics, Epidemiology and Public Health, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padova, Padova, Italy
[3]CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Antimicrobial resistance genes (ARGs) are widespread across natural and anthropogenic environments, yet environmental resistome surveillance remains largely restricted to specific matrices such as wastewater and clinical settings. Integrative bioindicator systems capable of capturing ARG distribution across diverse environmental compartments are therefore needed. Solitary bees of the genus *Osmia* are promising biological samplers: they interact with soil, plants, water, and airborne particles within a restricted foraging range, accumulate environmental particles on their densely setose body surface, and can be released across a wide range of habitat types.

Here we present the first evidence of solitary bees (*Osmia cornuta* and *O. bicornis*) as sentinels for environmental ARG surveillance. Small populations of both species were released at 26 sites across Catalonia (NE Spain) spanning seven environmental categories expected to differ in antimicrobial selective pressure (Hospital, Swine Farm, Urban Park, Agricultural, Coastline, River, Natural Park). Four matrices — bees, bee-surface material, pollen-nectar provisions, and nest-mud — were collected for both species at each site, resulting in 208 samples screened for 34 ARGs across seven antibiotic classes using multiplex PCR.

ARGs were detected in 43.8% of samples, with all 26 *O. bicornis* nesting-stations and 84.6% of O. cornuta stations testing positive. Twenty-four ARGs were detected, with tetracycline resistance genes dominating all matrices and environments. Multi-ARG profiles occurred in 27.4% of samples. ARG richness was fourfold higher in bees and provisions than in bee-surface material and nest-mud. Resistome composition differed significantly among sampling matrices and environmental categories, with bee samples showing the strongest environmental differentiation. Farm and hospital environments displayed the broadest resistome profiles, agricultural and coastline sites the simplest. *O. bicornis* samples consistently showed higher ARG richness and broader resistome composition than *O. cornuta*.

These results support solitary bees as promising integrative samplers of environmental AMR, enabling spatially targeted, multi-compartment monitoring of ARG distribution across heterogeneous landscapes.

OC 123 THE USE OF ENOS TECHNIQUES TO STUDY THE ATMOSPHERE INSIDE THE HIVE

Marek Gancarz[1,2,3], Robert Rusinek[2], Robert Socha[3,4], Paweł Żukowski[5], Aneta A. Ptaszyńska[5]

[1]Faculty of Production and Power Engineering, University of Agriculture in Krakow, Kraków, Poland
[2]Institute of Agrophysics Polish Academy of Sciences, Lublin, Poland
[3]Center for Innovation and Research on Prohealthy and Safe Food, University of Agriculture in Kraków, Kraków, Poland
[4]Faculty of Food Technology, University of Agriculture in Krakow, Kraków, Poland
[5]BeeLab UMCS, Deprtemnt od Cell Biology, Institute of Biological Sciences, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Lublin, Poland

Abstract

The aim of the study was to use an electronic nose to compare the air inside the hive depending on the bees' pollen-collecting environment. The study was conducted in hives from a single apiary located in five different locations. The Cyranose 320 device manufactured by Sensigent was used to compare reaction on volatile organic compounds inside hive. The Cyranose 320 is a fully integrated hand-held chemical vapour sensing instrument designed specifically to detect and identify complex chemical mixtures that constitute aromas, odours, fragrances, formulations, spills, and leaks. It is also used to identify simple mixtures and individual chemical compounds (Gancarz et al., 2017). Model 320 has 32 polymer-composite sensors. The conductivity of the polymer changes when molecules are absorbed at the conducting particle chain. Differences and similarities were demonstrated between the studied locations. It has been shown that the location of the hive influences the atmosphere inside, which is reflected in the response of the electronic nose sensors.

Gancarz M., Wawrzyniak J., Gawrysiak-Witulska M., Wiącek D., Nawrocka A., Rusinek R.: Electronic nose with polymer-composite sensors for monitoring fungal deterioration of stored rapeseed. International Agrophysics 2017, Vol. 31, 3, 317-325.

PURIFICATION, STANDARDIZATION AND BIOLOGICAL STUDIES OF PROPOLIS EXTRACTS FROM MT OLYMPUS

OC 124

Konstantinos Gardikis[1], Eleni Panagiotidou[1], Nikos Tsafantakis[2], Maria Nikolaou[2], Alexandros Papachristoforou[2]

[1]Research and Development Department, APIVITA SA, Industrial Park Markopoulo Mesogaias, Athens, Greece
[2]Laboratory of Applied Apiculture, Department of Animal Science, University of Thessaly, Larissa, Greece

Abstract

Propolis has been extensively studied for its diverse biological properties. This study focuses on the unique benefits and chemical composition of propolis sourced from Mount Olympus, renowned for its rich biodiversity. The propolis from this region is characterized by a high concentration of flavonoids, phenolic acids, and terpenes, contributing to its potent antioxidant, anti-inflammatory, and antimicrobial properties.

Several purification methods were developed to effectively separate propolis from waxes and other impurities such as centrifugation with and without cooling, specific pore-size filters and vacuum filtration.

The purified propolis was subjected to standardization of its chemical content using 1. Freeze drying methods to provide a pure solid raw material of propolis 2. Production of inclusion complexes in cyclodextrins. The target is the diminishing of the variability of the chemical content of propolis that leads to discrepancies in its biological efficacy.

Accelerated aging studies reveal the stable nature of produced propolis derivatives and non-cellular antioxidant assays (DPPH, ABTS) demonstrate significant radical scavenging activity. Initial antimicrobial and in vitro efficacy studies on human dermal keratinocytes reveal promising application in the field of pharmacy and cosmetology.

In conclusion, the propolis from Mount Olympus, with its unique chemical profile and potent biological activities, holds significant promise for developing new therapeutic and cosmetic applications. Further research and development, as well as scale up of selected formulations could lead to innovative products that harness the full potential of this natural substance.

OC 125 BEES IN PIXELS: MAPPING REDOX PROCESSES IN LIVE HONEY BEES WITH *IN VIVO* EPRI

Uroš Glavinić[1], Djura Nakarada[2], Marko Ristanić[1], Aleksa Mijatovic[1], Jevrosima Stevanović[1], Milos Mojovic[2], Zoran Stanimirović[1]

[1]Department of Biology, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia, Belgrade, Serbia
[2]Center for Physical Chemistry of Biological Systems, BioScope Labs, Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia, Belgrade, Serbia

Abstract

Honey bee (*Apis mellifera*) colony losses represent a major global concern driven by multiple stressors, including pathogens, pesticides, and environmental changes. One of the key indicators of health is redox status; however, its *in vivo* assessment in honey bees remains challenging. In this study, we present the first application of two-dimensional *in vivo* electron paramagnetic resonance imaging (EPRI) to monitor redox processes in live honey bees.

Newly emerged worker bees from a healthy, pathogen-free colony were maintained under controlled laboratory conditions and subjected to EPRI analysis seven days post-emergence. Bees were fed with a redox-sensitive, membrane-permeable spin probe (3-carbamoyl-PROXYL, 3CP), enabling noninvasive tracking of redox dynamics. Imaging was performed using an L-band EPR spectrometer, and spatial distribution of the probe was reconstructed using back-projection algorithms.

The obtained 2D EPR images revealed a predominant localization of the spin probe in the abdominal region, corresponding to the crop and digestive tract, with detectable signals also in the head and thorax. Temporal changes in signal intensity reflected ongoing redox processes, indicating active interactions between the probe and endogenous oxidants and antioxidants. Importantly, no adverse effects on bee survival were observed following the procedure, confirming the noninvasive nature of the method.

These findings demonstrate that *in vivo* EPRI enables spatially resolved, real-time monitoring of physiological processes in honey bees. The approach opens new possibilities for studying redox imbalance associated with diseases, environmental stress, and therapeutic interventions. Furthermore, the use of diverse spin probes could extend this methodology to assess pH, oxygen levels, and metabolic activity in bee tissues.

Overall, *in vivo* EPR imaging represents a promising tool for advancing our understanding of honey bee physiology and for developing innovative strategies to support pollinator health.

LOUD AND CLEAR! TOWARDS WILD BEE ACOUSTIC MONITORING

OC 126

Fernanda Herrera-Mesías[1], Antonio Cruz[1], Zeynep Tilki[2], Frederick Sarathchandra[3]

[1]Fondation faune-flore (FFF), Luxembourg, Luxembourg
[2]Université de Lorraine, Nancy, France
[3]enstic ltd, London, United Kingdom

Abstract

Bioacoustics is a discipline that records, stores, and analyses animal sounds to capture species-specific signals that enable non-invasive wildlife monitoring. Acoustic techniques are widely used in ornithology, herpetology, chiropterology, and marine biology. Insects can also be studied acoustically, but this has most often been confined to laboratory settings. Studies on wild insects that lack specialised sound-producing organs are rarer, largely due to the challenges in obtaining high-quality, repeatable field recordings.

Wild bees are a particularly compelling target for field bioacoustics because they are ecologically important, widely distributed, and difficult to monitor at scale with conventional survey methods.

Bee "buzzing" (wingbeat sound) is an airborne by-product of flight that is produced by all bees. This offers a potentially universal acoustic feature that can be recorded and monitored as an "acoustic fingerprint".

Here, we present preliminary results from a pilot experiment conducted in Luxembourg using customised anechoic insect bioacoustic chambers, aiming to test whether species-specific wingbeat "fingerprints" can be captured from wild bees and used for automated identification of Central European species.

Over the past three years, we have conducted in-field, in-vivo acoustic characterisation of multiple wild bee taxa and progressively refined our methods across different soundscapes. We have also carried out more focused investigations on selected target species, evaluating how intertegular span (ITS), body mass, and wing damage influence acoustic profiles.

Overall, these early findings indicate that acoustic identification of wild bees is feasible and promising, supporting the development of new automated monitoring approaches and non-lethal identification tools for pollinator research and conservation.

OC 127 INNOVATIVE, LOW-COST SENSOR SYSTEM FOR MONITORING HONEYBEE ACTIVITY

Bartos Hunor[1], Agnes Keresztesi[1,2], Robert Fiko-Dezso[1,3], Szilard Ambrus[1], Robert Szep[1,3,4]

[1]Research and Development Institute for Wildlife and Mountain Resources, Miercurea Ciuc, Romania
[2]Hungarian University of Agriculture and Life Sciences, Budapest, Hungary
[3]Sapientia Hungarian University of Transylvania, Cluj Napoca, Romania
[4]Alexandru Ioan Cuza University, Iasi, Romania

Abstract

Colony collapse disorder (CCD) is the modern time's most significant negative effect on honeybee's health and overall activity. Several issues are connected to the above-mentioned problem such as: mite attacks on beehives, microbial infections, climate change and entire human-bee connections (like honeybee's treatment and management). To understand the appearing concerns there is a need for a real-time monitoring application set-up which can bring the solution for the above-listed problems. Science is still far from fully understanding how a beehive operates, but the effects of biotic and abiotic stressors must be addressed.

Research and Development Institute for Wildlife and Mountain Resources has developed an innovative sensor set-up for monitoring the parameters which affect the honeybee's activity. The initiative aims to collect widespread data and to early-predict the deviations. Internal and external beehive parameters (temperature, humidity, gas concentrations, acoustic patterns, climate data) and the predictions based on these data can lead to the holistic understanding of honeybee's treats. The modularity of the developed prototype gives the countless opportunity, which can lead to the fine-tuning of the proposed system.

ASSESSING THE PRECISION OF HONEY GEOGRAPHICAL ORIGIN DETERMINATION USING NMR PROFILING

OC 128

Reet Karise[1], Risto Raimets[1], Indrek Reile[2], Stefan H. Kuhn[3]

[1]Estonian University of Life Sciences, Tartu, Estonia
[2]National Institute of Chemical Physics and Biophysics, Tallinn, Estonia
[3]Tartu University, Tartu, Estonia

Abstract

The geographical origin of honey is a critical parameter in quality assurance, authenticity assessment, and maintaining consumer trust. This study investigates the extent to which the precise origin of Estonian honey can be determined using advanced analytical methodologies. Traditional techniques—including melissopalynology and physicochemical characterization—were supplemented with modern approaches such as NMR-based honey profiling and modern statistical methods.

Based on Bruker's global honey database comprising over 28,500 honey samples, a model employing PCA–LDA coupled with Monte Carlo cross-validation was constructed for Estonia. The model achieved a classification accuracy of 96% for Estonian samples and 99% for non-Estonian samples, with prediction reliability exceeding 99%. Comparative assessment showed some misclassification involving Latvian and Finnish honeys, which we hypothesize may be influenced by similarities in botanical origin. Dominant floral sources included rapeseed and polyfloral mixtures. To further refine geographical discrimination, we added more data to the sample description (botanical origin, presence or absence of dominant plants species, honey physical parameters, geographic location and distance between sampling sites into the model. Our findings demonstrate that NMR profiling is a robust and sensitive tool for determining honey provenance, enabling reliable differentiation even among geographically proximate regions. Overall, the results highlight the potential of NMR-based analytics to enhance traceability and strengthen fraud prevention in the honey market.

OC 129 THINKBEE – A NEW TOOL TO SAFEGUARD BEES UNDER COMBINED PRESSURE OF PESTICIDES, CLIMATE CHANGE AND FOOD SCARCITY

Björn K. Klatt[1,2], Max Möritz[1], Matthias Albrecht[3], Annette van Oystaeyen[4], Ullrika Sahlin[2], Maj Rundlöf[2], Charlie C. Nicholson[5]

[1]Halmstad University, Halmstad, Sweden
[2]Lund University, Lund, Sweden
[3]Agroscope, Zurich, Switzerland
[4]Better[3]Fruit E.V., Aarschot, Belgium.
[5]Roger Williams University, Providence, Rhode Island, United States

Abstract

Bees are key providers of pollination as a vital ecosystem service for human well-being. However, bees are at risk due to multiple and interacting human-induced stressors, calling for new strategies to counteract ongoing bee declines. One of these key stressors are insecticides that may affect bees in agricultural landscapes both directly and in interaction with other stressors, particularly with extreme weather provided by global climate change and the lack of resources in intensified agricultural landscapes. However, insecticides are a diverse group of chemicals with different modes of action, which provides the interesting chance that specific pesticides are less harmful to bees than others in relation to the intensity of other stressors.

To proof this concept, we conducted a unique setup of large-scale laboratory and field experiments on up to six insecticides simultaneously at different temperatures and food availabilities to find combinations that are least harmful to bees. We found that some, but not all of the tested insecticides interacted with temperature and/or food availability, proofing the concept that specific insecticides are less harmful under specific conditions than others. These results were used to develop risk models that extrapolate our data to various theoretically possible conditions in agricultural landscapes. Finally, results of the risk models were implemented into the ThinkBee online tool. Farmers can use this tool for automatically gathering information about temperature and landscape food resources, which will be combined with a model-based risk matrix to give recommendations about which insecticide is least harmful to bumblebees under the specific conditions. Thereby, the ThinkBee tool is providing a new but yet to be discussed approach to safeguard bees in agricultural landscapes under differing climate and food conditions.

IN EUROPE, POLLINATORS ARE BETTER OFF DRINKING NECTAR THAN EATING POLLEN IN A MASS-FLOWERING CROP CONTEXT

OC 130

Marion Laurent[1], Stéphanie Bougeard[2], Camille Lucas[2], Nathalie Pierotti[1], Marie-Pierre Rivière[1], Anne-Claire Martel[1], Marie-Pierre Chauzat[3]

[1]Anses, Sophia Antipolis, France
[2]Anses, Ploufragan, France
[3]Anses, Maisons-Alfort, France

Abstract

Pollination is a crucial ecosystem service that underpins food production. However, global declines of insect pollinators have raised significant concerns among both the public and policy-makers. One of the main drivers of these declines is exposure to plant protection products (PPPs). In PoshBee, a Europe-wide study, data were collected from 128 sites across eight countries. Each site, planted with either oilseed rape or apple, hosted three honey bee colonies and three buff-tailed bumblebee colonies. PPPs were screened in nectar regurgitated by bees after foraging, as well as in pollen collected directly from crop flowers and from honey bee pollen traps.

The results revealed that both pollen and nectar samples from apple orchards contained higher concentrations of PPPs—primarily fungicides—than those from oilseed rape fields. Pollen showed both a higher number and higher concentrations of PPPs compared to nectar. PPPs were detected in pollen from flowers at 97% of sites. The fungicides cyprodinil and pyrimethanil were jointly detected in pollen matrices, while other fungicides were detected in nectar matrices. No co-occurrence of PPPs was observed across all four matrices (nectar from both species, pollen from flowers and pollen collected with traps). The highest average concentrations were recorded for boscalid, flonicamid, and pyrimethanil, all measured in pollen collected from flowers.

The number, type, and concentration of PPPs detected in bee-collected nectar were similar between honey bees and bumblebees. The choice of using either species as nectar samplers is discussed depending on study objectives and experimental conditions (ecology of bees, individual weights, foraging preferences). The results presented here are founded on a bee-based network and provide a good reflexion of the quality of the environment in which bee lives.

OC 131

MOLECULAR MECHANISMS OF CIRCADIAN REGULATION OF LOCOMOTOR ACTIVITY IN SEXUALLY MATURE REPRODUCTIVES OF HONEY BEE

Zhenxing Liu[1,2], Aleksandar Uzunov[1,3,4], Chao Chen[1]

[1]State Key Laboratory of Resource Insects, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, China
[2]National Nanfan Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China
[3]Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Skopje, North Macedonia
[4]Macedonian Academy of Sciences and Arts, Research Center for Environment and Materials, Skopje, North Macedonia

Abstract

Circadian clocks are conserved across insect species, enabling adaptation to daily environmental cycles. These clocks regulate rhythms in behavior and physiology and have been extensively studied in honey bee workers. However, little is known about circadian rhythm characteristics in reproductives, queens and drones. In this study, we investigated how circadian regulation shapes mating behaviors using *Apis mellifera* as a model. Our results show that mature queens and drones operate on a temporal niche distinct from that of workers, dictated by the demands of mating flights. Gene expression analyses revealed that, in queens and drones under normal light conditions, *Am-Cry* and *Am-Per* expression in the brain peaked at 21:00 local time. Integrated metabolomic and transcriptomic analyses further identified the dopamine signaling pathway as a key mediator regulated by day–night cycles, serving as a core pathway governing mating behavior. Together, these findings highlight the circadian clock as a critical regulator of reproductive behaviors in a species-specific manner, providing new insights into the temporal organization of reproduction in honey bees.

VIDEO TRACKING REVEALS PREDATOR-PREY INTERACTION PATTERNS IN *V. VELUTINA* AND *A. MELLIFERA*

OC 132

Joris Mattheijssens, Jan Verwaeren, Dirk C. de Graaf, Ellen Danneels

Ghent University, Ghent, Belgium

Abstract

The "Asian hornet", *Vespa velutina*, is an invasive species in Belgium, with the earliest observations dating back to at least 2011. This predator is increasingly impacting honeybee (*Apis mellifera*) hives, and eradication is likely impossible. To develop effective mitigation strategies, it is important to understand the hunting behaviour of *V. velutina*. Its flight patterns, as well as its hunting and fleeing behaviours, remain poorly understood, although some qualitative observations have been reported.

Hornets typically hover near the landing board for extended periods. Outgoing foragers, which fly quickly because they are well-rested and unladen, are generally ignored. Returning bees, which are slower due to fatigue and the load of pollen or nectar, are more frequently attacked in mid-air. Many attacks fail, after which the predator often changes its hovering position or even the hive it targets. Prey, on the other hand, attempt to evade attacks by flying along faster and more curved trajectories.

In this work, we aim to obtain quantitative insight into this predator–prey interaction by analysing video recordings around the landing board of a honeybee hive in Belgium, collected between June and October 2025, a record year for *V. velutina* observations. We reconstruct the trajectories of individual honeybees and hornets following a tracking-by-detection paradigm. Detection is performed using a neural network applied to individual video frames; key challenges include the small size of the insects and motion blur. Tracking is implemented using a multiple-hypothesis approach, where hypotheses are scored based on trajectory likelihood (distances, velocities, and curvatures) and appearance similarity (visual features). Additionally, we investigate coupling visual features with motion cues: insects typically move forward, and increased motion blur correlates with higher velocity. Remaining challenges include large inter-frame displacements, crossing trajectories, visual similarity between individuals, temporal variation in appearance, and high object density.

OC 133 SIX YEARS OF NATIONWIDE MONITORING: KEY INSIGHTS FROM THE ITALIAN BEENET PROJECT

Piotr Medrzycki, Marino Quaranta, Laura Bortolotti

CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

In the years 2019-2025 the Italian Ministry of Agriculture, Food Sovereignty and Forestry financed a huge nationwide monitoring project, BeeNet, with the objective to appraise the quality of the Italian agroecosystem through the assessment of honey bees and wild bees.

The honey bee monitoring network was based on more than 400 apiaries, each composed of 5 hives, located in all Italian regions and in most agricultural contexts. The colonies were periodically assessed visually and samples of beebread and adult bees were collected for the analyses. Residues of pesticides and presence of pathogens were assessed in laboratory. Part of the hives were equipped with sensors of temperature, weight and light.

The colony status and analytical results are put in correlation with the environmental factors like land use, type of agriculture, pesticide residues, climate.

As regards wild bees, 24 monitoring sites were established across 11 regions of Italy. Each site consisted of a 2 × 200 m transect, surveyed once per month during the growing season, with one hour dedicated to collecting all wild bees encountered. The aim was to assess whether patterns of abundance and species richness recorded at 12 sites located in semi-natural habitats within protected areas, compared with an equal number of sites in intensive agroecosystems, could provide useful insights into the health status of the agroecosystems under study.

Here we describe the effort dedicated to the BeeNet project and the job done. We summarise the numbers of monitoring stations, visual assessments, collected samples, completed analyses, determined wild bee specimens. We present the project's website where the main results are free for consultation. We list the publications resulting from the project and announce the imminent publication of the data papers.

ENVIRONMENTAL BIOMONITORING OF AGROECOSYSTEMS WITH BEEHIVES

OC 134

Silvina Niell[1], Estela Santos[2], Ivana Silva[1], Florencia Jesús[1], Fiamma Pequeño[1], Natalia Gérez[2], Rosana Díaz[2], Maria V. Cesio[2], Horacio Heinzen[2], Yamandú Mendoza[3], Belén Branchiccela[3], Hector Cancela[2], Gastón Notte[1]

[1]Universidad de la República, Paysandú, Uruguay
[2]Universidad de la República, Montevideo, Uruguay
[3]National Institute of Agricultural Research INIA, Colonia, Uruguay

Abstract

Findings of pesticides in beehives have been reported worldwide over the past decade. In Uruguay, between 2014-2017 and 2019-2020, biomonitoring campaigns with seasonal samplings of bees and beehives were carried out across 25 sites. They were selected with a gradient of anthropogenic impact, from "pristine" ecosystems to mixed agroecosystems. Land uses included rice, maize, sorghum, soybean, fruit orchards, horticulture, peri-urban areas, pastures and meadows, free-grazing livestock and forestry. Analyses of 634 samples of bees, wax, beebread and honey used QuEChERS-based multiresidue methods followed by GC-MS/MS and LC-MS/MS validated per European guidelines SANTE/11813/2017, covering 80-100 analytes. Pesticides were ubiquitously found at concentrations from <LOQ to 0.05 mg kg^{-1} in 96% of samples. Wax and beebread showed the highest frequency and multiple detections of pesticides per sample. Beeswax, as a persistent reservoir, poses exposure risk to nurse bees and larvae, underscoring the need for suitable risk assessment. Pesticide profiles at each apiary largely reflected surrounding land use, yet translating these findings into robust environmental quality indicators remains unresolved. To address this, machine learning classification models-Support Vector Machines (SVM) and Neural Networks (NN)-were explored to predict agroecosystem status ("affected" vs. "non-affected") based on pesticide load according to their land use. Input variables included biological colony-strength observations, number of pesticide detections per apiary, and indices constructed from hazard-quotient structures. Models were trained on 90% of the dataset and validated on the remaining 10%. Accurate predictions were obtained with some of the SVM and NN models assayed. These are promising results which indicate that machine learning models can assist in interpreting the environmental quality information obtained from bee-based monitoring networks.

OC 135 A BIOMIMETIC FRAMEWORK FOR MULTIMODAL HIVE STRESS DETECTION: ARTIFICIAL BEE COLONY OPTIMIZATION MEETS EXPLAINABLE AI

Muhammet E. Özkan, Sahin Aydin

Isik University, Istanbul, Turkey

Abstract

The decline in global honey bee populations demands intelligent, scalable monitoring solutions. Existing machine learning (ML) approaches for hive distress detection largely operate on isolated data modalities-audio or visual-failing to capture the multisensory complexity of a living apiary. Furthermore, the opaque nature of deep learning models limits their practical adoption among beekeepers who require interpretable, actionable insights.

This study proposes a biomimetic multimodal framework that addresses both limitations simultaneously. We integrate two complementary open-access datasets: the NU-Hive acoustic dataset for internal anomaly detection (swarming, queenlessness), and annotated Kaggle imagery for entrance-level *Varroa* presence and flight traffic analysis. Rather than fusing these modalities through computationally expensive static methods, we employ the Artificial Bee Colony (ABC) optimization algorithm to dynamically assign trust weights to each sub-model. Mirroring the decentralized foraging logic of *Apis mellifera*, our algorithmic scout and onlooker agents navigate the ensemble hyperparameter space-resolving conflicts when modalities disagree (e.g., normal visual traffic alongside distressed acoustic signatures).

To address the interpretability gap, we incorporate an XAI layer via SHAP value integration. This enables the system to explicitly surface the acoustic frequencies or visual features driving each distress prediction, translating complex model outputs into biologically meaningful signals for end users.

This contribution presents the theoretical architecture and methodological design of the proposed system. Complete empirical benchmarking - evaluating ensemble accuracy and false-positive reduction against baseline unimodal models - will be presented at the conference. Beyond precision apiculture, this framework demonstrates the broader potential of biomimetic optimization in multimodal environmental monitoring.

COMPARING POLLINATOR DIETS BY NON-INVASIVE METABARCODING: INSIGHTS FROM HONEYBEES, BUMBLEBEES, AND MASON BEES, AND PERSPECTIVES FOR A GLOBAL MONITORING NETWORK

OC 136

Benjamin Poirot, Claire Gay, Précillia Cochard

Apilab, Lagord, France

Abstract

Understanding how pollinators use floral resources is essential for describing trophic niches, assessing resource sharing, and developing biodiversity indicators. Here, we present a synthesis of recent studies using non-invasive DNA metabarcoding to characterize pollinator diets from biological materials that can be collected without capturing or killing individuals.

We first compare the diets of honey bees (*Apis mellifera*) and buff-tailed bumble bees (*Bombus terrestris*) using honey and wax-pot contents as environmental DNA sources. This approach revealed a high diversity of visited plants, as well as both shared and pollinator-specific taxa. It also highlighted an important methodological point: the inferred composition of diets and the estimated niche overlap strongly depended on the molecular marker used, underlining the need for cautious interpretation when comparing pollinators through different biological matrices.

We then extend this framework to the solitary bee *Osmia cornuta*, using nesting residual**s** as a non-lethal source of plant DNA. This approach provided access to the floral spectrum of a wild bee model while avoiding destructive sampling, and confirmed the value of combining markers to better describe visited plant communities.

Taken together, these studies show that honey bees, bumble bees and mason bees provide complementary information on floral resource use across ecosystems. Beyond species-level comparisons, we discuss how such multi-species and non-invasive approaches can contribute to a broader biosurveillance network, in which pollinators act as biological samplers of floral biodiversity across sites and regions. This perspective opens the way for more standardized and scalable monitoring of plant–pollinator interactions at larger spatial scales.

OC 137

PREDICTING *VARROA* POPULATIONS UNDER CLIMATE CHANGE AND DIFFERENT INTERVENTION TECHNIQUES USING A TWO-SPECIES INDIVIDUAL-BASED MODEL

Hollie Pufal[1], Ben Rowland[1], Steve P. Rushton[1], Annely Brandt[2], Lena Frank[2], Marin Kovačić[3], Janez Prešern[4], Giles E. Budge[1]

[1]Newcastle University, Newcastle, United Kingdom
[2]LLH-Bee Institute, Kirchhain, Germany
[3]Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
[4]Agricultural Institute of Slovenia, Ljubljana, Slovenia

Abstract

Varroa destructor poses a huge threat to the resilience of beekeeping on a global scale. Management interventions such as drone removal, queen caging, and chemical treatments are all implemented by beekeepers to ensure the mite population remains under levels that cause damage. In a changing climate the impact on the *Varroa* population and the efficacy of interventions is unknown. Investigating the impact of management interventions across different climates would be expensive, time consuming, and takes years to achieve, potentially slowing innovations in beekeeping management. Computational modelling is a very common approach to predict impacts of animal populations over time under changing conditions. Population models of honey bees and *Varroa* do exist, but many do not utilise underlying biology to drive the modelling, and do not offer flexibility to include climate data. Here we present an individual based model (IBM) that captures the underlying biological process of both the *Varroa* mite and the honey bee and how the two species interact, using weather as a driver, along with results from validation using real population data from Germany. With built in, adaptable functions for management practices the user can explore the impact of various management strategies on the *Varroa* populations, at different times of the year and under different climate conditions. The model is a useful prediction tool that could allow adaptability of management practices for beekeepers under a changing climate.

LABORATORY AND SEMI-INDUSTRIAL SCALE EVALUATION OF SOLVENT DECONTAMINATION METHODS FOR PESTICIDE REMOVAL FROM BEESWAX

OC 138

Daniel A. Ramírez, Ramón Arias, Karen Del Pilar Yañez, Beatriz Vivanco, Catherine Tessini

Universidad Técnica Federico Santa María, Valparaiso, Chile

Abstract

Beeswax is a key apicultural product that often accumulates agrochemical residues, posing risks to bee health and limiting its commercial use. This study evaluates, at laboratory and semi-industrial scales, solvent based cleaning methods for the removal of four commonly detected pesticides: coumaphos, amitraz, fluvalinate, and imidacloprid. At laboratory scale, three solvent systems were tested to treat 300 g of beeswax under controlled temperature and agitation conditions: ethanol water (50:50 v/v), isopropanol water (50:50 v/v), and butanol water (50:50 v/v). Removal efficiency for each pesticide and solvent system was determined, together with the physicochemical properties of the treated waxes. The isopropanol based system achieved removal efficiencies ranging from 28% to 95% and preserved the main physicochemical properties of beeswax. Based on these results, this solvent system was selected for scale up. Pilot scale experiments were performed using 20 kg of beeswax in a semi-industrial unit called CLEANWAX, developed and patented at Federico Santa María University in Chile. The process achieved removal efficiencies of 48.0% for fluvalinate, 62.2% for coumaphos, 75.5% for amitraz, and more than 78.3% for imidacloprid. Analysis of melting point, saponification, acidity, and esterification value, and FTIR spectroscopy before and after treatment showed no significant differences, confirming the preservation of key properties. These results demonstrate that solvent systems with controlled polarity are effective for beeswax decontamination and support the feasibility of scaling up this process to semi-industrial level.

OC 139 DEVELOPMENT OF A LOW COST MONITORING SYSTEM FOR STINGLESS BEES

Gabriel Reginatto-Silva, Tiago M. Francoy

University of São Paulo, School of Arts, Sciences and Humanities, São Paulo, Brazil

Abstract

The continuous and non-invasive observation of stingless bee colonies is essential to understand ecological, physiological, and behavioral aspects. Although monitoring systems are widely used for *Apis mellifera*, they cannot be directly applied to stingless bees, because Meliponini colonies have different structural architecture and their nests are smaller than those of honeybees. The present study aimed to develop a low-cost monitoring system capable of continuously recording internal nest parameters over prolonged observation periods. At the core of this hardware architecture, the ESP32 microcontroller was chosen due to its native Wi-Fi connectivity and compatibility with the Arduino IDE, which facilitates the programming. The system incorporates multiple sensor modules for data acquisition: the DS18B20 thermometer for temperature measurement and DHT22 for relative humidity (both can be placed inside and outside the nest to compare variation with the environment), a cell weight module positioned below the wooden box of the nest, and two infrared obstacle sensor module to count the entrance and exit of bees. The DS18B20 and DHT22 sensors can be put in multiple units and positioned in various regions of the nest to generate a thermal gradient map. Furthermore, the system supports customizable temporal configurations, allowing data collection intervals ranging from a high-frequency sampling rate (every 3 seconds) to a low-frequency recording (once per day). To ensure data redundancy and integrity, the acquired datasets are simultaneously recorded locally on a micro SD card and transmitted in real-time to a cloud-based spreadsheet. This device provides an accessible alternative for researchers and meliponists interested in the automated monitoring of stingless bee colonies. When compared with commercial dataloggers, the developed system exhibits a 90% cost reduction, maintaining the stringent functional requirements and data reliability necessary for long-term ecological studies.

OC 140

HONEY eDNA IS AN EFFECTIVE TOOL FOR DETECTING AND MONITORING INFESTATIONS OF THE PINE TORTOISE SCALE (*TOUMEYELLA PARVICORNIS*, COCKERELL) PEST

Anisa Ribani[1], Nicolò Di Sora[2], Valeria Taurisano[1], Maria Letizia Calabri[1], Gabriela Camargo Zyrianoff[1], Silvia Turco[2], Mario Contarini[2], Stefano Speranza[2], Luca Fontanesi[1]

[1]Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy.
[2]Department of Agriculture and Forest Sciences, Tuscia University, Viterbo, Italy

Abstract

Environmental DNA (eDNA) is considered a non-invasive methodology that can facilitate the detection and monitoring of invasive species that would be difficult to identify with other approaches. Honey contains eDNA derived from all organisms encountered by honey bees during foraging in the ecological landscape. This matrix represents a valuable source of information on plant-sucking insects that produce honeydew, which is used by honey bees as a sweet feed source. In this study, we used honey eDNA to develop an assay to detect the pine tortoise scale, *Toumeyella parvicornis* (Cockerell), a harmful invasive pest alien to Europe that in the recent years has caused a serious threat to many stone pines (*Pinus pinea*). A few pine tortoise scale specimens were collected from infested trees and two species-specific primer pairs targeting COI and 16S mitochondrial genes were designed to confirm species identification through PCR and sequencing. These specific primer pairs were then used in PCR assays targeting *T. parvicornis* DNA traces recovered from honey produced in areas with known and yet undeclared pine tortoise scale infestations. A total of 864 honey samples produced over 8 years (2018-2025) across central-southern Italy were analysed. Overall, the Lazio and Campania regions carried the most positive samples for *T. parvicornis* (>50%), followed by Toscana, Abruzzo, Marche and Puglia regions. Interestingly, this pest was also identified in unexpected areas where it had never been detected before. The results showed an increased number of positive samples in the more recent years (2024-2025), in line with the rise in outbreaks across Italy. Given the current difficulty in detecting early-stage *T. parvicornis* infestations, this approach could be implemented into pest early detection and monitoring systems. These results demonstrate the usefulness of honey eDNA in the surveillance of environmental invasive pests, representing a promising tool for large-scale ecosystem monitoring.

OC 141

NEW TOOL FOR DECODING THE LANGUAGE OF HONEYBEES

Freddie-Jeanne Richard[1], Sylvain E. Galopin[2], Guillaume Touya[3]

[1]INRAE, Unité Abeilles et Environnement 406, Avignon, France
[2]AgroParisTech, Palaiseau, France
[3]Université Gustave Eiffel, Géodata, IGN, Lastig, Marne-la-Vallée, France

Abstract

Pollinating insects play a vital role in the functioning of their ecosystems. They contribute, in particular, to maintaining floral diversity and to the production of seeds and fruits.
Knowledge about their activity, and more specifically the dynamics of their activity in relation to the landscape, remains insufficient, even though it is essential for the preservation of the species concerned.
Our project focuses on the geolocation of bees by decoding their waggle dances into spatial coordinates. Bees are able to communicate the direction and distance of their resources by waggle, thus indicating foraging locations to their fellow bees. To do this, the bees are filmed undisturbed inside a connected hive, the GeoDanceHive. The dances are decoded and converted into maps of probable foraging sites. The first maps produced will be presented. To refine and validate the decoding, we calibrated the observations of dance behavior using feeders placed at different distances. Ultimately, this will allow us to geolocate bee foraging locations in different landscapes and over time (daily, annual) without using GPS. We will also present our initial findings on the effects of an insecticide on communication between individuals within the hive. This system will enable us to better understand honeybee resource use over time and the effects of various stressors.

INTEGRATING UAV REMOTE SENSING AND AUTOMATED VIDEO MONITORING TO QUANTIFY PLANT-POLLINATOR DYNAMICS IN AGROECOSYSTEMS

OC 142

Ernesto Rossini[1,2], Pau Enric Serra-Marin[3], Agata Morelli[1], Lucia Lenzi[1], Giovanni G. Bazzocchi[1], Milena T. Stefanova[2], Michele D'Ostuni[1], Giuseppina Pennisi[1], Francesco Orsini[1], Anna Traveset[3], Giorgio Prosdocimi Gianquinto[1]

[1]Department of Agricultural and Food Sciences, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy
[2]Italian National Agency for New Technologies, Energy and Sustainable Economic Development - ENEA, Bologna, Italy
[3]Mediterranean Institute of Advanced Studies (IMEDEA, CSIC-UIB), Esporles, Spain

Abstract

Pollinator declines pose a major threat to both agricultural productivity and ecosystem functioning. Current monitoring approaches remain largely based on labour-intensive field surveys, limiting their spatial and temporal scalability. In this study, we developed and tested a semi-automated framework integrating unmanned aerial vehicles (UAVs) and automatic camera systems (ACS) to quantify floral resources and pollinator interactions within an agroecological field. Floral resource availability was estimated from high-resolution UAV imagery using a combination of reflectance-based methods, while pollinator activity was quantified through deep learning algorithms for object detection applied to video data collected by ACS units. Automated measurements were validated with vegetation quadrats to quantify floral resources and focal observations of pollination activity, conducted across six field campaigns during the spring season. We then linked floral resource availability to pollinator visitation rates using generalized linear mixed models, allowing us to extrapolate pollinator activity across the agroecosystem. This integrative approach enabled the assessment of temporal dynamics and potential phenological mismatches between flowering intensity and pollinator activity. Our results demonstrate that automated sensing technologies can provide reliable estimates of both floral resources and pollinator activity, with detection algorithms achieving high accuracy under field conditions. Moreover, pollinator activity closely tracked temporal fluctuations in flowering intensity, supporting the use of remotely sensed floral metrics as predictors of pollination dynamics. Overall, this study highlights the potential of combining UAV remote sensing, ACS and detection algorithms to develop scalable, reproducible, and efficient frameworks for assessing pollinator activity, plant phenology and potential mismatches. These approaches can complement traditional field surveys and support more effective adaptive management strategies to conserve pollination services in agricultural landscapes under global change.

OC 143 EXPLORING WILD BEE SPECIES ECOLOGY THROUGH BIAS-AWARE SPECIES DISTRIBUTION MODELLING IN LATVIA

Betija Rubene[1,2], Ainārs Auniņš[2], Jānis Gailis[1], Andris Avotiņš[2]

[1]Latvia University of Life Sciences and Technologies, Institute of Plant Protection Research "Agrihorts", Jelgava, Latvia
[2]University of Latvia, Faculty of Medicine and Life Sciences, Department of Ecology, Riga, Latvia

Abstract

Wild bee communities are closely linked to landscape structure and the availability of diverse habitat resources, yet land-use intensification and landscape homogenisation are increasingly altering these ecological relationships. In Latvia, understanding species-environment associations of wild bees is constrained by limited and spatially biased occurrence data, which limits ecological inferences and their direct application in land-use planning.

In this study, we explored ecological patterns for a range of wild bee species across Latvia using countrywide species distribution models (SDMs) based on maximum entropy analysis. In addition to climatic predictors, the models included an extensive set of habitat descriptors representing landscape composition and structure at a fine spatial resolution of 100 m. To expand the representation of species occurrences across environmental gradients, we combined structured research records with citizen science observations. To account for uneven sampling effort and spatial clustering in the data, we applied a bias-aware modelling workflow, using the target-group approach. Model tuning and validation incorporated spatially structured cross-validation and independent testing datasets to ensure robust ecological inference. Beyond statistical performance, model outputs were interpreted by experts from an ecological perspective.

The resulting species-specific responses to environmental predictors and habitat suitability maps highlight the key factors shaping the distribution of wild bee species and identify landscapes with high potential for supporting diverse bee communities. This study provides new insights into the ecological niches and habitat requirements of wild bees in Latvia. The results can support the identification of priority areas for wild bees and provide valuable information for habitat management planning, contributing to the maintenance of pollination services.

OPTIMIZATION OF IOT IN-HIVE SENSOR PLACEMENT FOR ENHANCED DETECTION OF BEE COLONY HEALTH CHANGES

OC 144

Dagmara Stasiowska[1,2], Maciej S. Bryś[3], Bartosz Kądziołka[2], Michał Wierzbiński[2], Krzysztof Olszewski[4], Aneta Strachecka[3], Sebastian Cibor[2]

[1]Faculty of Space Technologies, AGH University of Krakow, Kraków, Poland
[2]Apisense Sp. zo.o., Kraków, Poland
[3]Department of Invertebrate Ecophysiology and Experimental Biology, University of Life Sciences in Lublin, Lublin, Poland
[4]Subdepartment of Apidology, Institute of Biological Basis of Animal Production, University of Life Sciences in Lublin, Lublin, Poland

Abstract

Currently developed honey bee colony monitoring systems increasingly rely on in-hive sensors to detect environmental stressors and early symptoms of colony health changes. However, the effectiveness of such systems strongly depends on the sensor localisation inside the hive, influencing the signal quality, sensitivity to colony-specific dynamics, and the ability to detect abnormal patterns associated with disease or anthropogenic stressors

In this study, we evaluate the impact of sensor positioning on the sensitivity and diagnostic performance of the IoT in-hive monitoring system. Several sensor positions and orientations were tested, considering brood area proximity, hive center, and peripheral zones. Measured parameters included temperature gradients, humidity, acoustic activity, and gas resistance.

The results indicate a dependency between sensor localization and signal quality and stability. Beekeepers' preference toward the on-frame installation option was additionally noted. Optimized positioning improved the stability of the collected data, which simplifies its later analysis, especially in the case of temperature and humidity data. These findings contribute to the design of more reliable IoT solutions for beekeeping and support the development of scalable digital tools for supporting beekeepers in everyday work and for early detection of honey bee colony health issues.

OC 145 DEVELOPMENT OF ANTIFEEDANT TECHNOLOGIES VIA INHIBITION OF PROTEINS IN *VARROA DESTRUCTOR* SECRETED SALIVA

Daniel R. Swale, Sanghyeon Kim

University of Florida, Gainesville, United States

Abstract

Varroa destructor is an obligate ectoparasite and a major contributor to global colony losses of western honey bees (*Apis mellifera* L.). Salivary secretions that modulate host immune responses and facilitate feeding are critical for successful parasitism of bee hosts, yet gaps in knowledge regarding the functional relevance of individual secreted proteins to feeding and parasitism remain. To bridge this gap in knowledge and technology, we integrated proteomic and transcriptomic analyses to characterize the sialome of *V. destructor* and to identify salivary proteins involved in feeding physiology. Among the highly expressed candidates, five secreted salivary proteins, VdOBP2, VdAV422, VTP, VdCHIsal, and VdKazal, were selected for further investigation. Tissue-specific expression of these genes was quantified by qPCR, revealing abundant transcription in the salivary glands of both mobile and reproductive mites. Electrical penetration graph technique (EPG) studies were performed to quantify shifts in feeding behaviors and RNAi-mediated knockdown of the secreted proteins *VdAV422*, *VTP*, and *VdCHIsal* significantly ($P < 0.05$) reduced probing and overall feeding time. This altered feeding behaviors resulted in significantly ($P < 0.01$) reduced mite ingestion and inhibition to feeding. Furthermore, knockdown of *VdAV422* and *VdCHIsal* markedly decreased mite fertility. These data demonstrate that specific salivary proteins are essential for efficient feeding and reproduction of *V. destructor*. The comprehensive proteome–transcriptome dataset of the salivary gland and secreted saliva combined with the functional assays provide a valuable foundation for defining 1) *V. destructor*-bee interactions, 2) mechanisms of *V. destructor* parasitism, and 3) technology development to reduce mite-feeding induced damage.

CROSS-CULTURAL REQUIREMENTS ENGINEERING FOR TRUSTABLE OPEN PLATFORM FOR HONEY CHAINS

OC 146

Gönül S. Tuzlu[1], Mehmet N. Aydin[2], Zeynep Berktas[1], Sahin Aydin[3], Samet Okuyan[4], Serhat Solmaz[4], Gökhan Akdeniz[5]

[1]Kadir Has University, Istanbul, Turkey
[2]Bogazici University, Istanbul, Turkey
[3]Işık University, Istanbul, Turkey
[4]Apiculture Research Institute, Ordu, Turkey
[5]Aegean Agricultural Research Institute, Izmir, Turkey

Abstract

As the global honey market faces increasing issues of fraud, the TOP4HoneyChains project seeks to establish a trustable open platform for smart honey value chains. This study strategically focuses on Türkiye, one of the world's leading honey producers and a major consumption market, and Germany, the largest consumer market for honey in Europe. By comparing these two pivotal markets, the research aims to identify how cultural contexts shape the socio technical requirements of digital traceability technologies.The research employs a qualitative design based on 42 semi structured interviews (21 in Germany, 21 in Türkiye). Using Requirements Engineering (RE) principles, the study identifies 'trust anchors' that inform the platform's design. Thematic analysis was used to categorize findings into quality constructions, trust mechanisms, and willingness to pay.

Findings reveal a significant divergence in trust models. In Germany, trust is 'system based,' relying on institutional certifications and retail regulations, with traceability viewed as a baseline expectation. Conversely, in Türkiye, trust is 'relational and socially embedded,' where quality is intrinsically linked to beekeeper identity. A key finding is that Turkish consumers seek a personal connection with the producer; thus, digital traceability must be designed to offer 'social proof' such as beekeeper profiles and video narratives rather than mere numerical data. This segment shows a higher technology acceptance and a clear willingness to pay (5–25%) for such transparent information.

The study concludes that a 'one size fits all' approach is insufficient for global honey chain platforms. The TOP4HoneyChains infrastructure must adopt culturally tailored interfaces; emphasizing institutional compliance for the German market, while prioritizing relational transparency and social proof for the Turkish market. These insights provide a strategic roadmap for integrating small scale beekeepers into the digital economy through user centric, cross cultural design.

Keywords: Consumer Behavior, Digital Honey Traceability, Requirements Engineering, Trust, Sustainability, Food Fraud

OC 147 WHICH TRACEABILITY DATA MATTER MOST IN SMART HONEY VALUE CHAINS? CROSS-COUNTRY EVIDENCE FROM BEEKEEPERS

Ewa Wanda Ziemba, Dariusz Grabara, Rafał Kita

University of Economics in Katowice, Katowice, Poland

Abstract

The honey sector faces growing pressure to ensure transparency, traceability, and verifiable quality data in response to persistent concerns about authenticity, fraud, and consumer trust. Meeting these expectations requires better data integration and coordination across the chain, making digital solutions essential for building smart honey value chains. One such solution is a digital traceability honey platform, understood here as a socio-technical infrastructure that integrates and shares verified data on beekeepers, apiaries, honey, and beekeeping practices with downstream actors and consumers. This study examines which traceability data categories should be prioritized in such a platform. Based on relevance ratings from 1,253 beekeepers in Poland, Turkey, and Latvia, it identifies which data categories matter most, which priorities are shared across countries, and which vary by national context. The analysis covered 21 traceability data categories spanning beekeeper-, apiary-, honey-, and beekeeping practice-related data. The findings point to a common cross-country core centered on honey type, laboratory-based honey quality testing, apiary region, apiary location type, honeybee diseases and treatment, feeding of bee colonies, and honey harvesting. At the same time, categories such as non-formal beekeeping education and awards or certificates showed stronger cross-country variation, while others had lower priority across all three countries. Overall, the results support a modular platform design that combines a common interoperable core of traceability data with configurable national elements. The study provides evidence-based guidance for strengthening transparency, trust, authenticity, and data-driven coordination in the honey sector.

Acknowledgment

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Welfare in bees and ONEHEALTH

OC 148 LONG BEFORE THEY WERE 'EMERGING': RETROSPECTIVE DETECTION OF HONEY BEE VIRUSES

Daniela Arredondo[1,2,3,4], Pablo Zunino[1,3,4], Karina Antúnez[1,3,4]

[1]Instituto de Investigación Una Salud, Prorectorado de la Universidad de la República, Montevideo, Uruguay
[2]Programa de Desarrollo de las Ciencias Básicas MEC-UdelaR, Montevideo, Uruguay
[3]Laboratorio de Microbiología y Salud de las Abejas, Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, MEC, Montevideo, Uruguay
[4]Centro de Investigación en Ciencias Ambientales (CICA), Instituto de Investigaciones Biológicas Clemente Estable, MEC, Montevideo, Uruguay

Abstract

The honey bee is a key pollinator in natural and agricultural ecosystems. However, beekeepers worldwide are facing high colony losses driven by multiple interacting stressors, including RNA virus infections. Many honey bee viruses persist in a subclinical state, meaning that their apparent "emergence" may reflect detection rather than recent introduction. In combination with other factors, such as poor nutrition, high *Varroa destructor* infestations, or the presence of fungal or bacterial infections, these viruses can significantly contribute to colony decline. Using next-generation sequencing, our group characterized the viromes of *V. destructor*-resistant and susceptible colonies, reporting for the first time in Uruguay the presence of *Apis mellifera* Filamentous Virus (AmFV), Apis rhabdovirus -1 (ARV-1), and Lake Sinai Virus (LSV). This work aimed to detect these three viruses using a cost-effective molecular approach (qPCR) and perform a retrospective analysis to establish whether their presence is long-standing. We analyzed 202 samples from individual honey bee colonies collected across Uruguay from three independent studies: (i) 28 DNA samples from a regional survey (2004–2005), (ii) 74 cDNA samples from a nationwide survey (March 2011), and (iii) 100 DNA and 93 cDNA samples from a nationwide survey conducted between March and June 2021. Our results revealed the presence of AmFV as early as 2004-2005, with a prevalence of 74.1%, increasing to 82.0% in 2021. ARV-1 showed consistently high prevalence over time (83.8% in 2011 and 87.1% in 2021). In contrast, LSV exhibited a marked increase, from 37.8% in 2011 to 98.9% in 2021. These results indicate that while some viruses have been stably circulating, others have undergone substantial changes in prevalence. In the absence of effective treatments for viral infections in honey bees, molecular surveillance is essential to understand virus circulation and temporal dynamics.

IDENTIFYING CULTURAL AND CHEMICAL CONTROLS TO MANAGE *TROPILAELAPS* MITES IN HONEY BEE COLONIES

OC 149

Léna Barascou[1], Dan Aurell[1], Heather C. Broccard-Bell[2], Pichet Praphawilai[3], Bajaree Chuttong[3], Geoffrey R. Williams[1]

[1]Auburn University, Auburn, United States
[2]NOD Apiary Products Ltd, Trenton, Canada
[3]Chiang Mai University, Chiang Mai, Thailand

Abstract

Tropilaelaps mercedesae is an emerging threat to western honey bees, *Apis mellifera*, that challenges colony health through rapid reproduction, feeding on brood, and transmission of viruses. This research evaluated practical interventions for *T. mercedesae* control (cultural and chemical). As cultural control, brood frames were removed to induce a broodless period intended to interrupt mite reproduction and survival; as chemical control a single formic acid pad (FormicPro™) was applied. These interventions applied on their own and in combination created the following treatment groups: (1) Brood Break; (2) Formic Acid (3) Brood Break + Formic Acid; and (4) Untreated Control. Following the 21-day treatment period, a clean-up treatment was applied to all colonies consisting of queen caging for 24 days followed by two oxalic acid applications to eliminate remaining mites. *Tropilaelaps* infestation levels were assessed on Days 0 and 21 by uncapping and examining 200 worker brood cells per colony. Mite fall was monitored every three days throughout the experiment using bottom-board sticky boards. Colony strength and condition, including adult bee population, capped worker brood, and food resources, were evaluated through visual inspections on Days 0, 21 and 45. Post-treatment results (Day 21) indicate higher *Tropilaelaps* infestation in Untreated Colonies (2.84%) compared to the Brood Break (0%), Brood Break + Formic Acid (0.05%) and Formic Acid (0.07%) groups. Interestingly, when cells were inspected 4-5 days after formic acid application, all mites found in brood cells were dead; whereas all mites in the control colonies were alive, which explains the mechanism of action. Lower populations of adult honey bees and worker brood were observed in Brood Break and Brood Break + Formic Acid colonies compared to Control and Formic Acid colonies on Day 21. While some mites were detected post-treatment, these findings support brood interruption and formic acid treatment as effective strategies for *Tropilaelaps* control.

OC 150 INTERSPECIFIC LEARNING IN BEES ENHANCES A PARASITE RESISTANCE TRAIT

Alexis L. Beaurepaire[1,2], Alix Klein[2], Elisa Putteman[2], Preeyaporn Phongutthа[3], Rujira Ponkit[3], Sanchai Naree[3], Guntima Suwannapong[3], Vincent Dietemann[1], Peter Neumann[2]

[1]Swiss Bee Research Centre, Agroscope, Liebefeld, Switzerland
[2]Institute of Bee Health, University of Bern, Liebefeld, Switzerland
[3]Burapha University, Bang Saen, Thailand

Abstract

Although animals can learn to avoid parasites by observing the behavior of other species, there is limited evidence for interspecific learning in relation to parasite resistance. Since the signals and ecological niches of eastern honey bees (*Apis cerana*) and western honey bees (*Apis mellifera*) are very similar, it is possible that interspecific learning could be used to promote their parasite resistance traits. Here, we demonstrate that the grooming behavior of *A. mellifera* against the ectoparasitic mite *Varroa destructor* can be enhanced through interspecific learning from original hosts, *A. cerana*. In a laboratory assay in Thailand, newly emerged *A. mellifera* workers ('learners') were paired with either a conspecific or a heterospecific *A. cerana* worker ('demonstrators'). The learners' grooming behaviours were assessed before and after eight learning bouts, during which their paired demonstrators had been experimentally infested. The results show that learners paired with *A. cerana* demonstrators displayed a lower grooming latency and more pronounced grooming behaviors after the social learning bouts than those paired with conspecifics. If these results can be replicated in colonies under field conditions, they could provide practical solutions to the challenges posed by ectoparasitic mites, particularly in Asia where original resistant and introduced susceptible host species coexist. More broadly, the data suggest novel avenues to promote resilient beekeeping through social learning and to study the mechanisms underlying parasite resistance in social insects.

EFFICACY AND SAFETY OF API-BIOXAL SOLUTION FOR CONTROL OF *VARROA DESTRUCTOR* IN *APIS MELLIFERA*: A CLINICAL STUDY

OC 151

Michela Bertola[1], Martina Gallo[2], Annalisa Zonca[2], Mattia Schiavo[3]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, NRL for honey bee health, Legnaro, Italy.
[2]FAREFARMA S.r.l., Invorio, Italy
[3]Chemicals Laif S.p.A., Vigonza, Italy

Abstract

Varroa destructor is the most significant threat affecting honey bee (*Apis mellifera*) colonies worldwide, causing substantial economic losses in beekeeping. Effective and safe control strategies are therefore essential for sustainable beekeeping. This study evaluated the efficacy and safety of an oxalic acid-based veterinary medicinal product (API-BIOXAL 44.2 mg/mL solution) under field conditions.

A randomized controlled field trial was conducted in two apiaries in Northern Italy. Overall, thirty-two colonies were enrolled and randomly assigned to treated (n=16) and control (n=16) groups. To ensure brood interruption, queens were caged prior to treatment. API-BIOXAL was administered once at the recommended dose (5 mL per comb), while control colonies received a placebo. Mite infestation levels were assessed using adhesive boards combined with digital counting. Efficacy was calculated as mite mortality, while safety was evaluated based on bee mortality, colony strength, and honey production. A follow-up treatment was applied to all colonies to estimate residual mite populations.

The treatment demonstrated high efficacy, with mean mite reduction of 95.33% and 89.82% in the two apiaries, respectively, and an overall efficacy of 92.57%. No statistically significant differences were observed between treated and control groups in terms of bee mortality or colony strength, indicating a favourable safety profile. Colony performance parameters, including adult bee population and honey stores, remained comparable between groups.

In conclusion, API-BIOXAL showed high efficacy (>90%) against *V. destructor* and was well tolerated by honey bees under summer field conditions with brood interruption by queen caging. These results support its use as a reliable tool for integrated varroa management control.

OC 152 ENHANCED EFFICACY OF OXALIC ACID AGAINST *VARROA DESTRUCTOR*: LABORATORY ASSESSMENT OF AN ADJUVANT-BASED FORMULATION

Michela Bertola[1], Franco Mutinelli[1], Mattia Schiavo[2]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, NRL for honey bee health, Legnaro, Italy-[2]Chemicals Laif S.p.A., Vigonza, Italy

Abstract

This laboratory study evaluated the efficacy of different oxalic acid (OA) formulations against *Varroa destructor* under controlled conditions. Specifically, a 30% OA solution was tested with and without adjuvant (0.5%) and compared to glycerol and untreated controls.

Adult honey bees naturally infested with *V. destructor* were collected from broodless colonies in Northern Italy and allocated into experimental cages (≈300 bees per cage). A total of four treatment groups were tested in triplicate: OA with adjuvant (OA-A), OA without adjuvant (OA), glycerol (G), and untreated control (C). Treatments were administered via impregnated sponge strips, ensuring continuous contact exposure over 96 hours. Cages were maintained under controlled conditions (33°C, 60% RH), and mite mortality was assessed at multiple time points. Final mite counts were obtained through ethanol washing of bees. Efficacy was calculated as cumulative mortality and corrected using Abbott's formula. Statistical analysis was performed using a generalized linear model (GLM) with binomial distribution.

Results showed a significant effect of treatment on mite mortality (x^2=156.9; $p < 0.001$). The OA-A group achieved 100% efficacy across all replicates, while OA alone reached 95.2% efficacy. Both treatments were significantly more effective than glycerol (31.8%) and control (25.9%) groups. No statistically significant difference was observed between OA and OA-A (p=0.059), although the adjuvant-treated group consistently showed complete mite mortality. No bee mortality was recorded during the experiment.

These findings confirm the high efficacy of oxalic acid-based treatments under laboratory conditions and suggest a potential additive effect of the adjuvant. Further studies are needed to validate these results under field conditions and to assess long-term effects on colony health.

HONEYBEE BLOOD BEYOND THE MICROSCOPE: FROM MORPHOLOGICAL TO MARKER-BASED CLASSIFICATION OF HEMOCYTES

OC 153

Merel Braeckman, Lina De Smet, Bart Devreese, Dirk C. de Graaf

Ghent University, Ghent, Belgium

Abstract

The global decline of honeybee populations is a pressing issue, yet their cellular immunity—specifically the blood cells, or hemocytes—remains significantly understudied. In contrast, the blood cells of other farm animals have been extensively characterized, leading to a deeper understanding of their immune systems. One of the key challenges in studying honeybee hemocytes is their morphological similarity, which makes it difficult to distinguish their functions. For instance, differentiating between plasmatocytes and prohemocytes, or oenocytoids and granulocytes, often requires advanced microscopy techniques such as interference or phase contrast microscopy. Additionally, researchers debate the existence of hemocyte subtypes like spherulocytes and coagulocytes. A standardized classification system is crucial to determine the hemocyte types present in honeybee blood and their roles in immunity.

In our study, we aimed to characterize honeybee hemocytes based on the proteins present on the outer surface. This strategy, known as the Cluster of Differentiation (CD) system, has been widely applied to various cell types across different organisms. We enzymatically shaved the surface proteins from the hemocytes and identified them using mass spectrometry. Based on these findings, monoclonal antibodies were generated against selected candidate markers. By establishing a panel of antibodies that react with different hemocyte subtypes, researchers can investigate objectively the impact of stress factors like heat or nutritional stress on hemocyte populations. A better understanding of honeybee hemocytes could pave the way for new strategies to support colony health and resilience against environmental threats.

OC 154

EVALUATING THE EFFICACY OF A RNAI-BASED *VARROA DESTRUCTOR* CONTROL PRODUCT (NORROA™) IN THE SOUTHEASTERN UNITED STATES

Selina Bruckner[1], Smith Easton[1], Skyler Demi[1], Agostina Giacobino[1], Geoffrey R. Williams[1], Adam Pachl[2], Brian S. Manley[2]

[1]Auburn University, Auburn, United States
[2]Greenlight Biosciences, Medford, United States

Abstract

Around the world, honey bee colonies are suffering from effects of *Varroa destructor*. Beekeepers are encouraged to utilize a combination of different management strategies for *V. destructor* throughout the year. While there are mechanical and cultural management strategies for *V. destructor*, chemicals are extensively used due to their efficacy and ease of use. This has resulted in the development of resistant *V. destructor* populations, especially to synthetic chemical treatments. Thus, there is a need for novel, innovative management strategies to reduce *V. destructor* infestation levels in honey bee colonies. RNA interference is widely used in other agricultural commodities but has largely been absent in apiculture. Norroa™ is filling this void. Delivered via a sucrose-based solution, Norroa™ disrupts *V. destructor* reproduction thereby slowing their population growth and maintaining low infestation levels. As a newly registered product in the United States, there is a need to evaluate the product's performance at different times of the year and climatic regions. To address this, the objectives of this work were to 1) evaluate the efficacy of Norroa™ in full-sized colonies and 2) compare its performance across summer, fall and spring in the southeastern United States. We will discuss how the efficacy of Norroa™ varies across seasons and how initial *V. destructor* infestation levels may affect the product's performance.

HONEY AS A NON-INVASIVE MATRIX FOR THE MOLECULAR DETECTION OF *APIS MELLIFERA* PATHOGENS

OC 155

Giovanni Cilia, Rossella Tiritelli, Gian Luigi Marcazzan, Antonio Nanetti, Cecilia Costa

CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Honey bees (*Apis mellifera* L.) are threatened by multiple environmental pressures, including habitat degradation, pesticide exposure, climate change, and infectious diseases. Pathogens such as viruses, microsporidia, and trypanosomatid parasites can significantly compromise colony health and have been associated with large-scale losses. Conventional pathogen surveillance is typically based on sampling bees or brood, procedures that can be invasive and logistically demanding. Recent molecular approaches have highlighted honey itself as a useful matrix for pathogen detection because it is continuously exposed to the internal hive environment and may contain traces of microbial DNA and RNA originating from infected bees.

In this study, 679 honey samples of both multifloral and monofloral origin were collected directly from beekeepers across all Italian regions and analysed to assess the presence of eight major honey bee pathogens: DWV, CBPV, ABPV, BQCV, KBV, *Nosema ceranae*, *Crithidia mellificae*, and *Lotmaria passim*. Pathogen detection and quantification were performed using quantitative PCR assays. Overall, 97.5% of the analysed samples contained at least one pathogen. The most frequently detected agents were DWV (81.7%), *N. ceranae* (56.1%), and CBPV (56.0%). In contrast, neither KBV nor *C. mellificae* were detected. Co-infection was common: approximately 77% of the honey samples contained genetic traces of more than one pathogen. Significant differences were found in pathogen prevalence and load according to both botanical origin and geographic area, suggesting that environmental and floral factors may influence pathogen distribution.

These results demonstrate that molecular analysis in honey is an effective, non-invasive approach for large-scale surveillance of honey bee pathogens. Such monitoring could support improved epidemiological assessments and disease management strategies in beekeeping. Further investigations are needed to clarify the relationship between pathogen signals detected in honey and the actual health status of colonies.

OC 156 COGNITIVE EFFECTS OF CHLORANTRANILIPROLE ALONE OR IN MIXTURE WITH A PYRETHROID IN HONEY BEES

Loïc Colin-Duchevet[1], Apolline Sangely[1], Pierre Charnet[2], Jean-Christophe Sandoz[1]

[1]Evolution, Genome, Behavior and Ecology, CNRS, IRD, University Paris-Saclay (UMR9191), Gif-sur-Yvette, France
[2]Institut des Biomolécules Max Mousseron, Université de Montpellier, CNRS, ENSCM, Montpellier, France

Abstract

Chlorantraniliprole is a diamide insecticide targeting insect ryanodine receptors, which are involved in intra cellular calcium release in neurons and muscles. In turn, this calcium release triggers neurotransmitter exocytosis and muscle contraction. Despite the presence of these receptors within neurons, possible effects of this insecticide on cognitive function have not been addressed yet. Honey bees are vital pollinators and serve as a valuable model for studying insect cognition, through the use of appetitive learning. Here, we assessed the sublethal effects of chlorantraniliprole on bees' olfactory perception, learning and memory. Given that bees are exposed to multiple pesticides while foraging, we examined the effect of chlorantraniliprole alone or in combination with a pyrethroid insecticide to determine potential cocktail effects on cognition. To this aim, we used the conditioning of the proboscis extension response (PER) and assessed the effect of pesticide exposure on several aspects of bee cognition. Non-associative learning was assessed through habituation of the PER, while associative learning and olfactory generalization were evaluated using absolute conditioning. Finally, bees' olfactory discrimination abilities were measured through differential conditioning. Chlorantraniliprole was found to strongly affect bees' habituation and olfactory discrimination performances. Both antagonist and additive cocktail effects were found using both pesticides together. These results indicate that low-dose chlorantraniliprole contamination may negatively impact bees' ability to find fruitful floral sources while foraging, and in turn impair their pollination service.

TEMPORAL SHIFTS IN ACARICIDE RECEPTOR GENE MUTATIONS IN *VARROA DESTRUCTOR* FROM PORTUGAL AND SPAIN

OC 157

Maíra Costa[1], Antonio Pérez-Pérez[2], Carlos A. Yadró García[1], Ana R. Lopes[1], Raquel Martín-Hernández[3], Maria Alice Pinto[1], Mariano Higes[3], Dora Henriques[1]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[2]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Centro de Investigación Apícola y Agroambiental de Marchamalo, (CIAPA), Laboratorio de Patología Apícola, Marchamalo, Spain
[3]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Centro de Investigación Apícola y Agroambiental de Marchamalo, (CIAPA), Laboratorio de Patología Apícola, Marchamalo, Spain

Abstract

Varroa destructor is an ectoparasitic mite and a major threat to the honey bee (*Apis mellifera*), causing severe colony losses worldwide. Control relies primarily on synthetic acaricides, but their efficacy can be compromised by mutations in target genes that confer resistance. Key targets include the β-adrenergic octopamine receptor (*Octβ2R*), potentially linked to amitraz, and the voltage-gated sodium channel (*VGSC*), associated with pyrethroid resistance. We conducted a temporal analysis of mutation frequencies in *V. destructor* populations from Portugal and Spain, comparing historical samples (2010) with recent collections (2023/2025). DNA was extracted from individual mature female mites and sequenced using Sanger methodology with gene-specific primers. Sequences were visually inspected in BioEdit. In 2010, mutations theoretically linked to amitraz resistance were absent. The L925V mutation in *VGSC*, associated with pyrethroid resistance, was already present in some samples, suggesting selection from widespread pyrethroid use. Between 2023 and 2025, nearly all populations carried the F290L mutation in *Octβ2R*, whose role in amitraz resistance remains uncertain. Pyrethroid-resistance genotypes evolved toward M918L/L925V combinations, consistent with potential "super-resistance," although further confirmation is required. These findings demonstrate clear temporal shifts in acaricide receptor gene mutations in *V. destructor*. Changes likely reflect historical acaricide use and selective pressures, and may involve host–parasite coevolution. The high prevalence of F290L underscores the need to identify its selective factor to clarify amitraz involvement. Understanding these temporal patterns is critical for designing effective integrated pest management strategies, mitigating resistance development, and protecting honey bee health in Europe.

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OC 158

GENOMIC PROFILING OF DEFORMED WING VIRUS VARIANTS AND ARTIFICIAL INFESTATION SUGGEST POTENTIAL HOST/VIRUS COADAPTATION IN *APIS MELLIFERA UNICOLOR*

Emma Couranjou[1], Hélène Delatte[1], Benjamin Pélissié[1], Nicolas Blot[2,1], Alexandre L'Hostis[1], Gérard Lebreton[1], Frank Schurr[3], Christophe Simiand[1], Virginie Diévart[4], Anne Bonjour-Dalmon[4]

[1]CIRAD, UMR PVBMT, Saint-Pierre, France
[2]UCA, LMGE, Aubière, France
[3]ANSES, Biot, France
[4]INRAE, UR406, Avignon, France

Abstract

Among the many biotic and abiotic threats faced by honeybees, the Deformed Wing Virus (DWV), with its spread facilitated by the invasive acarian *Varroa destructor*, exerts important pressure on colony health. Two main, potentially recombining variants, DWV-A and DWV-B, are widely distributed and differ in their impact on honeybee health. Since the first detection of *V. destructor* in Réunion Island in 2017, high DWV prevalence and viral loads have been observed in the endemic South-West Indian Ocean (SWIO) honeybee *Apis mellifera unicolor*, although variant genetic identity remains uncharacterized. This recent tropical invasion parallels the vector's 2020 arrival on the temperate French island of Ushant, providing a unique opportunity to compare post-*Varroa* shifts in DWV diversity and its impact for colony health. First, we used nanopore sequencing to describe the genomic profile of the endemic variants and compare them to previously sequenced variants from various geographical origins. Multi-reference mapping analyses revealed a stronger similarity of Reunion's isolates to DWV-B rather than DWV-A variants, whereas Ushant's isolates show a mixture of DWV-B and DWV-A variants. Second, we assessed variant virulence on *A. m. unicolor* colonies from Reunion using an artificial infection experiment that compared the impact of a DWV-B reference variant, two isolates from Ushant, and one isolate from Reunion. Pink-eyed pupae were injected with standardized viral doses mimicking *Varroa* transmission and monitored for 9 days within a confined BSL-3 laboratory. We observed strong differences between treatments regarding both emergence rates and symptom severity, suggesting that infection severity in *A. m. unicolor* might be modulated by DWV genomic diversity. Overall, local DWV isolates had less impact on endemic honeybee populations than exogenic isolates, suggesting a pattern of local coadaptation between the virus and its host in SWIO.

THE COST OF TREATMENT: HIDDEN EFFECTS OF OXALIC ACID ON HONEY BEE PHYSIOLOGY AND RISKS FOR BEEKEEPING PRACTICE

OC 159

Jiří Danihlík, Eliška Beranová

Palacký University Olomouc, Olomouc, Czechia

Abstract

Oxalic acid (OA) is a traditional active substance used against the *Varroa destructor* mite. Its popularity increased significantly with the introduction of long-term carriers such as OA-glycerin (OA-G) strips. OA remains a crucial acaricide for Integrated Pest Management (IPM); however, its sublethal impacts and practical risks are often overlooked.

In adult bees, long-term OA-G exposure acts as an immune stimulant, significantly enhancing hemolymph antimicrobial activity and elevating key antimicrobial peptides. Conversely, the developing brood faces a severe physiological cost. Gene expression analysis of prepupae reveals significant developmental trade-offs under OA stress: while certain immune genes are upregulated (e.g., antimicrobial peptides), the expression of the crucial storage protein vitellogenin is markedly suppressed. Furthermore, multi-omics (proteomic and metabolomic) profiling demonstrates that OA forces adaptive metabolic reprogramming in larvae. This drives increased activity in the central energy metabolism (TCA cycle) to cope with the chemical burden.

This physiological burden on the brood is highly alarming when considered in the context of beekeeping practice. Moreover, prolonged exposure combined with suboptimal efficacy creates enormous selection pressure on the mites, a situation dangerously similar to the development of amitraz resistance. This raises the risk of rapid *Varroa* adaptation, potentially through cuticle strengthening, detoxification, or the involvement of oxalotrophic bacteria.

Ultimately, while OA-G bolsters adult immunity, it imposes high metabolic costs and immune compromises on the brood, highlighting a hidden developmental price for *Varroa* management that must be considered in colony health strategies and the sustainable use of OA.

We gratefully acknowledge our colleagues from Masaryk University Brno, Bee Research Institute Dol, University Hospital Olomouc (Czechia), and Aarhus University in Slagelse (Denmark) for their collaboration on this research topic. This work was supported by projects QL24010241, QL26020146 and LUAUS24085.

OC 160 COMBINING BROOD INTERRUPTION AND OXALIC ACID TREATMENT FOR SUMMER CONTROL OF *VARROA DESTRUCTOR* IN *APIS MELLIFERA* COLONIES

Victoria Ferland[1], Laurence Plamondon[2], Pierre Giovenazzo[1]

[1]Université Laval, Québec, Canada
[2]CRSAD, Québec, Canada

Abstract

In Canada, honeybee colonies experience substantial winter losses, largely driven by *Varroa destructor*. Because winter bees are produced in late summer, when varroa populations typically peak, effective summer control is critical for overwinter survival. However, the most effective summer treatment strategy in a North American beekeeping context remains unclear, as oxalic acid, one of the most widely used acaricides, has limited efficacy in colonies containing brood because mites reproducing within capped brood cells are protected from treatment. This study evaluates whether combining brood interruption, induced using an innovative queen-ringing technique, with oxalic acid dribble treatment improves varroa management.

In July 2025, 39 honeybee colonies were assigned to one of four groups: untreated control, brood interruption alone, oxalic acid alone, or brood interruption combined with oxalic acid. Colonies were monitored throughout the season for mite infestation, colony strength, honey production, effects of ringing on queen performance, winter survival, and viral loads.

Results indicate that the combined treatment was the most effective strategy for reducing mite levels below the recommended economic threshold of 10 varroas per day. No long-term effects on colony strength were detected. However, contrary to previous studies, brood interruption was associated with lower end-of-summer honey production and higher queen mortality. Summer treatment had no significant effect on viral loads measured at the end of summer or in autumn.

These results provide new insight into the combined use of queen ringing and oxalic acid as a summer treatment for *Varroa destructor*. They also support the development of cost-effective integrated pest management strategies that may reduce reliance on commonly used synthetic acaricides, limit resistance risk, and promote more sustainable apiculture.

ABUNDANT RESOURCES, LIMITED GROWTH: A NUTRITIONAL PARADOX OF MASS-FLOWERING CROP

OC 161

Zuzanna M. Filipiak[1], Bożena Denisow[2], Ernest Stawiarz[2], Natalia Kamińska[1]

[1]Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Kraków, Poland
[2]Laboratory of Plant Biology, Department of Botany and Plant Physiology, University of Life Sciences in Lublin, Lublin, Poland

Abstract

Mass-flowering crops, such as rapeseed, create short-lived but abundant pulses of floral resources. Although these crops may briefly alleviate food limitation, their nutritional imbalance may simultaneously impair larval development, especially in solitary bees. Bee populations rely on the successful production of numerous healthy offspring each year; therefore, understanding how crop pollen affects larval development in bees is essential.

To test this, we studied the effects of rapeseed pollen on larval development in the solitary bee *Osmia bicornis*, which incorporates rapeseed into its larval provisions when available. Using experimentally manipulated diets with different rapeseed proportions, we measured larval survival, adult emergence, and body mass in both sexes. A higher proportion of rapeseed pollen in the larval diet caused strong reduction in adult body mass, while larval survival and adult emergence remained unaffected. Therefore, rapeseed pollen can constrain growth, even when survival-based measures detect no cost.

To place this pattern in ecological context, we compared our results with published field data. Field studies show the opposite combination: higher body mass, but lower emergence, with increasing rapeseed representation in pollen provisions. This apparent contradiction may arise from scale-dependent mechanisms. In the field, rapeseed abundance likely enhances maternal provisioning capacity, leading to larger offspring, while simultaneously imposing nutritional imbalance that reduces successful survival to adulthood. Nutritional imbalance, together with other environmental factors, may result in selective filtering, whereby fewer individuals emerge, but those that do are larger.

The effects of mass-flowering crops on pollinators can differ depending on whether food quantity or quality is considered, highlighting the importance of accounting for both when evaluating agricultural landscapes.

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OC 162 NUTRITIONAL MODULATION OF HONEYBEE (*APIS MELLIFERA*) HEALTH: THE ROLE OF POLLEN QUALITY UNDER *NOSEMA CERANAE* AND *VARROA DESTRUCTOR* INFECTION

Laura Fourel[1], Vincent Piou[2], Michel Bocquet[3], Philippe Bulet[3], Frédéric Delbac[1], Angélique Vetillard[2], Marie Diogon[1], Hicham El Alaoui[1]

[1]Clermont-Auvergne University, Clermont-Ferrant, France
[2]Paul Sabatier University Toulouse 3, Toulouse, France
[3]Grenoble-Alpes University, Archamps, France

Abstract

The global decline of honeybee colonies threatens pollination services and biodiversity. Key contributors include the ectoparasitic mite *Varroa destructor* and the gut microsporidian parasite *Nosema ceranae*, both originating from the Asian honey bee (*Apis cerana*), and now commonly infecting the Western honey bee (*Apis mellifera*) since the late 20th century. Their presence is associated with profound physiological disruptions, including alterations in lipid metabolism and immune responses, gut dysbiosis, and reduced foraging abilities, severely compromising colony survival.

Pollen, providing essential proteins and lipids, plays a crucial role in bee development, immunity, and health. In this context, diet quality may critically influence the ability of bees to cope with parasitic stress. Thus, we hypothesized that pollen availability and nutritional quality can modulate tolerance to single and co-infections by *N. ceranae* and *V. destructor* and may influence important physiological markers and bee survival.

Our results show that both infection and co-infection significantly impair bee health, as reflected by reduced fat body lipid, altered immune markers, and reduced survival. However, nutrient-rich polyfloral pollen diets attenuated these negative effects, improving immune responses and longevity. In contrast, pollen-deprived or monofloral diets exacerbate parasite-induced damage, revealing strong interaction between nutrition and parasite pressure and suggesting nutritional competition between parasites.

Overall, this work demonstrates the pivotal role of pollen quality in mitigating pathogen effects, offering insights in nutrition-based strategies to enhance honeybee resilience and support sustainable apiculture.

CAN THE SELECTION FOR *V.DESTRUCTOR* MITE RESISTANCE BE ENHANCED BY THE USE OF AN INNOVATIVE MANAGEMENT CONCEPT IN *A.MELLIFERA* BEEKEEPING?

OC 163

Lena Frank, Marina D. Meixner

LLH - Bee Institute Kirchhain, Kirchhain, Germany

Abstract

Varroa destructor remains the most significant ectoparasite of the western honey bee (*Apis mellifera*) and causes considerable losses to honey bee populations worldwide. The implementation of innovative management concepts could in the long term contribute to an improved selection for increased defence against *V. destructor,* without increasing colony losses or reducing the honey yield.

Here we compare *Apis mellifera* colonies under different management concepts over three years in terms of health, performance, population development and mating success. In an innovative management group, an artificial brood interruption in summer is induced, followed by an oxalic acid treatment in the brood-free state; however, routine winter treatment is not performed. Instead, *V. destructor* infestation development is carefully monitored, and a winter treatment applied only when necessary. The control group of conventionally managed colonies receives formic acid treatments in late summer and an oxalic acid treatment in winter. In addition, in the comparison group, drone brood is removed in spring, which is not carried out in the innovative approach. Starting in July 2021 two apiaries were set up with 8+8 colonies each, evenly distributed to the experimental groups based on colony strength and mite infestation. Population dynamics and parasitization were recorded over the course of the years.

The innovative approach led to higher infestation levels in the summer, but to lower levels following the summer treatment. At the same time, honey yields remained comparable, provided that winter treatment was carried out on colonies where infestation thresholds had been significantly exceeded. The colonies managed using the innovative approach also showed, on average, greater colony strength prior to winter and a higher overwintering index, without any adverse effects on drone fitness being observed.

OC 164 FROM CROPS TO DROPS: A COMPREHENSIVE STUDY OF THE LINKS BETWEEN PESTICIDE APPLICATION AND DETECTION IN MATRICES OF THREE POLLINATORS

Elva Fuentes[1], Marion Laurent[1], Marie-Pierre Rivière[1], Marie-Pierre Chauzat[1,2]

[1]Unit of Honey Bee Pathology, Sophia Antipolis Laboratory, ANSES, Biot, France
[2]Laboratory for Animal Health, ANSES, Paris-Est University, Maisons-Alfort, France

Abstract

In a *One Health* context, a better understanding of the fate of pesticides in the environment and in wildlife is crucial to appreciate the risks to humans. Still, few studies investigated the links between in-field applications of pesticides and their detection in pollinator matrices, especially considering multiple substances under natural conditions. In the PoshBee project, samples from five matrices (bees, beebread, nectar, pollen from *Apis* pollen traps and from flowers) were collected in 128 sites across eight European countries. Three sentinel species were studied for their contrasted ecology: the honey bee (*Apis mellifera*), the bumble bee (*Bombus terrestris*) and the red mason bee (*Osmia bicornis*). Farmer surveys were conducted, providing information on the pesticides applied on the studied crops (oilseed rape and apple orchards). Multiresidue analyses revealed that 31% of all searched pesticides (167 substances among 542 screened) were detected in at least one pollinator matrix. Most of the substances detected were fungicides, boscalid being the most frequent across all matrices. In oilseed rape fields, the herbicide metazachlor had the highest doses applied while in apple orchards it was the fungicide sulphur. Metazachlor, screened in all matrices (except nectar), was not detected in any. Considering beebread from the three species, per site there was on average two substances both applied and detected, two applied substances not detected, two applied substances not screened, while 16 not applied were detected, and almost 300 substances were neither applied nor detected or screened. Overall, our results point out a mismatch between applied and detected substances in most cases. Detecting not applied substances questions the time spent conducting partial farmer surveys, and highlights the potential contamination by pesticides long after and far from their application. Moreover, screening a wide range of neither applied nor detected substances, questions the costs associated with laboratory analyses.

DISRUPTION OF CENTRAL CARBON METABOLISM MODULATES INFECTIVITY IN *LOTMARIA PASSIM*

OC 165

Tamara Gómez-Moracho[1,2], Mariano Higes[3], Luis Miguel De Pablos[1,2]

[1]Department of Parasitology, Biochemical and Molecular Parasitology Group CTS-183, Granada, Spain
[2]Institute of Biotechnology, University of Granada, Granada, Spain
[3]Laboratorio de Patología Apícola, Centro de Investigación Apícola y Agroambiental (CIAPA), IRIAF – Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Consejería de Agricultura de la Junta de Comunidades de Castilla-La Mancha, Marchamalo, Spain

Abstract

The trypanosomatid parasite *Lotmaria passim* is highly prevalent in *Apis mellifera* colonies worldwide. The parasite colonizes the honey bee hindgut, where it differentiates from the promastigote to the haptomonad form. During this transition, *L. passim* secretes Extracellular Polymeric Substances (EPS) forming a biofilm-like layer that covers the honey bee gut epithelium where haptomonads embed. Although *L. passim* reduces honey bee survival, the mechanisms underlying its pathogenicity are still unknown.

Carbohydrate metabolism provides essential precursors for the synthesis of the EPS, which has been associated with virulence-related traits in diverse microorganisms. Here, we investigated the role of central carbon metabolism in *L. passim* virulence by performing loss-of-function assays using CRISPR-Cas9 targeting genes involved in the carbohydrate-metabolic pathways. We characterised several parasite phenotypes, including growth dynamics, biofilm production and infectivity and virulence in honey bees. In addition, we analysed the metabolome to identify overexpressed metabolic pathways in different *L. passim* mutants. Disruption of carbohydrate metabolism genes altered parasite growth, biofilm production and infectivity, leading to less infective phenotypes.

These findings suggest that central carbon metabolism plays a major role in parasite differentiation and host colonisation, providing new insights into the molecular mechanisms underlying host-parasite interactions in honey bees.

OC 166 METABOLIC CHANGES IN HONEY BEE DRONE REPRODUCTIVE ORGANS IN RELATION TO *VARROA DESTRUCTOR* AND AGING

Gabriela Hofericová, Jiří Danihlík

Department of Biochemistry, Palacky University, Olomouc, Czechia

Abstract

Honey bee drones (*Apis mellifera*) are essential for colony reproduction and genetic diversity, as their successful mating with the queen ensures the long-term survival and vitality of the population. However, their reproductive health is frequently compromised by the mite *Varroa destructor*, which leads to reduced body weight and smaller reproductive organs, specifically the testes, seminal vesicles, and mucus glands. These anatomical impairments limit the production and storage of viable sperm, directly threatening queen mating success and colony stability. Our study investigates metabolic disturbances within the reproductive organs of drones from *Varroa* infested colonies. As these organs are the primary site for sperm maturation, their biochemical composition provides an indirect measure of sperm quality. These tissues are naturally rich in proteins and essential carbohydrates, such as glucose, fructose, and trehalose, which are critical for sperm motility and viability. *Varroa* infestation induced alterations in these metabolites potentially leading to an unsuitable metabolic environment for sperm maturation. To examine these disruptions at the biochemical level, we performed high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) to characterize the carbohydrate and amino acid profiles. The analysis revealed a distinct metabolic shift within the reproductive system of drones from infested colonies. This was marked by a reduction in key energy sources, such as trehalose, alongside significant changes in the composition and concentrations of amino acids. Furthermore, our focus on the impact of age showed similar biochemical differences between newly emerged and older, mature drones. Together, these findings suggest that both *Varroa* infestation and age significantly alter the biochemical environment of the reproductive organs, potentially reducing sperm quality and compromising overall colony fertility.

This work was supported by projects Ministry of Agriculture, Czech Republic QL24010241 & QL26020146 and by IGA_PrF_2026_021.

SILENCING THE MITE: FIELD PERFORMANCE OF AN RNAI-BASED CONTROL FOR *VARROA DESTRUCTOR*

OC 167

Cameron J. Jack, Devan Rawn

University of Florida, Gainesville, United States

Abstract

RNA interference is a promising next-generation tool and has been demonstrated to control invertebrate pest populations in other systems. The novel product Norroa™ (active ingredient vadescana) is the first of its kind marketed to beekeepers for the control of *Varroa destructor*. Our recent explorations in honey bee colonies during different seasons and hive configurations provides relevant information regarding the treatments safety and proper use. In 2023, during a nectar dearth, and again in 2024 during a nectar flow, field trials were conducted to test the effectiveness of Norroa™ in limiting the growth of *V. destructor* infestations. In each season, 36 honey bee colonies were established for testing. During the nectar dearth, mite numbers started higher (4.56 mites/100 bees) and increased more in control colonies compared with the treated colonies, but differences were not significant. During the nectar-flow, Norroa™ maintained *V. destructor* populations at or below the initial infestation rate (2.31 mites/100 bees) for twelve weeks, compared with the control group that grew significantly higher than treated colonies. To understand the effects of Norroa™ better, a final trial was conducted uncapping honey bee pupae and examining mite reproduction. Mites from colonies that had been treated with Norroa™ were significantly less likely to lay an egg, or have any offspring emerge from the eggs laid. These trials demonstrate the promise of a new tool for beekeepers to control *V. destructor*.

OC 168 BROOD HYPERTHERMIA FOR VARROA CONTROL REDUCES ANTIMICROBIAL PEPTIDE EXPRESSION IN ADULT HONEY BEES

Lenka Jerele[1], Eva Hrtánková[2], Maja I. Smodiš Škerl[1], Jiří Danihlík[2]

[1]Agricultural institute of Slovenia, Ljubljana, Slovenia
[2]Palacký University Olomouc, Olomouc, Czechia

Abstract

Brood hyperthermia is increasingly used as a biotechnical method to control *Varroa destructor,* exploiting the mite's greater sensitivity to heat compared with developing honey bee brood. Short-term exposure to elevated temperatures can effectively reduce mite populations without causing brood mortality. As resistance to synthetic acaricides continues to rise, such thermal treatments represent a promising sustainable alternative; however, their effects on honey bee physiology, particularly immune function, remain poorly understood.

In this study, we evaluated the immediate effects of heat treatment on pupae and the long-term effects on adult *Apis mellifera carnica* workers. Late-stage pupae were exposed to 42°C for 2 hours, while control brood was maintained at 34°C. Twenty-four hours after treatment, a subset of pupae was either left untreated or subjected to immune stimulation by cuticular puncture with a sterile needle. Pupae were sampled 24 hours later to assess their immune response. The remaining brood was allowed to emerge, and newly emerged workers were maintained in laboratory cages. At 11 days of age, adult bees were either left untreated or immune stimulated using the same procedure and sampled 24 hours later. Gene expression of four antimicrobial peptide genes—*defensin-1, abaecin*, *hymenoptaecin*, and *apidaecin*—as well as selected heat shock protein genes, was quantified by qPCR.

Adult bees originating from heat-treated pupae exhibited significantly lower basal expression of all four antimicrobial peptide genes compared with controls. However, mechanical injury induced a strong upregulation of these genes in both groups, with a comparable magnitude of response. These results indicate that while brood hyperthermia reduces resting immune investment of adult workers, it does not impair the functional capacity to mount a robust humoral response. This suggests that early-life thermal stress alters the immune set-point without compromising the bee's ability to defend against acute challenges.

NUTRITIONAL CONTEXT SHAPES SULFOXAFLOR EFFECTS IN BUMBLE BEE DEVELOPMENT

OC 169

Margret Jürison, Pilleriin Teras, Kaarel Pent, Vivika Väli, Risto Raimets, Sigmar Naudi, Angela Ploomi, Marika Mänd, Reet Karise

Estonian University of Life Sciences, Tartu, Estonia

Abstract

Pollen is the primary source of proteins, lipids, and micronutrients required for bee larval growth, development, and adult physiological performance. During development, pollinators may simultaneously face nutritional limitations and exposure to agrochemicals, yet the combined effects of these stressors on brood remain poorly resolved. We reared bumble bee larvae under controlled conditions on pollen diets differing in protein-to-lipid ratios and chronically exposed them to the insecticide sulfoxaflor via their food, quantifying larval growth, developmental timing, survival to adulthood, and adult body mass to assess diet-dependent pesticide effects. Larval performance was strongly shaped by pollen nutritional composition, with multifloral pollen supporting higher overall survival and more consistent growth than monofloral (oilseed rape and faba bean) diets. Sulfoxaflor exposure affected development across all diets, but its effects were strongly context-dependent. Across all diets, sulfoxaflor exposure increased emergence probability, with the strongest effect under lipid-rich oilseed rape, despite poorer performance under control conditions. Under multifloral pollen, it's exposure imposed measurable costs, including prolonged development, reduced growth, and lower adult body mass. Under faba bean, sulfoxaflor effects differed between traits, with prolonged development at lower exposure and reduced growth at higher exposure. Rather than exerting uniformly negative effects, sulfoxaflor responses were strongly shaped by nutritional conditions, reflecting shifts in diet-dependent trade-offs between survival and development. Pesticide risk therefore cannot be fully understood without considering nutritional context, as diet quality can fundamentally alter developmental responses to exposure.

OC 170

MODULATION OF HYGIENIC BEHAVIOUR BY THE SOCIAL CONTEXT IN *APIS MELLIFERA*

Grégory Lafon, Louise Jeandroz, Emilien Rottier, Benjamin Basso, Fanny Mondet

INRAE, Avignon, France

Abstract

The hygienic behaviour of honey bee workers contributes to the social immunity of colonies. The ability of workers to detect and remove unhealthy or dead brood prevents the transmission of brood diseases inside the colony. It is a very important part of the defence against parasites such as the deadly mite *Varroa destructor*, indeed colonies bred for hygienic behaviour show lower levels of *Varroa* infestation. Selecting colonies for their hygienic behaviour represents a very promising path toward containing the infestation from *Varroa*; however, the way this behaviour is regulated within the colony is still poorly understood. Here we decided to investigate the influence of social context on workers' hygienic performances, postulating that individual honey bees might be able to amplify the signal from damaged brood and thus recruit more workers to clean the brood. To test that hypothesis, we developed a new behavioural assay that allows to decouple the number of bees present in the experiment and the number of bees actually involved in hygienic behaviour. We showed that the social context does affect hygienic behaviour and that an increased number of surrounding bees increases the hygienic performances of worker bees. These results deepen our understanding of hygienic behaviour mechanisms at the colony level, and open new perspectives to unravel how individual honey bees become involved in such social immunity related tasks.

NORROA™: A NOVEL MODE OF ACTION FOR CONTROLLING VARROA MITES (*VARROA DESTRUCTOR*)

OC 171

Brian S. Manley, Adam Pachl

GreenLight Biosciences, Inc., Durham, United States

Abstract

The parasitic varroa mite (*Varroa destructor*) is widely regarded as the most significant threat to honey bee health and global beekeeping. These mites infest developing larvae, pupae, and adult bees, and the combined stress, along with the numerous viruses they transmit, weakens colonies and often leads to colony death if left unmanaged. Current options for managing varroa mites are limited. Some approaches, including cultural control practices, can be difficult to implement and may negatively affect bees and colony performance. In addition, many mite populations have developed resistance to several existing chemical treatments, underscoring the need for new control tools with novel modes of action.

Norroa, which contains the active ingredient vadescana, is a novel solution for controlling varroa mite populations and provides a much-needed new mode of action. Vadescana is a double stranded RNA (dsRNA) molecule specifically designed to target *Varroa destructor* and has been classified in IRAC Group 35 (RNA interference–mediated target suppressors). Unlike most existing varroacides , which focus on adult phoretic mites, Norroa uniquely targets reproductive foundress mites in the brood cells, preventing them from producing offspring. This phenotype has been demonstrated in both laboratory and field studies. Extensive trials conducted across Canada, Europe, New Zealand, and the United States have shown that Norroa can effectively reduce mite populations for up to 18 weeks throughout the production season when applied at the appropriate time, before mite levels reach local treatment thresholds.

OC 172 PATHOGEN DYNAMICS ASSOCIATED WITH HIVE TRANSLOCATION IN AN ISLAND POPULATION OF *APIS MELLIFERA*

Lorena Martínez-Mayor[1], David Lugo[2], Álvaro Urueña[1], Pilar De la Rúa[1]

[1]Universidad de Murcia, Murcia, Spain
[2]Universidad de La Laguna, Tenerife, Spain

Abstract

Hive translocation, also known as migratory beekeeping, is widely used to improve honey bee productivity by relocating colonies to areas with better floral resources. However, this practice may influence pathogen dynamics, particularly in isolated ecosystems such as islands. This study evaluates the impact of hive translocation on the prevalence of two intracellular pathogens in honey bees: *Nosema ceranae* (microsporidian) and *Lotmaria passim* (trypanosomatid), on the locally adapted Canarian black honey bee.

A total of 60 hives of 6 different apiaries (30 translocated and 30 stationary) were monitored at three time points: before relocation (T0), two weeks after (T1), and two months after (T2). Worker bees were sampled from the inner frames and pathogens were detected with PCR techniques targeting specific pathogen DNA fragments. Statistical analyses were performed to see the differences in pathogen prevalence between translocated and stationary hives using non-parametric tests. Results showed 40 positive samples for *Nosema* spp., with 34 identified as *N. ceranae*. Trypanosomatids were detected in 35 samples, including 11 confirmed as *L. passim*. Mixed infections occurred primarily in stationary colonies. No significant differences between colony types were observed at T0 or T1 for *N. ceranae*. However, at T2, translocated colonies showed significantly lower prevalence ($p = 0.0143$). Although *L. passim* followed a similar trend, differences were not statistically significant.

Contrary to expectations, hive translocation did not increase pathogen prevalence. Instead, results suggest that improved environmental conditions such as higher floral availability and milder temperatures may reduce stress and pathogen transmission in translocated colonies. In contrast, stationary colonies exhibited higher pathogen prevalence and increased mixed infections over time, indicating potential chronic stress and immune suppression.

These findings highlight the importance of environmental conditions in honey bee health and suggest that, under certain circumstances, translocating hives may have protective effects against pathogens.

PREVALENCE AND SEASONALITY OF PATHOGENS IN MANAGED HONEY BEE COLONIES IN FOUR EUROPEAN CLIMATE REGIONS AND LINK WITH COLONY MORTALITY

OC 173

Severine Matthijs[1], Susanne Fischer[2], Marc O. Schäfer[2]

[1]Belgian National Reference Laboratory for Bee Diseases, Sciensano, Brussels, Belgium
[2]Institute of Infectiology, Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany

Abstract

An overview of the prevalence and seasonality of diseases and parasites in managed *Apis mellifera* was obtained through sampling colonies from 14 European countries during three years (in spring, summer & autumn) in the framework of the Horizon 2020 project B-GOOD (Giving Beekeeping Guidance by cOmputatiOnal-assisted Decision making).

From 2020 to 2022, 1580 samples from 352 colonies, originating from research institutes and beekeepers in four European climate zones (Atlantic, Boreal, Continental, Mediterranean) were examined. Sampled bees were analysed for the presence of 11 known honey bee pathogens using microscopy, bacterial culture and molecular methods, taking into account reported seasonal occurrence of the different pathogens. All samples were tested for the viruses DWV-A, DWV-B, BQCV and SBV, and the level of infestation with *Varroa destructor* was determined. All spring and summer samples were also analysed for *Nosema apis* and *Nosema ceranae*. Additionally, spring and autumn samples were tested for ABPV and CBPV. Infection with *Paenibacillus larvae* and *Melissococcus plutonius* (cause of American and European foulbrood) was checked in autumn samples only. Information about colony survival allowed us to deduce the impact of these pathogens on honey bee colony mortality.

The results give an insight in the disease and parasite burden of managed honey bee colonies in Europe. Differences in the levels of diseases and parasites were observed between the different climate regions and over the seasons. The most prevalent viruses were BQCV, DWV-B and SBV, although with variable viral loads in the different regions. *Nosema ceranae* was frequently detected while *Nosema apis* seems nearly vanished in Europe. The co-occurrence of various pathogens and the relationship between pathogen infection and colony mortality showed important differences between the four studied climate zones, underlining the impact of the climate on pathogen prevalence and disease outcome.

OC 174 CHRONIC RADIOACTIVE EXPOSURE IN FUKUSHIMA IMPAIRS COGNITION IN TWO POLLINATOR SPECIES

Coline Monchanin[1], Lacombrade Mathilde[1], Kristine Abenis[1], Matthias Desmolles[1], Blandine Mahot-Castaing[1], Golefert Loïc[1], Nicolas Dubourg[2], Olivier Armant[2], Kenji Nanba[3], Lihoreau Mathieu[1]

[1]Research Center on Animal Cognition (CRCA), Toulouse, France
[2]ASNR, Service de Radioprotection des Populations et de l'Environnement (SERPEN), Saint Paul lez Durance, France
[3]Institute of Environmental Radioactivity, Fukushima, Japan

Abstract

In March 2011, a 15-meter tsunami struck the Fukushima Daiichi nuclear power plant, triggering a nuclear accident. Massive releases of cesium, a radionuclide with a half-life of 30 years, permanently contaminated the soil, and several areas are still designated "exclusion zone". In collaboration with the University of Fukushima, our research investigates the behavior and morphology of the domestic honey bees (*Apis mellifera*) and the wild giant hornet (*Vespa mandarinia*) along a cesium contamination gradient. To assess environmental radiation levels, we attached dosimeters to insects and released them at three selected sites, confirming contrasting levels of radiation exposure. We then trained these insects to associate a colored stimulus with a sugar reward in a Y-maze. For both species, learning performances declined as radio-contamination increased. Morphological analysis revealed asymmetries in wing length and shape in most radio-contaminated insects. Additionally, honey bees from the most contaminated sites exhibited reduced spontaneous walking activity and decreased sensitivity to sucrose. Altogether, our results suggest that the legacy cesium contamination can impact the behavior and morphology of both species, with potentially broader ecological consequences through impaired ecosystem services. Our work emphasizes the importance of understanding the long-term environmental impacts of radiation exposure with wider implications regarding the environmental monitoring, conservation efforts, and public health.

NUTRITIONAL STRESS, PATHOGEN LOAD, AND BEHAVIOR IN AT-RISK BUMBLEBEES

OC 175

Amélie Morin[1,2], Frédéric McCune[1,2], Valérie Fournier[1,2], Mathilde L. Tissier[2,3,4]

[1]Université Laval, Québec, Canada
[2]Quebec Center for Biodiversity Science, Montreal, Canada
[3]Université de Strasbourg, CNRS, IPHC, Strasbourg, France
[4]Université du Québec à Montréal, Montreal, Canada

Abstract

Wild pollinators are declining globally, with 28% of bumble bee species at risk in North America. Declines are driven by interacting stressors affecting physiology, behavior, and nutrition, and early vulnerability may be detected through sublethal responses. However, standardized nonlethal approaches integrating health metrics in wild bumblebees are scarce. We developed a non-destructive protocol assessing behavior, pathogen load, and nutritional status in field-captured individuals.

Assessments were conducted across castes in agricultural landscapes, comparing at-risk and common species. Each captured individual underwent a behavioral test, fecal collection, and corbicula pollen sampling. Behavior was assessed via standardized open-field assays to compare locomotion and spatial use across species, castes, and conservation status. Pathogens were detected in fecal samples, targeting *Nosema* spp. and *Crithidia* spp. by microscopy.

Several North American at-risk species were documented, including *Bombus bohemicus*, *B. fervidus*, *B. occidentalis*, *B. pensylvanicus* and *B. terricola*. Automated tracking of 382 individuals revealed a species-specific behavioral phenotype in *B. terricola*, with reduced locomotion and altered spatial use compared to *B. impatiens*. Among 134 fecal samples, 31% carried gut pathogens, and *Nosema* spp. spore loads were higher in *B. terricola*, indicating disproportionate pathogen burden.

Multidimensional biochemical analyses of pollen were conducted to characterize the nutritional landscape, investigating how diet composition – including macronutrients, vitamins, minerals, and other bioactive compounds – interacts with bumble bee behavior and pathogen loads. Preliminary patterns indicate substantial nutritional variation among floral resources, consistent with constraints contributing to observed differences in pathogen loads and behavioral responses.

Together, these results show that *B. terricola* experiences elevated pathogen load, a behavioral profile consistent with reduced performance, and exposure to variable nutritional conditions in agricultural landscapes. The framework provides a powerful approach to detect early health impairment in wild pollinators and guides conservation, including selection of floral resources that enhance nutrition and mitigate disease risk.

OC 176 THE IMPACT OF THE PARASITIC FLY *BRAULA COECA* ON HONEY BEE QUEEN EGG-LAYING AND VIRAL INFECTION

Tzion Movshovitz-Doitsh[1,2], Elad Bonda[1], Yael Mandelik[2], Hagai Y. Shpigler[1]

[1]Agricultural Research Organization (ARO), Volcani Institute, Rishon LeZion, Israel
[2]The Hebrew University of Jerusalem, Faculty of Agriculture, Food and Environment, Rehovot, Israel

Abstract

Braula coeca is an obligate kleptoparasitic fly that completes its life cycle within honey bee (*Apis mellifera*) colonies. Adult flies can be frequently found on the queen, where feeding usually occurs. Despite its presence on queens, little is known about its effects on queen fecundity or its potential role in virus transmission. This study examines two possible routes by which *B. coeca* may affect queen fecundity and viral spread: (1) alterations in queen egg-laying performance and (2) viral presence associated with the fly presence. Egg-laying was monitored in 30 queens maintained under controlled conditions. Nineteen queens were infested with flies and 9 clean queens served as controls. Egg production was recorded daily for twelve days. Egg-laying data were analyzed for differences among three infestation levels. Queens with high infestation (6 or more flies) showed markedly reduced egg production, whereas queens with moderate infestation (4–5 flies) exhibited higher egg-laying compared with control queens. Queens with low infestation (1–3 flies) showed egg-laying levels similar to those of control queens. These results suggest a non-linear pattern in queen egg-laying across infestation levels.

To investigate viral dynamics, we collected 40 queens and their associated flies during autumn queen replacement from apiaries in the Hebron Hills and the central coastal plain of Israel for molecular screening of five common honey bee viruses using qPCR. Preliminary screening detected the presence of Deformed Wing Virus (DWV) and Black Queen Cell Virus (BQCV) in fly samples. Further analyses aim to quantify viral loads and assess potential associations between virus detection in the flies and the infected queen. Using reproductive and molecular approaches, our findings suggest that *Braula coeca* infestation influence queen reproductive and may also be associated with honey bee viruses. The findings highlight the need for further investigation of this overlooked parasite.

NEW QPCR ASSAY FOR GENOTYPING *PAENIBACILLUS LARVAE* REVEALS SWEDISH ERIC V ISOLATES

OC 177

Anna Nilsson[1], Emilia Semberg[1], Ásgeir Ástvaldsson[2], Eva Forsgren[1]

[1]Swedish University of Agricultural Sciences, Uppsala, Sweden
[2]Swedish Veterinary Agency, Uppsala, Sweden

Abstract

Paenibacillus larvae, the bacteria causing the honeybee brood disease American foulbrood (AFB), can be divided into five enterobacterial repetitive intergenic consensus (ERIC) genotypes: *P. larvae* ERIC I-V. The five genotypes varies in virulence and can be classified as slow (ERIC I), medium fast (ERIC II) and fast (ERIC III-V) killers. ERIC I and II are the most prevalently reported genotypes affecting honeybee health. ERIC III and IV have not been reported for decades and ERIC V was only recently described.

The aim with this study was to develop a fast and simple qPCR assay for identification of the five genotypes as a complement to the earlier published time-consuming repetitive element PCR (rep-PCR). Primers were developed to target genotype unique sequences and tested against reference strains representing each of the genotypes. Additionally, we screened a total of 249 *P. larvae* isolates from Swedish AFB outbreaks using the new method. The isolates were previously assigned an ERIC-genotype using whole genome sequencing, and the result was consistent with previous results for 246 samples. Three samples were positive for two genotypes.

In 2024, we isolated *P. larvae* from commercially available pollen patties where bacterial colonies clearly differed phenotypically from *P. larvae* ERIC I and II. We used the new qPCR assay to assign the bacteria to ERIC V. This was further confirmed with whole genome sequencing. This is the first confirmed finding of *P. larvae* ERIC V in Sweden.

In conclusion, this newly developed qPCR assay is a fast and reliable tool for genotyping a large number of *P. larvae* isolates.

OC 178 BEES AND MULTIPLE STRESSORS: INVESTIGATING THE IMPACTS OF NUTRITION AND PESTICIDES ON SOLITARY BEES

Megan Reilly[1], Yasmin Bonner[1], Alison O'Reilly[2], Dara A. Stanley[1]

[1]University College Dublin, Dublin, Ireland
[2]Lund University, Lund, Sweden

Abstract

Bees exist in complex environments where they encounter multiple stressors regularly, such as pesticide stress or nutritional stress. Previous research investigating the effects of these stressors have mostly focused on social species and single stressors (e.g. neonicotinoid insecticides and honeybees). Most bee species are solitary with drastically different life histories to social species, which may affect their sensitivity to pesticides and other stressors. Using a systematic review, we quantified what research has been conducted on how pesticide exposure and nutrition impact both social and solitary bees, and highlight a number of research gaps. Then, using an experimental study, we investigated the impacts of varying nutrition and insecticide exposure in a cage experiment on the foraging behaviour of *Osmia bicornis*. In more diverse diets, bees took longer to forage on average when exposed to insecticide as opposed to monofloral buckwheat treatments. Together, this work provides valuable insights into the gaps in pesticide and nutrition research, and how solitary bees are affected by their interaction. Results are discussed in the context of future research and policy directions.

NANOMATERIALS AND BIOPESTICIDES AS EMERGING CONTAMINANTS: IMPACTS ON WILD BEE SURVIVAL AND BEHAVIOUR.

OC 179

Manuel Roppo Valente[1], Giulia Russo[1], Livilla A. Maggi[1], Stefano Domina[1], Wendalina Tigani[2], Fabio Candotto Carniel[2], Simone Tosi[1]

[1]Department of Agricultural, Forest and Food Sciences, University of Torino, Torino, Italy
[2]Department of Life Sciences, University of Trieste, Trieste, Italy

Abstract

Emerging contaminants are chemical compounds of concern because of their potential impact on human health and the environment. Their increasing use, driven by innovative physicochemical properties, raises questions about their possible effects on pollinators through contamination of environmental matrices. In this study, we separately investigated the impact of two categories of emerging contaminants: a nanomaterial (Graphene Oxide, GO) and three biopesticides (Coffee Production Waste, CPW; *Cannabis* inflorescence extract; *Cannabis* oil extract complexed with cyclodextrins), on two wild bee species, *Bombus terrestris* and *Osmia bicornis*. These bee species are essential for maintaining biodiversity and food production and serve as bioindicators of environmental health. Exposure studies to GO were conducted via aerial dispersion, and its potential toxicity was assessed through oral and contact administration in bumble bee microcolonies at three concentrations (0 ug/g, 50 ug/g e 500 ug/g). Our results showed that GO was detected on the bodies of bumble bees. We also demonstrated that intermediate GO exposure significantly increased food consumption in bumble bee microcolonies. Chronic oral toxicity of biopesticides was tested on *Osmia* exposed to two CPW concentrations (low and high) and a single concentration for each of the two *Cannabis* extracts. Our results indicated a significant increase in mortality at the highest CPW extract level, and a significant reduction in food consumption after exposure to *Cannabis* oil. Preliminary results showed that CPW caused a significant increase in hyperactivity, while *Cannabis* inflorescence extract significantly increased weakness and reduced "still" behaviours. This study provides innovative methods and the first insights for the risk assessment of emerging contaminants potentially affected wild bees, highlighting lethal and sublethal impacts of concern. Further studies are required for a more comprehensive evaluation of their potential effects on beneficial organisms.

OC 180 IMPROVING *VARROA DESTRUCTOR* RESISTANCE IN HONEY BEES USING LARGE-SCALE METAGENOMICS ANALYSES

Victor Rossier[1,2], Thibault Leroy[3], Markus Neuditschko[2], Vincent Dietemann[1,4], Benjamin Dainat[1]

[1]Swiss Bee Research Centre, Agroscope, Bern, Switzerland
[2]Animal GenoPhenomics, Agroscope, Posieux, Switzerland
[3]GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet Tolosan, France
[4]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

Abstract

The ectoparasitic mite *Varroa destructor* remains the greatest biotic threat to managed honey bees in the Northern Hemisphere. Selecting for host resistance to this parasite is considered the most sustainable solution. However, breeding programs have shown limited success, partly due to the low heritability of *V. destructor* resistance traits. We hypothesized that failing to account for microbiome effects may bias and underestimate these heritability values. Indeed, the honey bee microbiome plays an important role in colony health, indirectly impacting the colony's ability to resist *V. destructor*. Moreover, specific pathogens or symbionts could directly affect the expression of resistance traits by modulating sensory perception, learning and memory, or immunity. Using large-scale publicly available metagenomic data, we characterized the microbiome (including prokaryotic and eukaryotic pathogens, gut microbiota, DNA viruses, and plant eDNA) of 1442 European colonies. After identifying microbiome taxa and principal components significantly associated with *V. destructor* load and resistance traits (recapping and decreased mite reproduction), we incorporated these factors as covariates to update genomic heritability estimates for *V. destructor* load and resistance traits. Both mite load and recapping were significantly associated with microbiome composition, albeit to a lesser extent than geography or sampling date. Numerous taxa showed significant associations with these traits. Notably, the core gut symbionts *Lactobacillaceae* and *Bifidobacteriaceae* were negatively associated with mite load, whereas non-core bacteria such as *Bartonellaceae* and pathogens including *Morganellaceae* and *Trypanosomatidae*, showed positive correlations. This suggests that *V. destructor* either disrupts the microbiome, or that the mite proliferates preferentially in colonies harbouring particular microbial communities. Finally, by including microbiome principal components and relevant taxa as covariates, we report increased genomic heritability estimates for *V. destructor* load and resistance traits. These findings indicate that integrating microbiome information into breeding frameworks could strengthen selection programs for *V. destructor* resistance and ultimately improve honey bee health.

BIOMARKERS OF HONEYBEE HEALTH AND ENVIRONMENTAL CONTAMINANTS IN SUBURBAN APIARIES OF BOLOGNA, ITALY

OC 181

Cecilia Rudelli[1,2], Giulia Andreani[2], Roberta Galuppi[2], Gloria Isani[2]

[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna "Bruno Ubertini", Brescia, Italy
[2]Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

Abstract

Honeybees play a crucial role not only for beekeeping but also in biodiversity maintenance. Furthermore, they are excellent bioindicator organisms for assessing environmental contamination.
This study aimed to: (i) monitor honeybee health through the analysis of haemolymph proteins and antioxidant biomarkers, and (ii) evaluate the environmental contamination in the study area by analyzing essential (Cu, Fe and Zn) and non-essential (Cd) trace elements as well as the presence of microplastics in honeybees.
Eight apiaries located in the province of Bologna (Italy) and characterized by different levels of anthropization were considered. Sampling was performed at two times, in May (t1) and November 2025 (t2). Haemolymph proteins were separated by SDS-PAGE and quantified using ImageLab software, while total protein concentration was determined using the Bradford method. Antioxidant biomarkers were measured using commercial colorimetric assay kits. Trace elements were quantified using atomic absorption spectrometry, while microplastics were detected through Nile Red staining followed by fluorescence microscopy. Differences between sampling times were evaluated using the Wilcoxon signed-rank test, while differences among apiaries were assessed using Kruskal–Wallis and Dunn post hoc tests. Correlations were evaluated using Spearman's correlation.
Haemolymph proteins showed significant differences between sampling times and among apiaries, with total proteins and vitellogenin being significantly higher at t2 than at t1. Concentrations of Fe, Zn and Cu were homogeneous among apiaries, likely reflecting their homeostatic regulation. The Cd concentration was below the LOD (0.05 µg/ml) in all the apiaries. Microplastics were detected in honeybees from all apiaries without significant differences among them. A negative correlation ($p<0,05$) was observed between haemolymph antioxidant biomarkers and vitellogenin, apolipophorin and total proteins, suggesting a possible compensatory response to oxidative stress.
Overall, this study provides an integrated picture of the physiological status of honeybees and the level of environmental contamination in suburban apiaries from the Bologna province.

OC 182 BUMBLE BEE BROOD REMOVAL INDUCED BY EXPOSURE TO A BACTERIUM-BASED BIOPESTICIDE

Michelle Scheffler[1], Karoline Wüppenhorst[1], Doreen Babin[2], Abdulrahim T. Alkassab[1], Silvio Erler[1,3]

[1]Julius Kühn Institute, Institute for Bee Protection, Braunschweig, Germany
[2]Julius Kühn Institute, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany
[3]Technische Universität Braunschweig, Zoological Institute, Braunschweig, Germany

Abstract

Social immunity, the behavioural defence in social insects, includes the removal of sick or diseased individuals from their colonies. This specific behaviour is well described for ants, honey bees and termites. However, for bumble bees only limited information for corpse removal is known. Here, we used full-size *Bombus terrestris* colonies to investigate if brood exposed to a bacterial biopesticide (*Bacillus thuringiensis* ssp. *aizawai*, strain: ABTS-1857), especially their larvae, will be actively removed by worker bumble bees. Bacteria-exposed colonies removed the most larvae within 3.5 weeks post-exposure compared to both: (1) colonies that received untreated sucrose solution and (2) colonies with mechanically wounded larvae. Colony strength seems to play an important role as stronger colonies of all groups removed more larvae than smaller ones. The majority of the removed larvae were of lighter than 100 mg and of white colour. A subset of removed larvae of all treatment groups were desiccated or showed signs of coloured dots. All experimental manipulations did not affect colony development or the production of new sexuals. Here, we show that bumble bee colonies may regulate larvae removal to guarantee successful colony development and to minimize potential burden by affected individuals. The underlying mechanisms, e.g. chemical ecology of bumble bee brood-worker interaction and substances initiating brood removal, need further investigation.

SUSTAINABLE CONTROL OF *VARROA DESTRUCTOR* USING FORMIC ACID-BASED TREATMENT - EFFECTS ON HYGIENIC BEHAVIOR AND COLONY HEALTH IN HONEYBEES

OC 183

Zoran Stanimirović[1], Marko Ristanić[1], Jevrosima Stevanović[1], Tamara Cvetković[1], Jovana Stamenović[1], Branislav Vejnović[2], Uroš Glavinić[1]

[1]University of Belgrade - Faculty of Veterinary Medicine - Department of Biology, Belgrade, Serbia
[2]University of Belgrade – Faculty of Veterinary Medicine - Department of Economics and statistics, Belgrade, Serbia

Abstract

Varroa destructor remains one of the most significant drivers of honey bee (*Apis mellifera*) colony losses worldwide, underscoring the urgent need for control strategies that are both effective and sustainable. This study evaluated the acaricidal efficacy of a formic acid-based treatment approach, with particular emphasis on its potential to modulate colony-level defense through hygienic behavior. A total of 60 colonies were assigned to three experimental groups: a formic acid-treated group (n=30), a positive control group treated with amitraz (n=15), and an untreated negative control (n=15). Treatment efficacy was assessed by monitoring daily mite fall, while hygienic behavior was evaluated before and after treatment using the pin-killed brood assay. The formic acid-based treatment achieved a high mite mortality rate (88.37% ± 0.23), approaching that observed in the amitraz-treated group (94.30% ± 0.95). The treatment was well tolerated, with no queen losses and only mild, transient brood effects, accompanied by short-term bearding during the initial phase of application. Notably, a significant increase in hygienic behavior was recorded in the treated colonies (PCC from 96.69% to 99.01%, $p<0.001$), while a decline was observed in the untreated group and no significant changes occurred in the amitraz-treated colonies. These findings demonstrate that formic acid-based treatment strategies can provide substantial *Varroa* control while simultaneously enhancing an important component of social immunity. Such dual action supports their role as valuable tools within integrated and sustainable *Varroa* management frameworks aimed at improving colony resilience.

OC 184 INVESTIGATION OF *LOTMARIA PASSIM* INFECTION DYNAMICS, AND THE EFFECT OF INFECTION ON HONEY BEE SURVIVAL AND FOOD CONSUMPTION

Jevrosima Stevanović[1], Uroš Glavinić[1], Branislav Vejnović[2], Marko Ristanić[1], Patrycja Pluta[3], Robert J. Paxton[3], Zoran Stanimirović[1]

[1]University of Belgrade - Faculty of Veterinary Medicine, Department of Biology, Belgrade, Serbia.
[2]University of Belgrade - Faculty of Veterinary Medicine, Department of Economics and Statistics, Belgrade, Serbia
[3]Martin Luther University Halle-Wittenberg, Institute for Biology, General Zoology, Halle (Saale), Germany

Abstract

Lotmaria passim is a trypanosomatid parasite whose relationship with its host, *Apis mellifera*, remains insufficiently elucidated. In this study, the dynamics of *L. passim* infection was investigated along with the bee survival and food consumption. For a laboratory experiment, 600 newly emerged honey bees were allocated to 12 cages (50 bees per cage), divided into two groups (each consisting of 6 cages): one intended for the infection with *L. passim* (Lp group) and the other to serve as a negative control (C group). Bees in the Lp group were individually inoculated on the 2nd day after emergence with a 20% sucrose solution containing motile, flagellated *L. passim* (ATCC PRA-422). From the 3rd day onwards, the Lp group received 30% pure sugar solution *ad libitum*; the C group received this solution throughout the experiment, which lasted 24 days. Bee survival was monitored daily; dead bees were removed and their number recorded. Food consumption was estimated by weighing feeders every 24 h. For monitoring *L. passim* infection dynamics, bees were sampled daily, and used for DNA extraction and quantification of *L. passim* infection by qPCR. No significant changes were observed in bee survival and diet consumption between the Lp and C groups. With respect to the dynamics of *L. passim* infection in the Lp group, the increase was not linear. From 9 to 13 days p.i., the infection level did not vary significantly. A significant ($p < 0.01$) increase between days 13 p.i. and 14 p.i. was observed. From day 14 p.i. until the last day of the experiment (day 23 p.i., bees 24 days old), the level of infection remained consistently high.

HOST BARRIERS LIMIT VIRAL SPREAD IN A SPILLOVER HOST: A STUDY OF DEFORMED WING VIRUS IN *BOMBUS TERRESTRIS*

OC 185

Tabea Streicher[1,2], Pina Brinker[1,3,4], Simon Tragust[1], Robert J. Paxton[1,4]

[1]Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
[2]State Institute of Bee Research University of Hohenheim, Stuttgart, Germany
[3]Institute of Evolutionary Ecology University of Regensburg, Regensburg, Germany
[4]German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany

Abstract

The transmission of pathogens from reservoir to recipient host species, termed pathogen spillover, can profoundly impact plant, animal, and public health. However, why some pathogens lead to disease emergence in a novel species while others fail to establish or do not elicit disease is often poorly understood. There is strong evidence that deformed wing virus (DWV), an (+)ssRNA virus, spills over from its presumed reservoir host, the honey bee *Apis mellifera*, into the bumble bee *Bombus terrestris*. However, the low impact of DWV on *B. terrestris* in laboratory experiments suggests host barriers to virus spread in this recipient host.

To investigate potential host barriers, we followed the spread of DWV genotype B (DWV-B) through a host's body using RT-PCR after experimental transmission to bumble bees in comparison to honey bees. Inoculation was per os (oral administration), mimicking food-borne transmission, or by injection into the bee's haemocoel, mimicking vector-based transmission.

In honey bees, DWV-B was present in both faeces and haemolymph within 3 days of inoculation per os or by injection. In contrast, DWV-B was not detected in *B. terrestris* haemolymph after inoculation per os, suggesting a gut barrier that hinders DWV-B's spread through the body of *B. terrestris*. DWV-B was, however, detected in *B. terrestris* faeces after injection and feeding, albeit at a lower abundance than that observed for *A. mellifera*, suggesting that *B. terrestris* sheds less DWV-B than *A. mellifera* via faeces when infected. Barriers to viral spread in *B. terrestris* following oral infection may reduce DWV's impact on this spillover host and limit its contribution to the community epidemiology of DWV.

OC 186 *APIS MELLIFERA* WINTER COLONY LOSSES: INSIGHTS FROM 20 YEARS OF MONITORING HONEY BEE HEALTH IN GERMANY

Tabea Streicher[1], Elke Genersch[2], Marina D. Meixner[3], Christoph Otten[4], Stefan Berg[5], Gertje Petersen[6], Marc O. Schäfer[7], Kirsten S. Traynor[1]

[1]State Institute of Bee Research, Stuttgart, Germany
[2]Länderinstitut für Bienenkunde Hohen Neuendorf e. V., Hohen Neuendorf, Germany
[3]Landesbetrieb Landwirtschaft Hessen, Bieneninstitut Kirchhain, Kirchhain, Germany
[4]Dienstleistungszentrum Ländlicher Raum Westerwald-Osteifel, Fachzentrum Bienen und Imkerei Mayen, Mayen, Germany
[5]Bayerische Landesanstalt für Weinbau und Gartenbau, Institut für Bienenkunde und Imkerei, Veitshöchheim, Veithöchheim, Germany
[6]LAVES Institut für Bienenkunde, Celle, Germany. [7]Friedrich-Loeffler-Institut (FLI) - Bundesforschungsinstitut für Tiergesundheit, Nationales Referenzlabor für Bienenkrankheiten, Riems, Germany

Abstract

Honey bee colonies face numerous stress factors that can lead to colony decline and winter mortality. In Germany, the "Deutsches Bienen Monitoring" (DeBiMo) program has monitored the health of approximately 1,200 colonies for over 20 years. Colonies are examined three times annually, in spring, summer, and fall, to assess their population and to monitor their pathogen and parasite loads. In addition, honey quality and pesticide residues in stored pollen (bee bread) are analyzed to monitor environmental exposure. This provides a unique long-term dataset on colony growth, disease incidence, and environmental influences.

Results consistently identify *Varroa destructor* as the primary driver of winter losses, mainly through the transmission of Deformed Wing Virus (DWV) and Acute Bee Paralysis Virus (ABPV). Elevated mite levels in summer and autumn reliably predict winter mortality, underscoring the central role of parasite management. Long-term pesticide monitoring since 2009 shows that strict EU regulations effectively prevent high-risk exposure through pollen. Neonicotinoid residues have declined to near zero since the restriction of neonicotinoids, reflecting the success of changes in agricultural policy. Although pesticide residues remain detectable, particularly fungicides, the risk from consumption is generally low, especially compared to similar data from the United States, where 5.4% of all pollen samples had an elevated hazard quotient score indicating the potential for sublethal effects on colony health.

Emerging threats are becoming increasingly important. The invasive hornet *Vespa velutina* has spread into Germany, especially in the south-west, adding predation pressure to foraging bees. Future risks include potential invasions by the Small Hive Beetle (*Aethina tumida*) and the ectoparasitic mite *Tropilaelaps mercedesae*, now expanding its range from Crimea and Georgia towards Western Europe.

Overall, DeBiMo data show that *Varroa*-mediated viral infections remain the dominant cause of losses, while invasive species pose the next major challenge for European apiculture.

ASSESSMENT OF THE EFFICACY AND BROOD TOXICITY OF STINGLESS BEE PROPOLIS AND *MORINGA OLEIFERA* EXTRACTS AGAINST *TROPILAELAPS MERCEDESAE*

OC 187

Guntima Suwannapong[1], Sanchai Naree[2], Rujira Ponkit[1], Preeyaporn Phonguttha[1], Alexis L. Beaurepaire[3], James D. Ellis[4]

[1]Department of Biology, Faculty of Science, Burapha University, Chon Buri, Thailand
[2]Pharmacology and Biopharmaceutical Department, Faculty of Pharmaceutical Sciences, Burapha University, Chon Buri, Thailand
[3]Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland; Bee research center, Agroscope, Switzerland, Bern, Switzerland
[4]Entomology and Nematology Department, University of Florida, Gainesville, United States

Abstract

Tropilaelaps spp. are ectoparasite mites native to the Asian giant honey bee, *Apis dorsata*, and have now spread to three *Apis* species in Thailand, including the managed honey bee, *A. mellifera*, where they cause severe colony losses. Many bioactive natural extracts are increasingly being explored as alternative control agents against *Tropilaelaps mercedesae* with low toxicity to honey bees. We evaluated the effects of ethanolic extracts of Thai herb, *Moringa oleifera* leaves, and propolis from the stingless bee, *Tetragonula pagdeni*, on the survival rates of *T. mercedesae* and *A. mellifera* pupae under laboratory conditions. White-eyed pupae were individually placed into 24-well plates, followed by the addition of one *T. mercedesae* per well. Each well was then covered with wax foundation. A volume of 2 µl of *M. oleifera* and propolis extracts at concentrations of 100%, 75%, 50%, 25%, and 12.5% was applied onto the wax surface. Formic acid and ethanol at the same concentration as the extract served as positive and solvent controls, respectively, while distilled water was used as a negative control. Mortality of both *T. mercedesae* and pupae was recorded over seven days. Ethanolic propolis and *M. oleifera* extracts at higher concentrations (50–100%) resulted in approximately 50% mite mortality, while having minimal effects on honey bee pupal mortality (16.67%). The positive control (12.5% formic acid) caused complete mite mortality within one day, as well as resulting in high honey bee pupal mortality (50%). The negative control (distilled water) did not affect both mites and honey bee pupae. These findings suggest that ethanolic propolis and *M. oleifera* extracts might have potential as safer alternatives for *T. mercedesae* control with lower toxicity to honey bee brood.

OC 188 COMPARING LETHAL AND SUBLETHAL ENDPOINTS IN TWO BEE SPECIES EXPOSED TO FLUPYRADIFURONE

Rafaela Tadei[1], Sergio Albacete[1], Lisa Cabiddu[1], Riccardo Girodo[1], Sara R. P. Trejo[1], Agata Di Noi[2], Ilaria Caliani[2], Laura Giovanetti[2], Tommaso Campani[2], Silvia Casini[2], Jordi Bosch[3], Fabio Sgolastra[1]

[1]Department of Agricultural and Food Sciences, Alma Mater Studiorum Università di Bologna, Bologna, Italy
[2]Department of Environmental, Earth and Physical Sciences, University of Siena, Siena, Italy
[3]Centre for Ecological Research and Forestry Applications (CREAF), Bellaterra, Spain

Abstract

Current evaluation of pesticide safety for bees relies primarily on lethal endpoints, which may overlook sublethal and delayed effects that impair key ecological functions. Incorporating sublethal endpoints into pesticide regulation frameworks remains challenging due to the diversity of measurable responses and the limited understanding of their biological relevance. This study aimed to compare the sensitivity of multiple endpoints to sublethal exposure to the insecticide flupyradifurone in two bee species, *Osmia bicornis* and *Apis mellifera*. Newly-emerged females were orally exposed to flupyradifurone at species-specific median lethal doses (LD_{50}; 0.98 and 3 µg/bee, respectively) and three sublethal doses (LD_{50}/5, LD_{50}/10, LD_{50}/100). Following acute exposure, longevity and food consumption were monitored daily. Phototactic response and thermoregulation were assessed at 4 and 48 hours post-exposure. Midgut histological structure, biochemical biomarkers (AChE, CaE, GST, NA assays, and differential haemocyte counts), and ovary maturation (only in *O. bicornis*) were assessed at 48 hours. Longevity was reduced only at the LD_{50} in both species, and no effects on food consumption were observed at any dose. Thermoregulation was impaired at LD_{50} and LD_{50}/5, at 4 and 48 h in *A. mellifera* and at 4 h in *O. bicornis*. Negative effects on phototactic response were observed only at the LD_{50} at 4 hours in both species. Histological effects on midgut structure were more pronounced in *O. bicornis*, with all doses reducing tissue regenerative capacity and increasing cellular damage. In contrast, *A. mellifera* showed only a reduction in active nuclei at the LD_{50}. No effects on ovary maturation were detected. Comparatively, endpoints at lower levels of biological organization, i.e., physiological and cellular, were more sensitive than mortality, and stronger effects were detected in *O. bicornis*. These results emphasize the importance of expanding this approach to other pesticides and evaluating the biological consequences of sublethal effects.

TWO VIRAL VARIANTS, TWO TRANSMISSION STRATEGIES: CONTRASTING SEASONAL AND SPATIAL DYNAMICS OF DWV-A AND DWV-B IN MANAGED *APIS MELLIFERA* COLONIES

OC 189

Rossella Tiritelli[1,2], Allegra Sartore[2], Laura Zavatta[3], Sergio Albertazzi[1], Vittorio Capano[1], Valeria Caringi[1], Irene Guerra[1], Elena Tafi[1,4], Laura Bortolotti[1], Antonio Nanetti[1], Giovanni Cilia[1], Dolores Catelan[2]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]Unit of Biostatistics, Epidemiology and Public Health, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padova, Padova, Italy
[3]Department of Agricultural and Food Sciences, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy
[4]Department of Life Sciences, University of Siena, Siena, Italy

Abstract

Deformed wing virus (DWV) is one of the major viral pathogens of *Apis mellifera* and occurs mainly as two variants, DWV-A and DWV-B, which differ in virulence and transmission dynamics. This study presents a three-year nationwide molecular surveillance of Italian honey bee colonies aimed at characterizing the spatial and seasonal distribution of these variants in relation to apiary density and landscape composition.

This study was conducted within the national monitoring project BeeNet and included 380 apiaries distributed across all Italian regions. Each apiary comprised three colonies sampled four times per year from June 2021 to March 2024. Viral prevalence and abundance patterns were first explored descriptively. Then, the presence/absence of viruses was analyzed using Bayesian spatial and spatio-temporal models to estimate infection probability and identify environmental drivers. DWV-B emerged as the dominant variant (mean prevalence 73.7%), showing a pronounced peak in autumn (November) followed by a decline during winter. In contrast, DWV-A displayed an opposite seasonal pattern, peaking in summer and decreasing with increasing apiary density, suggesting a different, possibly environmentally mediated transmission pathway.

Spatial analyses revealed a clear geographical segregation: DWV-B was more prevalent in southern and insular regions, whereas DWV-A predominated in central and northeastern areas. Landscape variables further indicated that DWV-B is associated with intensive beekeeping and anthropogenic environments, while DWV-A is linked to more heterogeneous landscapes.

Overall, these results highlight distinct ecological and epidemiological profiles of the two DWV variants. DWV-B appears to be primarily driven by colony-level dynamics, likely associated with *Varroa destructor* and favored by warmer, stable conditions, whereas DWV-A may persist through more diffuse environmental routes. Understanding these differences is crucial for predicting viral dynamics under changing environmental and management conditions.

OC 190 VINEYARDS UNDER WATCH: HONEY BEES AS SENTINELS OF MULTI-CONTAMINANT EXPOSURE AND IMPACT

Léa Tison[1], Coline Monchanin[2], Sophie Cachet[1], Louis-Antoine Chaput[1], Thierry Dalix[3], Joséphine Delage[1], Céline Franc[4], Louis Gross[1], Gaëtane Le-Provost[1], Mathieu Lihoreau[2], Gilles de Revel[4], Denis Thiéry[1], Zoé Tourrain[1], Timothée Wattellier[1], Renghzuang Xie[2], Adrien Rusch[1]

[1]National Research Institute for Agriculture, Food and Environment (INRAE), UMR 1065 Santé et Agroécologie du Vignoble (SAVE), Villenave d'Ornon, France
[2]Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI); CNRS, University Paul Sabatier - Toulouse III, Toulouse, France
[3]National Research Institute for Agriculture, Food and Environment (INRAE), USRAVE, Villenave d'Ornon, France
[4]University of Bordeaux, UMR 1366 OENO, INRAE, Bordeaux INP, Bordeaux Sciences Agro, ISVV, Villenave d'Ornon, France

Abstract

Pollinators are chronically exposed to varying, often sublethal, concentrations of contaminants in our environments. Our research aims to assess the exposure and impacts of contaminants on bees used as sentinels in different landscape contexts subjected to diverse agricultural practices. Managed honey bees (*Apis mellifera*), their predators (*Vespa velutina*), as well as inter-row plants and wild pollinators, were sampled throughout the season in the Bordeaux vineyard region. Organic pesticide residues were extracted using adapted QuEChERS methods and analyzed by LC-MS/MS, while inorganic compounds were measured by ICP-MS and ICP-AES. We are also currently testing *in situ* walking behavior and learning abilities using FMPER (Free-Moving Proboscis Extension Response) on bees from the sentinel hives before, during, and after pesticide applications. We evidenced a clear footprint of the viticultural environment on these organisms, with on average eleven different organic pesticides detected at each site, as well as numerous inorganic compounds such as copper, whose concentration increased significantly in bees over the season and was found at higher concentrations in their predator, the Asian hornet. We further showed that copper concentrations in bees were lower when grasslands were more prevalent in the surrounding landscape, and that wild pollinators were more abundant in plots where sentinel bees were the least contaminated. The *in situ* behavioral tests (walking and learning) are currently being conducted, and the results will be presented at the conference, providing new insights into the sublethal effects of vineyard phytosanitary treatments on honey bee cognition. This study advances our understanding of contaminant transfer and bioaccumulation under natural conditions, as well as their consequences for the health of pollinators, predators, and ecosystems.

FROM SUBSTANCES TO SYSTEMS: A NEW PARADIGM FOR PESTICIDE ENVIRONMENTAL RISK ASSESSMENT AND ITS IMPLICATIONS FOR POLLINATOR PROTECTION

OC 191

Christopher J. Topping[1], James H. Williams[1], Johan Axelman[1], Xiaodong Duan[1], Noa Simon Delso[2]

[1]University of Aarhus, Aarhus, Denmark
[2]BeeLife European Beekeeping Coordination, Brussels, Belgium

Abstract

Current pesticide environmental risk assessment (ERA) is built around a single-substance, single-use framework structurally mismatched with the ecological reality it aims to protect. Pesticides do not act in isolation: their impacts on pollinators, groundwater, and biodiversity arise from cumulative exposure to multiple chemicals across landscapes and over time. Assessing products in isolation cannot address an inherently systemic problem. The result is a recurring pattern: substances are authorised, ecosystem-level effects emerge, and the regulatory process iterates through increasingly complex refinements, consuming resources without resolving the underlying problem.

We present a systems-based approach to ERA implemented within the PollinERA project and operationalised through the Regional Cumulative Assessment Group (RCAG) framework (Topping et al., 2026, Env. Sci. & Tech.). Rather than asking whether a given pesticide is safe, the framework asks: how much total chemical stress can a given ecosystem sustain while meeting its legal protection obligations? This inversion— from product-to-authorisation to ecosystem-to-allocation — enables direct linkage between ERA and real-world environmental and pesticide monitoring, eliminating iterative, case-by-case threshold refinements.

PollinERA's prototype demonstrates practical feasibility for non-*Apis* pollinators, integrating agent-based population models for multiple focal species (*Osmia bicornis*, *Pieris napi*, *Eristalis tenax*, *Sphaerophoria scripta*, *Nocrua pronuba*), QSAR-based predictive toxicology, environmental Cumulative Assessment Groups (eCAGs) to manage chemical mixture complexity, and a machine-learning emulator for rapidly landscape-scale population outcomes. The system is prototyped for Denmark and will be extended across Poland, Italy, and Sweden.

The RCAG framework can be translated into a concrete policy instrument: regional pesticide budgets calibrated to ecological thresholds, compatible with existing EU legislation (1107/2009/EC, 2009/128/EC, 2000/60/EC), and technically feasible today, using existing digital land-parcel and monitoring infrastructure.

This reorientation improves ecological relevance, reduces regulatory complexity, provides farmers with flexible, lower-impact pesticide options, supports resistance-management practices, and delivers a defensible, adaptive framework to help reduce pressure on pollinators while protecting effective crops.

OC 192 RADIOFREQUENCY ELECTROMAGNETIC FIELDS (RF-EMF): POTENTIAL IMPACTS ON HONEY BEES AND OTHER POLLINATORS

Manuel Treder[1], Marcus Müller[2], Michael Glück[1], Larissa Fellner[1], Sam J. England[3], Martin Hasselmann[4], Peter Rosenkranz[1], Kirsten S. Traynor[1]

[1]State Institute of Bee Research, University of Hohenheim, Stuttgart, Germany
[2]Communications Engineering Lab, Karlsruhe Institute of Technology, Karlsruhe, Germany
[3]Department of Evolutionary Morphology, Museum für Naturkunde – Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany
[4]Department of Livestock Population Genomics, Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

Abstract

The ongoing expansion of urban areas and wireless communication technologies has led to increasing levels of radiofrequency electromagnetic fields (RF-EMF) in environments inhabited by honey bees and other pollinators. These anthropogenic emissions, particularly in the 2.4 and 5.8 GHz frequency bands, are now ubiquitous, yet their biological relevance for pollinator health and behavior remains poorly understood.

In these studies, we investigated the effects of chronic RF-EMF exposure on key fitness-related parameters of the honey bee (*Apis mellifera*) and on pollinator foraging activity on attractive plants. To ensure controlled and ecologically realistic exposure conditions, we used a standardized RF-EMF setup developed in collaboration with the Communications Engineering Lab (Karlsruhe Institute of Technology), allowing consistent and reproducible radiation scenarios.

We assessed (i) brood development, adult longevity, and homing ability of honey bees, (ii) gene expression responses in three honey bee developmental stages, and (iii) flower visitation rates of different pollinator groups to attractive foraging plants under exposure to 2.4 and 5.8 GHz frequencies.

Our results demonstrate that long-term RF-EMF exposure significantly impairs the homing ability of foraging honey bees, suggesting effects on orientation or learning. In addition, preliminary analyses indicate changes in gene expression. In contrast, no significant effects were detected on brood development or worker longevity of honey bees. Furthermore, we observed a significant reduction in flower visitation rates of bumble bees in RF-EMF-exposed flowerpots.

Overall, our findings suggest that RF-EMF exposure acts as a sublethal environmental factor affecting the behavior of honey bees and other pollinators. Given the ecological importance of pollinators, further research is needed to better understand long-term implications under field-realistic conditions and to elucidate the underlying biological and biophysical mechanisms.

This work was funded in part by the Ministry of Food, Rural Affairs and Consumer Protection Baden-Württemberg, Germany.

PATTERNS OF PATHOGEN PREVALENCE SUGGEST RESISTANCE MECHANISMS IN WILD IRISH HONEY BEES IN IRELAND

OC 193

Alexandra Valentine[1], Cristina Botias[2], Arrigo Moro[3], Mick Verspuij[1], Helen Mooney[1], Raquel Martín-Hernández[4], Mariano Higes[5], Julia Jones[6], Grace P. McCormack[1]

[1]University of Galway, Galway, Ireland
[2]University of Alcalá, Madrid, Spain
[3]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy
[4]Center for Beekeeping and Agro-environmental Research (CIAPA), Marchamalo, Spain
[5]Community of Castilla-La Mancha, Marchamalo, Spain
[6]University College Dublin, Dublin, Ireland

Abstract

Varroa destructor, viruses transmitted by the parasitic mite, and other pathogens such as *Nosema* spp, contribute to massive honey bee colony deaths globally each year. At the same time, honey bee colonies are increasingly reported to be living in the wild. These free-living colonies (FLCs) are suspected by many to be escapees from nearby apiaries, as well as a reservoir for pests and diseases. They are, therefore, also considered a threat to managed colonies due to pathogen transmission, and are not expected to become established. Here, we compared the prevalence of 16 common parasites and pathogens in FLCs and managed colonies (60 and 40 colonies respectively) in Ireland and show that there was no significant difference between colonies in pathogen prevalence, apart from a higher frequency of *N. ceranae* in colonies untreated for miticides. Some FLCs survived for multiple years despite the presence of multiple pathogens in their worker bees. This suggests there may be selection for resistance or tolerance mechanisms in the Irish honey bee population.

OC 194 CHARACTERIZATION OF CANDIDATE TARGET-SITE INSECTICIDE RESISTANCE MUTATIONS IN *A. MELLIFERA*

Gilles Verbinnen[1], Lina De Smet[1], Jan Tytgat[2], Kathleen Carleer[2], Maria Alice Pinto[3], Dora Henriques[3], Daniela Lima[3], Fernanda Li[3], Matthew T. Webster[4], Demetris Taliadoros[4], Dirk C. de Graaf[1]

[1]Ghent University, Ghent, Belgium
[2]KU Leuven, Leuven, Belgium
[3]CIMO, Instituto Politécnico de Bragança, Bragança, Portugal
[4]Uppsala University, Uppsala, Sweden

Abstract

Insecticides are one of the many factors contributing to the decline of pollinator populations. Insect pest species, such as aphids and Lepidoptera, have developed widespread resistance to a large number of compounds. However, research on insecticide resistance in pollinators is rather limited and has mainly focused on metabolic resistance. Therefore, we screened 4,897 publicly available *Apis mellifera* whole genomes from around the world and an additional 1,092 European *A. mellifera* genomes for mutations in the exons of 17 insecticide target-site genes. In this study, we uncovered 154 non-rare, non-synonymous mutations. The potential functional impact of these mutations was assessed based on literature and in-silico prediction tools such as SIFT, I-Mutant2.0 and MusiteDeep. Selected mutations in the octopamine receptor β2, acetylcholine esterase 2, GABA receptor and nicotinic acetylcholine receptor were further evaluated for their potential as candidate resistance mutations using electrophysiology, in silico protein modelling, molecular dynamics simulations and cage experiments.

THE PRELIMINARY STUDY: MICROPLASTIC ACCUMULATION IN HONEYBEE ORGANS AND ITS EFFECT ON SURVIVAL UNDER COMBINED ENVIRONMENTAL STRESSORS.

OC 195

Paweł Żukowski[1], Marek Gancarz[2,3,4], Aneta A. Ptaszyńska[1]

[1]BeeLab UMCS, Department of Cell Biology, Institute of Biological Sciences, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Lublin, Poland
[2]Faculty of Production and Power Engineering, University of Agriculture in Krakow, Kraków, Poland
[3]Institute of Agrophysics Polish Academy of Sciences, Lublin, Poland
[4]Center for Innovation and Research on Prohealthy and Safe Food, University of Agriculture in Kraków, Kraków, Poland

Abstract

This study analysed the presence of microplastics (MPs) in selected organs of *Apis mellifera* and assessed their impact on survival under additional environmental stressors. The MPs were prepared from polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), low-density polyethylene (LDPE) and polystyrene (PS). They were then ground and sieved before being fluorescently labelled with Nile Red.

Four experimental groups were established: bees infected with *Nosema ceranae*; bees exposed to sublethal doses of acetamiprid; bees subjected to both stressors simultaneously; and a control group that was not exposed to any stressors. Each group included subgroups that were fed a sugar paste supplemented with one of the five types of MP (100 mg of MPs per kg) for ten days. Following the experiment, the following organs were collected, fixed in 4% paraformaldehyde and analysed using confocal laser scanning microscopy: the midgut with rectum and Malpighian tubules; the brain with hypopharyngeal glands; the abdominal sternites with fat body; and the thoracic muscles.

The highest MP accumulation occurred in the midgut and rectum, with minimal presence in other organs. Exposure to MPs alone did not significantly affect survival ($p>0.05$). However, a significant reduction in survival was observed in the *N. ceranae*-infected groups (PVC: $p=0.0023$; PS: $p=0.00049$) and in the combined acetamiprid–*N. ceranae* group (PP: $p=0.0015$; LD-PE: $p=0.032$; PS: $p=0.0075$).

These results suggest that ingested MPs can penetrate the gut wall and that their adverse effects primarily manifest under combined stressor exposure.

OC 196 ACUTE ORAL TOXICITY OF PFAS IN *APIS MELLIFERA*: A PRELIMINARY STUDY

Laura Zulian[1], Marianna Pauletto[2], Michela Bertola[1], Sergio Perandin[3], Gioele Scopel[4], Franco Mutinelli[1], Mauro Dacasto[2], Federica Gallocchio[4], Anna Granato[1]

[1]National Reference Laboratory for Honey Bee Health, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy
[2]Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro, Italy
[3]Independent Researcher, Montebelluna, Italy.
[4]Department of Chemistry, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

Abstract

Per- and polyfluoroalkyl substances (PFAS) are emerging organic contaminants with amphiphilic properties and high chemical-physical stability widely used in a variety of industrial applications. Their release and accumulation in the environment can impact human and animal health, including honey bees which are sensitive sentinel species in ecotoxicology. Knowledge about PFAS toxicity on honey bees is still limited; to date, very few studies have investigated the acute, sub-lethal and/or chronic effects of these harmful environmental contaminants.

This study aimed to assess the acute oral toxicity of three PFAS, namely PFOS, PFOA and PFBA in adult worker honey bees (RC IZSVE 06/23, funded by the Italian Ministry of Health).

Approximately 320 adult worker bees were collected weekly from an apiary located in a low-risk PFAS exposure area of the Veneto region. According to OECD 213 acute test guidelines, groups of 10 bees per cage were maintained in controlled environmental conditions (in the dark, at 25 ± 2 °C with 50-70% relative humidity). Six different concentrations of each PFAS, administered via a 50% (w/v) sucrose solution, were tested (n=4 replicates per concentration). In each experiment, both a positive (the insecticide dimethoate) and a negative control (sucrose solution) were included. Mortality and abnormal behaviour were recorded 4 h after test initiation and every 24 h up to 96 h.

Among the three PFAS studied, it was possible to determine the LD50 value only for PFOS; however, for the highest concentrations of PFOA and PFBA the lethality rate was around 27.5% and 20.0%, respectively. Behaviour abnormalities were observed including reduced movement, coordination problems, inability to fly and ultimately leading to starvation.

A deeper understanding of toxic effects of PFAS and their potential risks on biodiversity and ecosystems is crucial, as their widespread contamination has already been confirmed globally, thus representing a One Health issue.

Posters



Agroecology

AGAPE (AGRICULTURE, BEEKEEPING, ENVIRONMENT): A SERIOUS GAME TO PROMOTE DIALOGUE BETWEEN BEEKEEPERS AND FARMERS

P1

Fabrice Allier[1,2], Agathe Thannberger[1,2], Miguel Rotenberg[3]

[1]ITSAP-Institut de l'abeille, Avignon, France
[2]UMT ETTAP, Avignon, France
[3]PlayTime, Marseille, France

Abstract

Agricultural regions are rich in diverse actors and products. Beekeeping is one such product, generating numerous interactions at different scales and potentially strengthened through bee-friendly farming practices and a variety of agro-ecological infrastructures. In reality, however, interactions between actors (beekeepers and farmers) are sometimes nonexistent. Although they work in the same area, mutual support is rare, and they demonstrate a lack of mutual understanding of each other's work, even tensions, particularly regarding the impact of pesticides on bees. Farmers and beekeepers do not operate on the same spatial and temporal scales. Furthermore, the management structures specific to each activity are more topological for farmers and center-periphery for beekeepers.

To better understand these reasoning patterns and promote mutual knowledge and cooperation between farmers and beekeepers, a serious game has been developed. This innovative tool represents the dynamics of the agricultural landscape and its impact on bee colonies and their productivity. Specifically, within an imaginary territory, farmers and beekeepers carry out their technical activities (crop rotation, plant protection treatments, harvesting, site selection and hive migratory, and honey harvesting). The game design of this serious game encourage interaction to achieve sustainable production goals for grains, fruits, and honey, while simultaneously improving bee health and local biodiversity. Following this 2-hour workshop, a 1-hour debriefing session facilitates further exploration of the topics covered and the actions undertaken during the game. Participants also consider how to apply these positive interactions for bees to their own realities. We will also explore how this type of tool extends beyond research and is being implemented in agricultural advisory and development organizations.

P 2

HONEYBEE VISITATION TO HASS AVOCADO FLOWERS INCREASES WITH INTER-ROW FLOWERING VEGETATION

Sharon Asis[1], Yael Mandelik[1], Arnon Dag[2], Gil Eshel[3]

[1]The Hebrew University, Faculty of Agriculture, Rehovot, Israel
[2]Gilat Research Center, Volcani Institute, Gilat, Israel
[3]Soil Erosion Research Station, Ministery of Agriculture, Rishon LeZion, Israel

Abstract

One of the key challenges in agricultural pollination is promoting pollinator activity in intensively managed systems that lack sufficient floral and nesting resources. This limitation is especially critical in crops that are inherently unattractive to pollinators yet depend heavily on effective pollination, such as Hass avocado orchards. Honeybees (*Apis mellifera*) are the primary managed pollinators of Hass avocado, yet its nectar is relatively unattractive compared with competing floral resources outside the orchard. Here, we report the results of a two-year field experiment (2024–2025) testing the effect of maintaining inter-rows flowering vegetation on honeybee and wild pollinator visitation to avocado flowers.

The experiment was conducted in 22 plots across central and southern Israel. Two agroecological approaches were tested: (1) Service Crops (SC) — sown mixtures of annual flowering plants in Low vs High plant species richness; and (2) Spontaneous Vegetation (SV) — unmowed naturally growing plants, both compared to mowed control.

The two systems showed a significant increase in both honeybees and wild pollinators visits to avocado flowers in treatments compared with control. In each system a single structural variable of the inter-row vegetation was associated with this increased visitation activity. In SC, plant height was the strongest predictor and in SV, plant cover was the dominant predictor.

Honeybee visitation to inter-row flowers was, as expected, significantly and dramatically higher in treated plots but showed only small correlation to avocado visits In SC. The most visited flowers in the rows were *Silybum marianum*, *Sinapis alba* (*seeded*) and *Eruca sativa* (*seeded*).

These results demonstrate that maintaining flowering vegetation between avocado tree rows — whether sown or spontaneously growing — significantly increases honeybee visitation to avocado flowers. These findings challenge the common practice of mowing inter-row vegetation during bloom and provide an evidence-based rationale for integrating flowering habitats within intensive orchard systems to support pollination services.

FUNCTIONAL STRUCTURING OF *BOMBUS*–PLANT INTERACTIONS: IMPLICATIONS FOR POLLINATION ECOLOGY

P 3

Engin Bacak, Fatih Dikmen

Istanbul Üniversitesi, İstanbul, Turkey

Abstract

Plant–pollinator interactions are central to ecosystem functioning and agroecological stability, yet the role of functional traits in structuring these interactions remains insufficiently resolved at the community level. Here, we analyse a presence–absence dataset of *Bombus*–plant genus associations based on field collections from the Marmara Region (Türkiye), focusing on how pollinator morphology and floral traits shape interaction patterns.

The interaction network exhibited a nested structure with a limited number of plant genera forming a shared resource core. Asteraceae genera (e.g., *Cirsium*, *Centaurea*, *Carduus*) functioned as generalist hubs, supporting a wide range of Bombus species. In contrast, tubular-flowered genera (e.g., *Echium*, *Digitalis*, *Stachys*) showed more selective associations.

Clear functional differentiation emerged at the subgeneric level. Long-tongued lineages (e.g., *Megabombus*, *Thoracobombus*) were primarily associated with deep, tubular corollas, whereas short-tongued groups (*Bombus* s. str., *Pyrobombus*) exploited a broader spectrum of open or shallow flowers. This pattern reflects strong trait matching between proboscis length and floral morphology, promoting niche partitioning within the community. Cuckoo bumblebees (subgenus *Psithyrus*) displayed no clear floral specialization.

Our results demonstrate that *Bombus*–plant interactions are structured by functional traits rather than taxonomic identity alone. Generalist plant genera provide a shared resource base, while morphological specialization enables coexistence through resource partitioning. These findings highlight the importance of maintaining functionally diverse floral communities to support resilient pollinator assemblages in agroecological landscapes.

P 4

EFFECTS OF FLOWER STRIPS ON POLLINATORS AND BENEFICIAL INSECTS IN SUGAR BEET CROPPING SYSTEMS

Margot Beelaert, Louis Hautier

Plant and Forest Health Unit, Life Sciences Department, Walloon Agricultural, Gembloux, Belgium

Abstract

Floral resources are indispensable for pollinator nutrition but are often limited in intensive agricultural fields. This study aims to evaluate the effectiveness of establishing flower strips alongside sugar beet and chicory fields in enhancing functional biodiversity at the landscape scale, particularly by increasing the availability of floral resources and suitable habitats.

Fields with flower strips in Belgium, beneficial insects and pollinators were monitored during the summer of 2023 and 2024, both within the flower strips and at a distance of 50 m into the adjacent fields. The flower strip mixtures, selected by the farmers, consisted of mustard, ravenelle, phacelia, buckwheat, and sunflower. On average, we observed 8 times more pollinators (bees, bumblebees, hoverflies and butterflies), 7 times more predators (ladybirds, hoverflies, cantharids and lacewings) in the flower strips compared to areas located 50 m into the fields. In 2024, flower strips were particularly attractive to pollinators and flying predators, a pattern likely driven by the use of a more diverse, nectar-rich flower mixture. In addition, the unusually rainy spring may have reduced floral resource availability in the surrounding environment, further increasing the relative attractiveness of the flower strips.

These results demonstrate that flower strips can substantially enhance functional biodiversity in intensive agricultural landscapes by providing essential floral resources and habitat for pollinators and natural enemies of pests. By concentrating pollinators and predators, flower strips not only support ecosystem services such as pollination and biological pest control but also contribute to landscape-scale biodiversity conservation. Overall, our findings highlight the effectiveness of flower strips as a practical strategy for promoting both ecological resilience and agricultural sustainability. Encouraging farmers to adopt diverse, nectar-rich flower mixes could maximize these benefits, particularly in years when environmental conditions limit natural floral resources.

P 5

GENOTYPE AND MICROBIOME-DEPENDENT REGULATION OF NECTAR-RELATED GENES SWEET AND NEC IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

Chiara Braglia[1], Daniele Alberoni[1], Diana Di Gioia[1], Marco Roverso[2], Silvia Pettenuzzo[2,3], Luca Cappellin[2,3], Sergio Angeli[4]

[1]Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy
[2]Department of Chemical Sciences, University of Padova, Padova, Italy
[3]IASMA Research and Innovation Centre, Fondazione Edmund Mach, Food Quality and Nutrition Area, San Michele all'Adige, Italy
[4]Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Bolzano, Italy

Abstract

The decline in sunflower honey production observed in several European regions highlights the need to better understand the molecular regulation of nectar secretion. Nectar production depends on conserved pathways of sucrose metabolism and transport, in which *SWEET9* and *NEC1* play central roles. This study investigated i) the expression variability of these genes across 36 sunflower cultivars (25 hybrids and 11 non-hybrids), and ii) their modulation by plant growth–promoting bacteria (PGPB) under open-field conditions over two consecutive years. Gene expression was quantified by TaqMan qPCR using newly designed sunflower-specific primers and analysed through ΔCt-based robust linear models. Across cultivars, *NEC1* expression was highly conserved, with no significant differences relative to baseline genotypes in either hybrid or non-hybrid groups. In contrast, *SWEET9* showed pronounced genotype-dependent variability. Several hybrid cultivars exhibited significant upregulation (up to ~14-fold) compared to the hybrid baseline (LST907), while only two non-hybrid genotypes significantly diverged from Peredovick (non-hybrid baseline), indicating greater transcriptional variability among hybrids. PGPB treatments (*Bacillus*, *Lactobacillus*, and *Pseudomonas*-based consortia) induced gene- and variety-specific responses. *NEC1* was largely unaffected, whereas *SWEET9* displayed significant genotype × treatment interactions, including strong downregulation in LST under *Lactobacillus* treatment and marked upregulation in Peredovick under the same treatment. Nectar sugar analyses revealed treatment-dependent shifts in fructose, glucose, and sucrose content. BAC treatment doubled both fructose (0.013 mg) and sucrose (0.0053 mg) content in Peredovick and in LST varieties (fructose: 0.012 mg and sucrose: 0.004 mg) respect to the CTR. In 2025, *NEC1* expression positively correlated with nectar sugar concentration, while *SWEET9* showed negative correlations with normalized sugar levels. In conclusion, nectar-related gene regulation in sunflower is strongly genotype-dependent and differentially responsive to microbial inoculation, with *SWEET9* emerging as a key source of transcriptional plasticity.

P 6

GLOBAL CONTRIBUTION OF HOVERFLIES TO CROP POLLINATION

Martina A. Bramardi[1], Ana L. Pietrantuono[1], Fabrice Requier[2]

[1]INTA Bariloche - Instituto de Investigaciones Forestales y Agropecuarias Bariloche (INTA- CONICET), San Carlos de Bariloche, Argentina
[2]Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, Gif-sur-Yvette, France

Abstract

Approximately 90% of flowering plants depend on animal pollination for reproduction. Particularly insects provide an essential ecosystem service for conservation and agricultural production, where the diversity of pollinator species increases crop yields and the resilience of agroecosystems. However, most studies have focused on the pollination service provided by bees (Hymenoptera: Apidae), underestimating the contribution of other taxa. Hoverflies (Diptera: Syrphidae) are a widespread and diverse group of pollinating flies, that improves fruit set and quality, by their foraging behaviour.

The aim of this study was to quantify the global contribution of hoverflies (Diptera: Syrphidae) as crop floral visitors through a literature-based analysis, and to reveal potential gaps in pollination research. The CropPol database was cleaned, filtered and analysed, reviewing 201 studies on floral visitors across 49 crops in 34 countries.

We first found that only 49.2% of the studies included hoverflies in their sampling designs. Despite this, we recorded an unexpected large hoverfly diversity of 63 genera, with Eristalis ssp. being the most abundant. Overall, hoverflies were recorded as flower visitors for 70.5% of the worldwide crops. The crops with the highest relative abundance of these insects were wild mustard (*Brassica rapa*, 58.7%), strawberry (*Fragaria* x *ananassa*, 34.5%), and broad bean (*Vicia faba*, 30.4%).

These findings highlight the importance of hoverflies in crop pollination and also emphasise the gaps and taxonomic biases in pollination research, where hoverflies are often overlooked or insufficiently sampled. Therefore, further research is needed to improve our understanding of functional biodiversity and the contribution of different taxa to crop pollination.

THE EFFECT OF TIMING AND APPLICATION METHOD ON EFFICACY AND COLONY PERFORMANCE OF A SLOW-RELEASE FORMIC ACID MITE TREATMENT

P 7

Heather C. Broccard-Bell

NOD Apiary Products Ltd., Trenton, Canada

Abstract

Effective management of *Varroa destructor* requires the alignment of treatment timing with environmental conditions. For formic acid treatments, application strategy must also be considered. We tested a commercially-available slow-release formic acid treatment, Formic Pro. In North America, the product is registered under two application protocols: (1) simultaneous application of two strips, and (2) sequential application of single strips over two consecutive 10-day periods. During the 2025 beekeeping season, we conducted a field experiment at our research apiary in Quinte West, Ontario, Canada (n = 35, k = 5). *Varroa* treatments were applied either in the spring (May 20), or in summer (July 5). We evaluated the interaction between treatment timing and application method on mite control efficacy and colony performance. Colony-level outcomes, including mite drop and performance indicators, were monitored throughout the active season.

We found an interaction between treatment timing and application method, indicating that the relative efficacy of each protocol is context-dependent. Earlier in the season, the two methods performed identically; however, at the July timepoint, the two simultaneous strip method outperformed the sequential single strip method. Our findings highlight the importance of integrating both timing and application strategy when deploying formic acid treatments, and provide guidance for beekeepers seeking to optimize their *Varroa* control strategies.

P 8

LETTING BEES SPEAK: A COLONY-BASED APPROACH TO ASSESS CROP FLORAL RESOURCE AVAILABILITY

Lorenzo Corsi, Giorgio Sperandio, Matteo Pacella, Maria Chiara Battistelli, Abdalhadi M.A. Abulebda, Paola Riolo, Sara Ruschioni

Università Politecnica delle Marche, Ancona, Italy

Abstract

In agroecosystems, floral resource availability is commonly assessed through flower-level measurements of nectar and pollen. While these approaches quantify potential resource production, they often fail to reflect the actual availability and usability of these resources for pollinators under field conditions.

Here, we propose a colony-based approach that shifts the focus from flowers to pollinators, using honeybee colonies as functional bioindicators of floral resource provision. By confining colonies within insect-proof mesh cages containing a single sunflower accession, bees are forced to forage exclusively on one resource, allowing a direct assessment of how floral traits translate into resource collection and colony development.

The approach was applied to different sunflower varieties. Honeybee nuclei were placed within 8×24 m enclosed plots, each containing a single accession, ensuring controlled foraging conditions. Three colonies per treatment were monitored over time. At regular intervals (T_0–T_{27}), colony-level variables were assessed, including stored honey, pollen, brood development, and foraging activity. Changes over time ($\Delta=T_i - T_0$) were used to describe resource accumulation dynamics.

Results reveal clear differences among sunflower varieties in their capacity to sustain colony development and resource storage, indicating that floral resource production does not necessarily translate into effective resource use by honeybees.

These findings demonstrate that honeybee colonies can act as integrative bioassays of crop resource availability, providing a more ecologically relevant evaluation than flower-based measurements and offering a novel tool for designing pollinator-friendly cropping systems.

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BOMBO PROJECT: ASSESSING THE IMPACT OF AGRO-ENVIRONMENTAL INTERVENTIONS ON POLLINATOR BIODIVERSITY

P 9

Marco d'Agostino[1], Simone Flaminio[1,2], Antonio Papaleo[3], Teresa Lettieri[3], Danilo Mirandola[3], Laura Bortolotti[1]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]UMons, Mons, Belgium
[3]CREA Research Centre for Agricultural Policies and Bioeconomy, Bologna, Italy

Abstract

The conservation of biodiversity is a key priority within the CAP Strategic Plan (PSP) 2023–2027, with increasing attention to the protection of pollinators, particularly wild bees, and their habitats. The BOMBO project aims to assess and enhance the effectiveness of selected some important agro-environmental interventions by generating qualitative insights into their implementation and ecological outcomes.

The project is based on a field-oriented approach involving the selection of representative pilot farms participating in these measures. Within these farms, biodiversity will be monitored through the development and application of an innovative indicator focused on wild bees (BEE indicator). This will allow for a more precise evaluation of how agricultural practices influence pollinator diversity and ecosystem health.

In parallel, butterfly populations will be monitored using an adaptation of the standardized "Butterfly Monitoring Scheme Italy" protocol, enabling a comparative analysis between different biodiversity indicators. The integration of these datasets will contribute to understand the effectiveness of the agro-environmental intervention investigated

A key output of the project will be the development of practical guidelines titled "Pollinator-Friendly Farmer," designed to promote sustainable agricultural practices that support pollinator well-being. The project will conclude with a final conference to disseminate results and inform future policy and intervention design. BOMBO ultimately seeks to strengthen the evidence base for biodiversity-friendly farming and support the evolution of more effective agro-environmental policies.

P 10

PLANT GROWTH-PROMOTING RHIZOBACTERIA SHAPE SUNFLOWER VOLATILE PROFILES AND OLFACTORY DETECTION OF HONEY BEES

Claire Duménil[1], Riccardo Favaro[1], Chiara Braglia[2], Daniele Alberoni[2], Luca Cappellin[3], Diana Di Gioia[2], Sergio Angeli[1]

[1]Faculty of Agricultural, Environmental, and Food Sciences, Free University of Bozen-Bolzano, Bolzano, Italy
[2]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy
[3]Department of Chemistry, University of Padova, Padova, Italy

Abstract

In recent decades, anthropogenic pressures have degraded agroecosystem soils, reducing microbial contributions to plant health, ecosystem functioning, and biodiversity. Plant Growth-Promoting Rhizobacteria (PGPR) are widespread in the rhizosphere and enhance plant health and performance. Their effects may extend to the modulation of floral volatile organic compounds (VOCs), which mediate insect-plant communication. Enhancing beneficial plant–microbe interactions is therefore a key objective of sustainable crop management. However, evidence on the role of PGPR in shaping plant chemical ecology, particularly in relation to pollinator attraction, remains limited. We hypothesize that PGPR improve soil fertility and quality and directly influence plant–pollinator interactions by modifying floral VOC emissions as well as nectar and pollen composition, thereby contributing to pollinator health and ecosystem functioning. For this reason, we assessed how different PGPR communities affect the VOC profiles of sunflower cultivars and their detection by the honey bee, *Apis mellifera*. An ancient (Peredovik) and a modern high-oleic hybrid (LST-907) sunflower cultivar were grown under inoculation with different PGPR taxa (*Bacillus* spp., *Lactobacillus* spp., *Pseudomonas* spp.) in a randomized experiment conducted under both greenhouse and open-field conditions. Floral VOCs were collected from the headspace of blooming flowers using closed-loop stripping analysis and identified by gas chromatography-mass spectrometry (GC-MS). Electrophysiological responses of honey bee antennae were assessed using coupled gas chromatography–electroantennography (GC-FID-EAD). We found that different PGPR treatments induced distinct volatile profiles in both, with more than ten compounds eliciting antennal responses in *A. mellifera ligustica*. PGPR treatments also influenced nectar and pollen composition. These results suggest that plant-associated microbiota can modulate pollinator-relevant chemical cues and resource quality. This work is supported by the project "IMPLiCIT - Improving soil-plant-insect interactions to promote pollinators " funded by the MIUR Progetti di Ricerca di Rilevante Interesse Nazionale (PRIN) Bando 2022- grant 2022NMAPEL.

BEEKEEPING IN THE CONTEXT OF AGROFORESTRY AND APOIDEA IN THE NEW OPEN SPACES AFTER VAIA STORM: THE LIFE VAIA PROJECT

P 11

Paolo Fontana, Livia Zanotelli, Luca Toldo

Edmund Mach Foundation, San Michele all'Adige, Italy

Abstract

The Life VAIA project (Enhance the reforestation of damaged forests with Innovative Agroforestry), funded under the LIFE Programme 2014-2020 of the European Community, planned to apply innovative intervention techniques for the recovery of forests, after Vaia storm (October-November 2018) and other extreme events, with the purpose of promoting the adaptation of forest vegetation to climate change. Agroforestry is the highly innovative element of the project as a solution to deal, in the short and long term, with the damage caused by extreme weather events. This strategy allows investing in the production of organic products with minimal impact, increasing biodiversity and the sustainable use of resources. The destruction of many forest areas has left room for a new flora, which for some decades, until natural and guided reforestation, will be able to support both a growing community of pollinators and a more profitable beekeeping activity than a forest environment is able to do. In the Italian pilot sites, sedentary apiaries have been established and managed according to the Biodiversity Friend Beekeeping certification protocol to verify the quantity and quality of beekeeping production. It is from these experimental apiaries that the "Regeneration honey" was obtained. Regarding anthophilous Apoidea, from 2022 to 2024, a network of monitoring pan trap transects was set up in the Italian pilot sites (Altopiano di Piné in Trentino and Altopiano dei Sette Comuni and Altopiano del Cansiglio in Veneto) to study the population of these insects. The samples of anthophilous Apoidea sampled in all the pilot sites are being studied but what is emerging is a complex fauna that is very different between the sites. The sedentary beekeeping activity and the analysis of productivity and products will continue for the entire duration of the project and then be transferred to local beekeepers.

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ASSESSING POLLINATION EFFICIENCY AND HONEYBEE FORAGING DYNAMICS UNDER VARYING HIVE DENSITIES IN HASS AVOCADO ORCHARDS

Avi Gabai[1], Sharoni Shafir[1], Arnon Dag[2]

[1]The Hebrew University of Jerusalem, Faculty of Agriculture, Food, and Environment, Israel
[2]Volcani Institute Agricultural Research Organization, Gilat Research Center, Israel

Abstract

Avocado is a key subtropical crop with rapidly expanding global cultivation, relying on insect-mediated pollination for successful fruit set. In commercial orchards, honeybees are the primary managed pollinator, however, the relatively low attractiveness of avocado flowers often results in suboptimal pollination. Increasing hive density is widely used to mitigate this limitation, yet optimal stocking rates remain poorly defined and are typically based on grower intuition rather than empirical evidence. Concurrent increases in hive density and avocado cultivation may further intensify pressure on honeybee availability for avocado pollination.

Adopting a pollination ecology framework, this study evaluates the effect of hive density on honeybee activity and pollination outcomes in commercial avocado systems. Multi-year field experiments are being conducted across five 'Hass' orchards in Israel, each comprising three plots (2–5 ha) separated by at least 300 m. Three hive density treatments are applied: 2.5, 3.3, and 5.0 hives ha^{-1}. The pollinizer cultivar 'Ettinger' is integrated across all sites.

Honeybee activity is quantified using standardized visual counts. Pollination success is assessed via pollen deposition on stigmas, fruit set, and final yield, alongside measurements of colony strength and health. To link foraging behaviour with landscape context, waggle dance decoding, honey sugar composition, and mapping of co-occurring floral resources are conducted.

Preliminary results show that visitation rates differed markedly between cultivars: mean visitation to 'Ettinger' flowers was more than 3 times that to 'Hass' (11±0.9 vs. 3±0.3 visits $tree^{-1}$ 2 min^{-1}, respectively). However, across sites, increasing hive density did not enhance visitation to 'Hass', despite increased overall bee abundance, and did not affect colony strength.

We found that reducing hive density did not decrease honeybee activity in avocado orchards, potentially due to limited floral attractiveness of avocado flowers, dominance of competing resources, or the spatial scale of the density manipulation.

POLLINATION SERVICES ALONG A LANDSCAPE GRADIENT IN SMALLHOLDER BEAN SYSTEMS OF THE AMBOSELI ECOSYSTEM, KENYA

P 13

Stella M. Gachoki[1,2,3], Dennis Kimiti[3], George Sunte[3], Victor Mose[3], Fabrice Requier[1,2]

[1]Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, Paris, France
[2]International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya
[3]African Conservation Centre (ACC), Nairobi, Kenya

Abstract

In the Amboseli agro-pastoral landscape of Kenya, smallholder agriculture is expanding into areas historically dominated by pastoralism, yet the effects of landscape context on pollination services and crop yields remain poorly understood. We investigated animal-mediated pollination services on yellow beans (*Phaseolus vulgaris* L.) along a vegetation greenness gradient, a crop of importance for the food security of local communities. We hypothesize that pollinator visitation and pollination services increase with vegetation greenness gradient in the Amboseli ecosystem. To test this hypothesis, we selected 11 spatially independent farms (farms ≥ 2 km apart) along a vegetation greenness gradient and planted a drought-tolerant variety of yellow beans (KALRO KAT B1) under standardized management. Before flowering we established four pollination treatments (open pollination, open with hand pollination, pollinator exclusion, and pollinator exclusion with hand pollination) each treatment having at least four replicates per farm. During bloom we conducted 20-minute focal observations of flower visitors on each farm across three periods (n = 363 observed flower visitors). At maturity we harvested labelled pods and measured seed number and seed mass per pod (n = 581 pods) to quantify pollination services and potential deficits. Our results suggest that pollination services increase initial pod success while flower visiting insects varies along the landscape gradient. By integrating community ecology, field experiments, and social data, this study quantifies pollination contributions to bean yield and reveals where and how pollinator-friendly management could most effectively sustain yields in rapidly changing agro-pastoral landscapes.

P 14

RESEARCH ON BEE (HYMENOPTERA: ANTHOPHILA) SPECIES COMPOSITION IN LATVIAN ARABLE LAND

Jānis Gailis, Niks Ozols, Zane G. Grase, Liga Zemeca, Sabine Mocane, Betija Rubene, Inta Jakobija

Latvia University of Life Sciences and Technologies, Institute of Plant Protection Research 'Agrihorts', Jelgava, Latvia

Abstract

Since 2024, we have been conducting research in arable land to understand what kind of bee community can develop under conditions of unstable foraging resource availability. Some crops provide pollinators with large amount of pollen and nectar; before and after flowering of the crops or in cases of cereals growing, only weeds (if not controlled) can provide foraging resources. We selected four fields in two regions of Latvia: Zemgale, where the landscape is dominated by arable land, and Vidzeme, where the landscape is dominated by forest biotopes. In the first year, all fields were sown with faba beans (*Vicia faba*), and in the second year with cereals. We collected bee material with blue, white and yellow pan traps three times during the vegetation season. In the first year, the number of species was noticeably higher – 62, 51, 72 species in May, June and July, respectively, compared to 56, 37, 58 species in the corresponding months of 2025. In the first year, the bees had plenty of foraging resources in the fields (weeds bloomed in all months, and beans in June), but in the second year, there were almost none (weeds were not present). Also, weather was more favourable (warmer, less precipitation) for bee's activity during the first year. Consistently, one of the dominant species was *Apis mellifera*. *Lasioglossum pauxillum* and *Lasioglossum morio* were the most common wild species in 2024 at all study sites throughout the season. In the second year, the most common wild species in Zemgale were *Lasioglossum calceatum* and *L. pauxillum*, and in Vidzeme – *L. calceatum*, *Halictus maculatus* and *Bombus sylvarum*. In both years, several representatives of the genus *Andrena* also appeared among the most common species in May. Several species of *Nomada* and *Sphecodes* were also found, but the density of kleptoparasitic individuals was low.

ASSESSING THE IMPACT OF ENTOMOPATHOGENIC NEMATODES FORMULATIONS ON HONEYBEE ADULTS AND LARVAE

P 15

Aikaterini Gerasimatou, Alexandros Rigos, Evangelos Moutsaras, Spyridon Vlogiannitis, Athanassios Antonopoulos, Dimitrios Lazarakis, Antonios Tsagkarakis

Agricultural University of Athens, Athens, Greece

Abstract

Entomopathogenic nematodes (EPNs), such as *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, are widely used as biological control agents against a range of agricultural pests. While generally considered environmentally safe, their potential non-target effects on pollinators remain insufficiently explored. This study evaluated the impact of selected EPN formulations on adults and larvae of honeybees (*Apis mellifera*) under controlled laboratory conditions. Caged bee, as well as plate reared larvae experiments were conducted to assess mortality following both contact and oral exposure to commercially relevant nematode formulations. Bees were exposed to treatments simulating field-realistic application routes, and survival was monitored over time. Our results indicate that certain formulations caused significantly increased mortality compared to controls. The observed effects varied depending on the nematode species, formulation type, and exposure route, with some treatments inducing notable acute toxicity in adult bees. These findings raise concerns about the assumption that EPN-based biopesticides are universally safe for non-target organisms, particularly pollinators. The mechanisms underlying the observed mortality remain unclear but may involve interactions between nematode-associated symbiotic bacteria and the bee immune system, or formulation-specific factors that enhance exposure risk. Given the increasing reliance on biological control agents in sustainable agriculture, our results highlight the need for more comprehensive risk assessments that include pollinators. Further research is required to clarify the conditions under which EPNs may pose a threat to honeybee health and to develop application strategies that minimize unintended impacts.

P 16

A HISTORY OF BEEKEEPING IN CYPRUS

Salih Gücel, Özge Özden

Near East University, Nicosia, Cyprus

Abstract

Cyprus, often referred to in antiquity as the "honey-colored island," boasts a beekeeping tradition that is as much a part of its geological identity as its copper mines. This abstract examines the evolution of Cypriot apiculture. Historically, beekeeping on the island dates back to the Neolithic period, with archaeological evidence suggesting that the indigenous honeybee, *Apis mellifera cypria*, has been managed for millennia. During the Classical and Roman periods, Cypriot honey was highly valued and widely exported throughout the Mediterranean. Its unique floral flavor was a result of the island's distinctive endemic plant species. A defining characteristic of traditional Cypriot beekeeping is the "tziverti"—a horizontal earthenware cylindrical hive. These clay pipes, often stacked in stone walls to provide thermal insulation against the Mediterranean heat, represent a sophisticated historical adaptation to the local climate. Throughout the Lusignan and Venetian periods, honey and beeswax remained essential to the island's economy, used both as sweeteners and in the production of candles for religious ceremonies. In this study, historical documents regarding beekeeping on the island of Cyprus and also literatures were reviewed and observation visits were made to some known historical apiaries.

WHICH *PHILADELPHUS* SHRUBS ARE SUITABLE FOR POLLINATOR-FRIENDLY GREEN SPACES?

P 17

Jacek Jachuła[1], Karolina Tymoszuk-Rydzewska[2], Hubert Rydzewski[3], Aleksandra Splitt[1]

[1]The National Institute of Horticultural Research, Skierniewice, Poland
[2]Maria Curie-Skłodowska University in Lublin, Lublin, Poland
[3]Botanical Garden of Maria Curie-Skłodowska University in Lublin, Lublin, Poland

Abstract

Ornamental plants are not only pleasing to the eye, but can also be beneficial to pollinators. A key challenge when designing greeneries lies in balancing plants' aesthetic value with their usefulness for pollinating insects. Private gardens, backyards, parks and other green spaces offer valuable opportunities for enhancing the diversity of food resources available to pollinators. However, for many plant species and cultivars, data on floral rewards is scarce. To address this, detailed research on the availability of nectar and pollen provided to insects, is essential.

We conducted studies on several *Philadelphus* species and cultivars that are popular on the gardening market. *Philadelphus* shrubs flowered from early June to the third decade of the month. The most common flower visitors were honey bees (45–50%), bumble bees (30–37%), and butterflies (13–21%), while solitary bees and beetles were observed only occasionally. Sugar reward per 1 flower varied greatly between the species and cultivars and ranged from 0.38 mg (*P. falconeri*) to 3.53 mg (*Philadelphus* 'Belle Etoile'). The mass of pollen per 1 flower ranged from 0.52 mg (*P. falconeri*) to 1.82 (*Philadelphus* 'Kalina').

Ornamental *Philadelphus* species/cultivars differ markedly in the amounts of nectar sugars and pollen they provide to insect pollinators. These findings offer valuable guidance for garden owners and architects. The key message from this study is that by following the similar aesthetics, one can choose plant species/cultivars that are better suited to pollinators and create pollinator-friendly green spaces.

This research was funded by the Ministry of Science and Higher Education of Poland as a basic subsidy, grant number 7.7.26.

P 18

EQUATIONS FOR CALCULATING FOOD RESOURCES FOR POLLINATORS IN HABITATS AND LANDSCAPES

Jacek Jachuła[1], Aleksandra Splitt[1], Magdalena Michalak-Tomczyk[2]

[1]The National Institute of Horticultural Research, Skierniewice, Poland
[2]The John Paul II Catholic University of Lublin, Faculty of Medicine, Department of Physiology and Toxicology, Lublin, Poland

Abstract

Pollinator-friendly management of agricultural areas requires identifying which habitats provide sufficient nectar and pollen resources, in terms of their amount, spatial distribution and temporal availability.

To calculate nectar and pollen resources, one first needs to compile data on habitat plant species composition, species flowering phenology and the abundance of blooming, followed by measuring nectar sugar and pollen production per floral unit. Such data can be collected during fieldwork or extracted from existing databases, e.g. published by Filipiak et al. 2022 (DOI:10.1002/ecy.3705). The presented formulas enable resource estimations per habitat and scaling the results up for entire landscapes:

- Resource mass per 1m^2 for each species is determined by multiplying the number of floral units per 1 m^2 by the pollen or sugar mass per floral unit. Next, this value is adjusted by each species ground cover (%) within a habitat, and these values are summed across all species.
- The seasonal availability of pollinator food resources at the habitat level is estimated by multiplying the amount of sugar or pollen per flower unit, the abundance of flowering, the ground cover of each plant species and the factor for the particular flowering stage. The values are summed across all species.
- Total pollen resources at the landscape level are calculated by combining pollen production estimates from each habitat with the habitat coverage across the landscape.

The method was developed and applied by Jachuła et al. (2021, 2022) to illustrate the spatial and temporal distribution of pollinator food resources in southeastern Poland, and it is continuously used by our team to assess resources in other habitat types (e.g., forests of different alliances and urban areas). These calculations enable the identification and protection of resource-rich habitats.

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PESTICIDE CONCENTRATIONS IN BEE POLLEN COLLECTED BY HONEY BEES NEAR CROPLANDS

P 19

Christina Kast, Marion Fracheboud

Agroscope, Bern, Switzerland

Abstract

Honey bees are important pollinators for wild plants and crops. While foraging, they may be exposed to a variety of pollutants, including agricultural pesticides. The aim of this study was to investigate the exposure risk for honey bees when beehives are located near croplands. Ten study sites with varying proportions of oilseed rape (OSR), grain, and vegetable crops were included, and the proportion of each crop was determined within a two-kilometer radius around each apiary. From April to August, pollen samples were collected from four bee colonies per apiary one day a week over two consecutive years (2024 and 2025). Ultra-high-performance liquid chromatography coupled with mass spectroscopy allowed identifying 33 pesticides (out of 55 analysed pesticides), which included 18 fungicides, nine insecticides, five herbicides and a synergist. Frequently detected fungicides included azoxystrobin (max. 217 µg/kg), cyprodinil (max. 3370 µg/kg), difenoconazole (max. 154 µg/kg) and mandipropamid (max. 276 µg/kg). Herbicide levels were up to 202 µg/kg (pendimethalin). Acetamiprid, a neonicotinoid insecticide, was the most frequently detected pesticide (prevalence of 39%). The correlation (R = 0.75) between acetamiprid concentrations in pollen collected in April and the proportion of OSR near the apiary suggests that this insecticide being used to control pollen beetles in OSR fields, while detections in pollen collected later in the season might reflect applications on vegetables. The toxic exposure ratios (TER) were calculated for insecticides, such as spinosad (max: 33 µg/kg, TER 143), permethrin (max: 13 µg/kg, TER 843), lambda-cyhalothrin (max: 54 µg/kg, TER 1411), acetamiprid (max: 625 µg/kg, TER 1937), and indoxacarb (max: 5 µg/kg, TER 4275). The maximum concentrations of the pesticides in pollen were considered of low concern with regard to acute oral toxicity for adult honey bees; however, synergistic and sublethal effects were not evaluated.

P 20

BODY ELEMENTAL COMPOSITION REFLECTS BEHAVIOURAL ROLES IN CASTES AND SEXES OF BUFF-TAILED BUMBLEBEES (*BOMBUS TERRESTRIS*)

Ronalds Krams[1,2], Sergejs Popovs[2], Tatjana Krama[2], Indriķis Krams[2]

[1]Institute of Food Safety, Animal Health and Environment "BIOR", Rīga, Latvia
[2]Daugavpils University, Daugavpils, Latvia

Abstract

Ecological stoichiometry provides an important framework for understanding how physiological functions and ecological roles influence the elemental composition of organisms. In social insects, differences in behaviour, activity level and reproductive roles among castes may be reflected in their body elemental composition. We investigated variation in body carbon (C), nitrogen (N) and C/N ratios among castes, sexes and age groups of the buff-tailed bumblebee (*Bombus terrestris*). Commercial colonies originating from the same breeding line were placed in apple orchards in southeastern Latvia. During the reproductive phase of colony development, individuals representing ovipositing queens, young queens, workers and males were collected. Body elemental composition was determined using an elemental analyser. Differences among groups were analysed using linear mixed-effects models.

Body carbon concentration did not differ significantly among castes and sexes. However, nitrogen concentration and C/N ratios varied markedly. Workers and young queens exhibited significantly higher body nitrogen concentrations than ovipositing queens and males. In contrast, ovipositing queens and males showed higher C/N ratios. These differences likely reflect variation in behavioural roles and energetic demands within the colony. Workers and young queens are responsible for foraging, colony thermoregulation and defence, which require greater investment in flight muscles and protein-rich tissues. Ovipositing queens and males are less involved in energetically demanding activities, which may explain their lower body nitrogen concentrations.

Our findings demonstrate that elemental body composition in bumblebees reflects functional specialization associated with division of labour rather than environmental stress. Ecological stoichiometry may therefore provide a useful physiological indicator of behavioural roles in social insects.

POLLINATION AND COMMUNICATION ECOLOGY OF AN INVASIVE BEE, MEGACHILE SCULPTURALIS

P 21

Julia Lanner, Julia Witter, Stefan Dötterl

Department of Environment and Biodiversity, University of Salzburg, Salzburg, Austria

Abstract

In recent decades, an increasing number of bees have been introduced to Europe from outside their native ranges. Notably, at least two exotic species were recorded at the EurBee11 venue in Bologna. *Megachile sculpturalis* Smith 1853, native to Asia, has been introduced to both Europe and the USA. In novel environments, these introduced bees encounter unfamiliar plants as well as potentially co-introduced, familiar plant species.

We propose the solitary bee *M. sculpturalis* as a model system for studying the pollination ecology of invasive bees and how such bees interact with host plants in foreign habitats. We present an initial synthesis of current knowledge and report new results on the pollination ecology of *M. sculpturalis*, including the color and floral scent cues of its host plants. Using a multilocus metabarcoding approach, we investigated pollen sources at a global scale. Pollen profiles revealed complex, spatially dependent foraging: samples from the USA indicated a polylectic strategy characteristic of successful invaders, whereas European samples were often dominated by *Styphnolobium japonicum*, a large-flowered East Asian Fabaceae tree widely used as an ornamental in Europe.

Based on these findings, we selected 15 plant species to analyze floral scents, including physiologically active compounds, and the visual cues of inflorescences. The flowers of most host plants exhibited reflectance peaks around 450 nm and 680 nm and were UV-blue to the bees. Although terpenoids predominated in the floral scent samples, the scent chemistry was diverse, with many physiologically active compounds, confirming the generalist foraging behavior of *M. sculpturalis*. Together, these results refine our understanding of plant-pollinator interactions involving an invasive bee and identify priorities for future research.

P 22

CRACKING THE SECRETS OF POLLINATION USING BEE COGNITION

Juliane Mailly[1,2], Louise Riotte-Lambert[2], Mathieu Lihoreau[1]

[1]CRCA-CBI (CNRS), Toulouse, France
[2]CEFE (CNRS), Montpellier, France

Abstract

Predicting pollination is a key challenge for the conservation of natural ecosystems and sustainable food production. While ecology models typically assume pollen disperses randomly around flowers, behavioural studies demonstrate that individual pollinators do not forage randomly. In particular, a century of research shows how bees heavily rely on sensory cues, learning and memory, and communication to move across space and collect floral resources in an efficient manner. Here we will discuss how these complex, yet predictable, collective movement patterns shape pollen dispersal and plant reproduction. We will explore this idea with a series of field experiments on bee navigation and simulations of agent-based models, leading to new hypotheses about pollen dispersal, plant mating patterns and fitness. Such integration of behavioural mechanisms into pollination models is a powerful mean to predict pollination and mitigate forthcoming crises in the context of fast changing environmental conditions.

INTERACTIONS BETWEEN HONEYBEE AND ANT POPULATIONS IN AUSTRIAN AGRICULTURAL LANDSCAPES

P 23

Stefan Mandl[1,2], Thomas Druml[2,3], Berthold Heinze[4]

[1]Bienenhof Mandl GmbH, Traiskirchen, Austria
[2]BFW-Austrian Research Centre for Forests, Department for forest growth, silviculture and genetics, Vienna, Austria
[3]BBS, Nötsch, Austria
[4]BFW-Austrian Research Centre for Forests, Department for forest growth, silviculture and genetics, Vienna, Austria

Abstract

The impact of honeybee colonies on biodiversity and agricultural ecosystems has been the subject of controversial debate in the past, which has since led to the formation of opposing sides within the scientific community. In this preliminary study we aimed to determine the influence of honeybee populations on the occurrence of ant species as well as on the size of ant colonies. From 2010 to 2012, ant populations were counted at and near 12 apiary sites at various distances from the apiaries. The habitats for the ants remained consistent; only the influence of honeybees varied with distance from the apiary. The ant species as well as the number of individuals per species were documented at each of the 36 counting sites. In the presence of beehives, a significantly higher number of ant species was observed (3,5–7,5 species, mean=5,1) and on average 36,0-111,5 individuals (mean=54,5) per site. At distances of 150 m and 300 m from the apiary, significantly fewer ant species (from 1 to 4, mean 150m=2,6; mean 300 m=2,7) and significantly fewer individuals (from 6 to 25, mean 150 m=11,9; mean 300 m=10,3) were found. The counts showed that the presence of honeybee colonies has a positive effect on the occurrence of ant species as well as the size of ant colonies. Reasons for this coincidence might be the additional environmental structures provided by the bee hives/housing as well additional foraging possibilities for ant colonies near to apiaries. These findings also triggered further research work focusing on positive effects of honey bee colonies on biodiversity in Austria.

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SYNERGISTIC EFFECTS ON HONEY BEE LARVAE OF TWO PESTICIDES MOST FREQUENTLY DETECTED IN BEEBREAD

Piotr Medrzycki, Irene Guerra, Valeria Caringi

CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Pollen is the main protein source in the honey bee colony and one of the essential ingredients in the larval diet. Being pollen on flowers exposed to contaminants, it can also constitute one of the main exposure routes of the entire colony to agricultural pesticides. On the other hand, thanks to the bee inclination to collect and accumulate it in the hive in the form of beebread, we can easily use bees to monitor the quality of environmentally available pollen, by analysing beebread for pesticide residues.

A recently concluded Italian nation-wide monitoring project BeeNet, provided data on the presence of pesticide residues in beebread collected in different Italian regions and different agricultural contexts. With the present study we took under examination the two substances most frequently detected in beebread samples and we assessed in laboratory their synergistic effects on honey bee larvae. For this purpose we tested the acute toxicity, both of the single substances and their binary mixtures, according to the OECD TG 237. The compounds were administered to 3 days old *Apis mellifera* larvae, reared on standardized semi-artificial diet, and the mortality was assessed daily till pupation.

We observed significant synergistic effects on the larval mortality. The results provide serious concerns about the bee safety of commonly used products, which may seem harmless to bees when found alone. This shows the importance of the synergistic effects, which should be considered in the environmental risk assessment procedures.

MONEY RULES THE WORLD: GAPS IN OUR UNDERSTANDING OF CROP POLLINATION

P 25

Dawid Moroń, Agnieszka Gudowska

Institute of Systematics and Evolution of Animals Polish Academy of Sciences, Kraków, Poland

Abstract

Pollination of crop plants - including that performed by bees - is a key determinant of global food security. Yet our understanding of how thoroughly crop pollination has been studied remains limited, both for globally cultivated species and for those grown locally.

To assess global research intensity on crop pollination, we surveyed scientific literature published between 1950 and 2023 addressing pollinators of 95 crop species (both globally and locally cultivated) that rely on animal pollination. As a proxy for the level of knowledge on crop pollination, we counted the number of peer-reviewed articles devoted to pollinators of each crop species.

In total, nearly 2,200 bibliographic records concerning crop pollination were compiled from all continents except Antarctica. Publication growth rates were slower for locally cultivated crops than for globally important ones. On average, globally important crops have 3.5 times more pollination-related publications than locally cultivated crops. The mean impact factor of the journals in which these articles appeared did not differ significantly between global and local crops (≈2.5). The primary positive predictor of the number of pollination publications was the economic value of a crop's yield. By contrast, the degree of pollinator dependence showed no correlation with the number of scientific articles.

To address identified knowledge gaps, we recommend increasing research attention to (1) locally cultivated crops, which often play substantial economic and social roles within their respective regions, and (2) crops with relatively low yield value, irrespective of whether they are grown globally or locally. Improving our understanding of crop-pollination systems will enhance our ability to predict environmental change impacts and devise effective strategies for safeguarding food production.

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POLLEN RESOURCE USE BY HONEYBEES AND STINGLESS BEES IN AN AVOCADO ORCHARD REVEALED BY DNA METABARCODING AND SABER-MASSARRAY

Matthias A. Reese, Joel Nichols, Stephen J. Trueman, Rachele S. Wilson

Griffith University, Nathan, Australia

Abstract

Managed honeybees (*Apis mellifera*) and stingless bees support pollination in orchards of avocado (*Persea americana*), yet the floral resources exploited by these pollinators in avocado orchards remain poorly characterised. Avocado flowers exhibit protogynous dichogamy and are capable of both self- and cross-pollination, but with cross-pollinated flowers having higher fruit retention and improved fruit development. The extent to which pollinators transport cross-pollen from another cultivar remains largely unknown.

We investigated pollen sources and pollen movement within an avocado orchard by sampling honeybees and stingless bees at managed hives, collecting both departing and returning foragers. Honeybees, stingless bees and flies were also collected directly from 'Hass' avocado flowers at increasing distances (10–150 m) from a neighbouring block of 'Shepard' avocado trees. A tiered molecular approach was employed. The plant species visited by foragers were identified by DNA metabarcoding from individuals with visible corbicular pollen loads. SABER–MassARRAY determined the cultivars of avocado pollen carried by all sampled individuals.

This approach characterised the species and cultivars of pollen exploited by orchard pollinators and quantified pollen transfer among cultivars. This data provides a basis for understanding pollinator foraging patterns and cross-cultivar pollen movement in avocado production systems.

BEYOND THE PILOT: THE BETTER-B PROTOCOL REVEALS LANDSCAPE-DRIVEN RESILIENCE IN HONEY BEE COLONIES EXPOSED TO FLUPYRADIFURONE

P 27

Annelise Rosa-Fontana[1,2], Antonio Pérez-Pérez[2], Antonio Silva[3], José P. Sousa[3], Gilles Verbinnen[4], Dirk C. de Graaf[4], Lina De Smet[4], Nuno Capela[3], Claudia Pasça[5], Daniel S. Dezmirean[5], Raquel Martín-Hernández[2], Mariano Higes[2]

[1]Instituto Politécnico de Bragança, Bragança, Portugal
[2]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha, Marchamalo, Spain
[3]University of Coimbra, Coimbra, Portugal
[4]Ghent University, Ghent, Belgium
[5]Universitatea de Științe Agricole și Medicină Veterinară din Cluj-Napoca, Cluj Napoca, Romania

Abstract

This study presents the transnational validation of a standardized lab-to-field pipeline developed within the EU BETTER-B project to evaluate honey bee resilience across Spain, Portugal, Belgium, and Romania. Flupyradifurone (FPF) was selected as a strategic model stressor; despite its "bee-safe" classification and increasing use in Europe as a neonicotinoid alternative, current European Food Safety Authority (EFSA) guidelines highlight the urgent need to assess its long-term sub-lethal impacts under diverse environmental conditions. Following a successful proof-of-concept pilot, exposure was harmonized using centrally prepared solutions (36 ppm) fed *ad libitum* for 9 days. Landscape selection was standardized using CORINE Land Cover data to compare responses across Agricultural versus Forest or Urban gradients. Colony development and nutritional reserves were evaluated longitudinally over 42 days using raw values via Linear Mixed-Effects Models, with Landscape, Treatment, Day, and their interactions as fixed effects, and Hive as a random effect. FPF treatment had no significant main effect on Total Brood across Belgium ($p=0.945$), Spain ($p=0.142$), Portugal ($p=0.694$), or Romania ($p=0.409$). Instead, colonies were driven by environmental context, with Landscape exerting highly significant main effects across all nations ($p<0.001$). Pesticide impacts were strictly context-dependent and localized. Spain was the most sensitive ecosystem, showing significant treatment effects on Adult Bees ($p=0.0002$), Honey ($p=0.045$), and Pollen ($p=0.0005$), modulated by triple interactions ($p<0.03$). Elsewhere, FPF triggered localized interactions: Romania exhibited a Landscape x Treatment interaction for Total Brood ($p=0.018$) and a Treatment x Day interaction for Adult Bees ($p=0.042$); Belgium showed Landscape x Treatment interactions for Adult Bees ($p=0.009$) and Honey ($p<0.0001$); Portugal for Pollen ($p=0.047$). No other interactions occurred ($p>0.05$). These results confirm the BETTER-B framework as a robust tool for assessing bee resilience at a continental scale, demonstrating that environmental context and resource availability are more significant determinants of colony fitness than the tested pesticide exposure.

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PROTECTING BEES: A TOOL FOR PESTICIDE SELECTION BASED ON BEE TOXICITY

Fabio Sgolastra[1], Serena Alessandrini[2], Elisa Monterastelli[2], Claudio Porrini[1], Alberto Contessi[2]

[1]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy
[2]Osservatorio Nazionale del Miele, Castel San Pietro Terme, Italy

Abstract

The aim of this work was to provide farmers with a practical tool to minimize the impact of phytosanitary treatments with pesticides on bee health. To this end, a table was developed summarizing the toxic effects (LD_{50}) of active substances currently authorized in Italy for phytosanitary use on honey bees (*Apis mellifera*), along with their environmental persistence. When available, the same information was also reported for other bee species, namely *Bombus terrestris* and *Osmia* spp.

Toxicity classes were assigned based on the lowest LD_{50} values for acute oral or contact exposure. The following thresholds were applied to all three species: < 2 µg/bee = high toxicity; 2–100 µg/bee = medium toxicity; > 100 µg/bee = low toxicity. In cases where both oral and contact toxicity data were available, the lower value was used; if only one value was available, that value was considered. Additionally, the relative toxicity with respect to the most sensitive species was indicated.

The table was designed to be user-friendly: toxicity levels were represented using a three-color coding system to allow immediate interpretation of the data. Data were compiled from several databases, including ECOTOX, TOXNET, the PAN Pesticide Database, PPDB (Pesticide Properties Database), BPDB (Biopesticide Database), EFSA documents, and the Win BDF Agrochemicals Data Bank. A second table was also developed to report active substances for which sublethal effects on bees have been documented. This table was compiled through a dedicated literature review.

Both tables are made freely available to all interested users via the web (in three formats) and in print, and are updated annually. These tables are intended to serve as a valuable resource for promoting good agricultural practices and enhancing the protection of both managed and wild bee populations.

INNOVATING WITHOUT HARMING: SAFE-FOR-BEES EMULSIFIERS FOR SUSTAINABLE APPLE PRODUCTION

P 29

Maja I. Smodiš Škerl, Lenka Jerele, Nika Hillmayr, Matej Stopar

Agricultural Institute of Slovenia, Ljubljana, Slovenia

Abstract

Emulsifiers are widely used in agricultural formulations to improve spray stability and coverage. In apple production, new emulsifier-based formulations are being explored as alternatives for chemical fruit thinning. However, their potential effects on pollinators remain poorly understood. In this study, we evaluated the effects of standard thinning agents and novel emulsifiers proposed for apple fruitlet thinning on honeybee workers using chronic oral exposure assays. Adult workers were caged and fed sucrose solutions containing emulsifiers for 10 days. Mortality and cumulative food intake were monitored throughout the experiment. Treatment-dependent differences in survival and cumulative food consumption were observed, with significant differences among treatment groups detected on days 3 and 4 for survival (Kruskal–Wallis test, $p=0.043$ and $p = 0.010$, respectively) and on days 3–5 for food consumption ($p=0.012–0.021$). Post-hoc Dunn's test with Holm correction showed lower survival in the ammonium thiosulfate (ATS) group compared with the control, 1-naphthylacetic acid (NAA), and polysorbate 20 (T20) groups on several observation days. In addition, cumulative food consumption was significantly lower in the ATS group compared with metamitron (MM) on days 7 and 10 ($p=0.046$ and $p=0.027$, respectively). Nevertheless, survival remained relatively high across most treatments, including polysorbate-based emulsifiers, with no pronounced adverse effects detected under the applied exposure conditions. To additionally assess developmental sensitivity, in vitro larval feeding bioassays are currently ongoing to evaluate larval survival and weight gain following exposure to the same formulations. Overall, the results support the need to include pollinator safety assessment when developing new orchard thinning formulations and suggest that the tested emulsifier-based approaches show relatively low risk for adult honeybees at relevant exposure levels.

P 30

FLORAL RESOURCES AND PHENOLOGY OF SELECTED *AESCULUS* SPECIES AS SUPPORT FOR POLLINATORS IN URBAN ENVIRONMENTS

Karolina Tymoszuk-Rydzewska[1], Monika Strzałkowska-Abramek[2], Marta Dmitruk[2], Hubert Rydzewski[3], Bożena Denisow[2]

[1]Department of Botany, Mycology and Ecology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Lublin, Poland
[2]Department of Botany and Plant Physiology, University of Life Sciences in Lublin, Lublin, Poland.
[3]Botanical Garden of Maria Curie-Skłodowska University in Lublin, Lublin, Poland

Abstract

The ongoing pollination crisis, reflected in the decline of pollinator diversity and abundance, underscores the urgent need to enhance floral resources availability. Ornamental trees may play a dual role by providing aesthetic value and supporting biodiversity of pollinators. Consequently, evaluating the melliferous potential of such species is crucial for urban planning and conservation. This study focused on flowering phenology of selected *Aesculus* species, their pollinator assemblages, and quantified nectar and pollen production to determine their efficiency as vital food sources for urban insects.

Field studies were conducted in 2023–2025 in the Botanical Garden of Maria Curie-Skłodowska University in Lublin (Poland) and included five *Aesculus* taxa *A. hippocastanum* L.; *A. flava* Sol., *A. glabra* Willd., and *A. parviflora* Walter; *A. turbinata* Blume; and the hybrid A. × *carnea* Zeyh., including the cultivar 'Briotii'. Flowering phenology differed significantly among species but not between years. Most taxa flowered from mid-April to late May, whereas *A. parviflora* bloomed in July, extending the seasonal availability of floral resources. Floral display and nectar traits were strongly species dependent, with limited interannual variation.

The highest nectar production was recorded in *A. × carnea* (7.89 mg per flower) and 'Briotii' (6.56 mg per flower), while the highest pollen yield was observed in *A. × carnea* 'Briotii. In contrast, *A. parviflora* showed the lowest nectar and pollen output. At the canopy level, sugar resources were greatest in *A. glabra* (4.36 $g{\cdot}m^{-2}$), whereas pollen resources peaked in *A. turbinata* (1.10 $g{\cdot}m^{-2}$). Flowers of all species were intensively visited by bumblebees (*Bombus* spp.), honey bees (*Apis mellifera*).

The results demonstrate that *Aesculus* species differ substantially in their value as floral resources but collectively offer complementary benefits by extending forage availability across the growing season. Their incorporation into urban green infrastructure can contribute to pollinator conservation and enhance ecosystem resilience in cities.

THE MOST EFFECTIVE POLLINATOR OF *SCOPOLIA CARNIOLICA* AND ITS SIGNIFICANCE FOR REPRODUCTIVE SUCCESS OF A RARE FOREST SPECIES

P 31

Karolina Tymoszuk-Rydzewska[1], Mykhaylo Chernetskyy[2], Bożena Denisow[3]

[1]Department of Botany, Mycology and Ecology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Lublin, Poland
[2]Botanical Garden of Maria Curie-Skłodowska University in Lublin, Lublin, Poland
[3]Department of Botany and Plant Physiology, University of Life Sciences in Lublin, Lublin, Poland

Abstract

Scopolia carniolica is a rare, protected forest clonal herb in Central Europe. This study aimed to identify the most effective pollinators of *S. carniolica* and assess their role in reproductive success. Research was conducted in 2018–2019 and 2023 in two populations in SE Poland: a cultivated population in the Botanical Garden of Maria Curie-Skłodowska University in Lublin and a natural population in the Tomaszów Lubelski Forest District. We analyzed floral biology, floral rewards, pollinator assemblages, pollination efficiency, and the breeding system.

Flowers of *S. carniolica* exhibit traits promoting outcrossing, including movement herkogamy and incomplete protogyny. Dense non-glandular trichomes at the base of stamens likely function as a mechanical barrier restricting access to ineffective pollinators. The species is obligatorily xenogamous and strongly self-incompatible. Pollinator exclusion caused complete reproductive failure in both populations. Although geitonogamy induced initial fruit set, it ultimately resulted in late-acting self-incompatibility and non-viable seeds.

Among flower visitors, bumblebees (*Bombus* spp.) were the most effective pollinators. They carried 551.1 ± 379.8 pollen grains on their bodies. Honeybees (*Apis mellifera*) carried 323.8 ± 367.7 pollen grains, whereas solitary bees transported 82.8 ± 70.9 grains. Bumblebees deposited an average of 413.9 ± 354.4 viable pollen grains on stigmas, compared with 60.9 ± 73.9 grains deposited by honeybees and 2.1 ± 1.4 by solitary bees. The insect species visiting flowers significantly affected the amount of pollen deposited on stigmas. However, a single visit by any pollinator was insufficient to ensure fruit set.

These findings demonstrate that long-term survival of *S. carniolica* depends on efficient pollen transfer by bumblebees, emphasizing the importance of pollinator conservation for maintaining reproductive success and genetic resilience in this obligately xenogamous clonal species.

P 32

NATURAL VEGETATION ENHANCES WILD BEE DIVERSITY IN VITICULTURAL AGROSYSTEMS

Livia Zanotelli, Luca Toldo, Paolo Fontana

Technology Transfer Centre Edmund Mach Foundation, San Michele all'Adige, Italy

Abstract

Background. Bees (Apoidea Anthophila) serve as fundamental bioindicators in temperate regions. Despite the prevalence of viticulture, vineyards are often overlooked in pollinator studies due to the autogamous nature of *Vitis vinifera*.

Objectives. This study investigated bee richness and abundance within vineyard agrosystems in the Province of Trento (Italy) to evaluate how landscape composition influences wild bee communities.

Methods. Within three core viticultural areas, two contrasting 500-meter radius landscape contexts were compared: vineyards surrounded by natural vegetation (NV) and vineyards dominated by monoculture (VV). Sampling was conducted monthly using pan traps throughout the bee flight season over three consecutive years (2023–2025).

Results. A total of 2,719 specimens belonging to 114 species were identified. Both abundance and species richness were significantly higher in NV sites (1,862 specimens, 102 species) compared to VV sites (857 specimens, 56 species). Our data confirmed a constant bee presence throughout the vegetative season, identifying both early-spring and summer-active species.

Conclusions. The results demonstrate that wild bees are highly sensitive to the presence of spontaneous vegetation within the vineyard matrix, which provides essential trophic resources and nesting sites. This study highlights that extensive viticulture integrated within a mosaic of natural habitats has a positive impact on bee communities and, consequently, on overall biodiversity. Furthermore, monitoring Apoidea offers a more granular and site-specific perspective on local biodiversity than observing *Apis mellifera* and its related parameters alone.

Bee products, ecosystemic services and circular economy

P 33 ETHNOMEDICINAL, NUTRITIONAL, AND CULTURAL USES OF STINGLESS BEES IN SOUTH INDIA

Chet Bhatta[1], Charana Kumar[2]

[1]Radford University, Radford, United States
[2]Tumkur University, Karnataka, India

Abstract

Meliponiculture has long been part of the social, economic, and religious life of many ethnic communities worldwide, including in India. Stingless bees are crucial to tropical and subtropical ecosystems, where they play essential roles in agricultural production and biodiversity conservation. Several indigenous communities in South India practice traditional meliponiculture and utilize stingless bees for ethnomedicinal and various non-food purposes. Ethnoecological studies were conducted to document the traditional uses and cultural significance of stingless bees across thirteen districts in four states of South India. Data were collected through semi-structured interviews with stingless beekeepers and traditional honey hunters. In total, 44 specific uses were documented across eight categories: food, medicine, veterinary medicine, crafts, spiritual beliefs, religious rituals, cosmetics, and plant growth promoters. The Relative Cultural Importance (RCI) of stingless bees among South Indian ethnic communities was also assessed. The Brahmin communities showed the highest RCI, followed by the Kunbi communities. Most communities practice destructive honey extraction methods, except for Brahmin communities, who maintain traditional meliponiculture using hives made from coconut shells, bamboo poles, mud pots, and logs. Notably, informants reported—for the first time—the use of stingless bee pollen to treat sac brood disease in Asian honeybees. Overall, indigenous communities in South India utilize stingless bees and their hive products in diverse and meaningful ways. However, modern meliponiculture remains in its early stages and will require significant scientific and technological advancements to become eco-friendly, profitable, and sustainable.

WITHDRAWN

SUSTAINABLE BEEKEEPING – DEVELOPMENT OF A TIME AND RESOURCE-EFFICIENT BEEKEEPING PRACTICE

P 34

Annely Brandt[1], Lena Frank[2], Heiner Klingenberg[2], Oliver Lenz[3], Marcel Meisel[4], Valon Mustafi[4], Jürgen Parg[5], Wolfgang Scheele[6], Solveigh Siodlaczek[7], Sven Teichmann[8], Lothar Wehner[9], Ansgar Westerhoff[2], Anette Widmer[10], Ulrich Widmer[10], Saskia Wöhl[4]

[1]LLH-Bee Institute Kirchhain, Kirchhain, Germany
[2]bienenprojekte.de, Göttingen, Germany
[3]Imkerei Lenz, Gründau, Germany
[4]Center for Bees and Apiculture, Mayen, Germany
[5]Imkerei Glückshonig, Obernburg, Germany
[6]Imkerei Scheele, Trendlburg, Germany
[7]Comunis Projektbüro, Morschen, Germany
[8]Imkerei Teichmann, Glashütten, Germany.
[9]Imkerei Wehner, Nentershausen, Germany
[10]Taunusbiene, Aarbergen, Germany

Abstract

Climate change-induced changes in environmental conditions, rising operating and feeding costs as well as diseases and parasites, in particular *Varroa destructor*, are confronting beekeepers with growing economic and ecological challenges. Against this background, the EIP-AGRI project 'Sustainable beekeeping - resource-conserving and efficient beekeeping for a sustainable future' aims to develop and optimise a time- and resource-efficient management practice that is adapted to the local conditions.

The innovative approach of the project lies in the combination of several beekeeping management practices and their accompanying scientific investigation. These include early split colony formation with capped brood combs, omission of drone brood removal and reduced winter treatment against *V. destructor*. As part of the project, the innovative EIP-management approach will be compared with pre-existing individual management methods of the seven participating beekeeping operations. Material, labour time and energy consumption as well as honey yield and quality will be recorded and subsequently analysed from an ecological and economical business perspective. We will create an individual climate footprint for each beekeeping operation. The central objectives are to record and analyse economic key figures to increase the efficiency of resource use and to promote the exchange of experience and knowledge between the participating beekeepers and the beekeeping community.

An intensive exchange within the seven EIP-project beekeeping operations and with the beekeeping community will strengthen a shared culture of learning and innovation. The results are disseminated via lectures, training courses and events and supplemented by citizen science approaches.

P 35 APITHERAPY IN ITALY: SCIENTIFIC DEVELOPMENT, APPLICATIONS, AND REGULATORY CHALLENGES WITHIN A ONE HEALTH FRAMEWORK

Aristide Colonna, Gianluigi Bressan

Associazione Italiana di Apiterapia, Roma, Italy

Abstract

The Italian Association of Apitherapy was established in 2015 and operates at a national level with the aim of promoting, developing, and disseminating knowledge of apitherapy across its scientific, educational, and wellness-related applications.This work presents and explores the Association's activities, which range from dissemination and professional updates on the use of hive products, to research, the organization of training courses, the management of "wellness apiaries," the development of protocols, and broader initiatives related to apitherapy within a "One Health" perspective. Furthermore, the critical issues surrounding apitherapy in Italy are examined, particularly in the absence of specific regulatory frameworks, where apitherapy is currently considered an integrative approach to traditional medicine. Deepening the presentation, the Italian Association of Apitherapy pursues several objectives, first and foremost the promotion of the use of hive products as a complement to conventional medicine. To this end, it collaborates with numerous universities and research centers, including the Mario Negri Institute, the University Federico II, and the University of Eastern Piedmont. Using the "Wellness Apiary," the Association is also collaborating with the Bambino Gesù Hospital, taking advantage of the aromas inside the apiary itself to support children suffering from respiratory diseases, particularly asthma. Another important field is safe bee education ("apididactics"). Children are introduced to the life of bees and made aware of their importance. Furthermore, the Association is a member of APIMONDIA (an organization linked to the FAO), where I personally serve on the Scientific Committee of the Apitherapy Section. Last, but certainly not least among its many activities, the Association is also part of the IFA (International Federation of Apitherapy), which includes more than 50 countries, all dedicated to the study of hive products and their applications.

EFFECT OF TECHNOLOGICAL TREATMENT ON HYDROGEN PEROXIDE LEVELS AND VOLATILE ORGANIC COMPOUNDS PROFILES IN HONEY FROM DIFFERENT REGIONS OF ITALY

P 36

Clelia Covino[1], Daniela D'Esposito[2], Federico Manganello[1], Maurilia M. Monti[2], Liberata Gualtieri[2], Michelian Ruocco[2], Pier Paolo Danieli[1], Filippo Lazzari[1], Gennaro Di Prisco[2]

[1]Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Rome, Italy
[2]IPSP-CNR Institute for Sustainable Plant Protection, National Research Council, Portici, Napoli, Italy

Abstract

Honey is a complex natural product whose quality is influenced by various factors, such as botanical and geographical origins. However, some honey processing methods, such as those involving heating, can alter its chemical, physical and organoleptic properties, by reducing both its antimicrobial activity and the emissions of Volatile Organic Compounds (VOCs), which are responsible for honey aroma.

The antimicrobial activity of honey is due to the high content of sugars, mainly glucose and fructose, high free acidity, and the enzymatic activity of glucose oxidase (GOX). In diluted honey, GOX converts glucose in gluconic acid by accumulating hydrogen peroxide (H_2O_2), which acts as antimicrobial agent.

Here we investigated the effect of heating on H_2O_2 content and VOCs profile of honey samples from different geographical origins. Hydrogen peroxide concentrations were determined using a colorimetric assay by scoring the absorbance at 595 nm, providing an indirect assessment of the enzymatic activity responsible for its production, while VOCs profiles were characterized by Proton Transfer Reaction Quadrupole Interface Time-of-flight Mass Spectrometry (PTR-Qi-TOF-MS). Both H_2O_2 and VOCs levels can vary significantly across the honey samples and can be further influenced by heat treatment. The results may be useful for designing industrial-scale honey processing protocols for honey production, aimed at minimizing the loss of freshness, aroma, and antimicrobial activity.

P 37

BOTANICAL COMPOSITION OF LINDEN AND HONEYDEW HONEYS FROM HUNGARY

Helga Déri, Judit Horel, Márton Flórián, Tiborné Szonda

Institute for Farm Animal Gene Conservation, National Centre for Biodiversity and Gene Conservation, Gödöllő, Hungary

Abstract

Linden honey is the third most popular type among Hungarian consumers. The nectar secretion of *Tilia* flowers is highly sensitive to weather factors, which under extreme conditions can drastically reduce the honey yields. In the current study, we present the botanical origin of 11 honey samples collected between 2021 and 2023 during the linden flowering period. Although all samples contained linden pollen, its percentage met the minimum limit of 23% recommended by the Hungarian Standard and International Honey Commission in only five samples. 4 samples were a mix of nectar and honeydew honey, while 2 were classified as pure honeydew honey. In the latter 6 samples (honeydew group), the main sources of honeydew may have been maple species (*Acer* spp.), fruits belonging to the Rosaceae family (*Prunus* spp.), sunflower (*Helianthus annuus*), and crown vetches. However, linden honeys also included a small amount of honeydew. A total of 90 plant species were identified in the examined samples, two-thirds of which could serve as nectar sources for pollinators. Statistical analysis showed no significant difference between the two groups in this respect, since at least 50% of their plant species were identical. Regarding linden honeys, 10 nectar-producing and 2 nectarless species were identified in over 75% of the samples. Conversely, in the honeydew group, 5 nectar-producing and 5 nectarless species occurred in three-quarters of the samples. In the linden honeys, non-nectar-producing plants accounted for 2–17 %, which was significantly lower than the 17–67% found in the honeydew samples. Although the number of plant taxa identified in the honey samples shows that honey bees found various food sources besides *Tilia* species in early summer, it is assumed that due to the insufficient availability of linden nectar, honey bees also collected honeydew as a supplementary food source during this period.

ANALYSIS OF ANTIMICROBIAL ACTIVITY IN HONEY FROM *MELIPONA RUFIVENTRIS* AND *SCAPTOTRIGONA TUBIBA* (APIDAE: MELIPONINI)

P 38

Mayara Faleiros-Quevedo, Tiago M. Francoy

USP, São Paulo, Brazil

Abstract

The honey produced by stingless bees (Meliponini) is known for its distinct physicochemical characteristics and biological properties. Compared to honey from *Apis mellifera*, stingless bee honey presents higher moisture content, high acidity, and bioactive compounds that may influence its antimicrobial activity. However, these properties can vary significantly depending on the bee species and geographic origin. In this study, two honey samples produced by different stingless bee species were analyzed to evaluate physicochemical parameters and the presence of antimicrobial activity against the pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*, as well as to determine the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). Samples produced by *Melipona rufiventris* and *Scaptotrigona tubiba* exhibited high inhibitory effects against the bacterial strains tested. The honey from *M. rufiventris* presented MIC values ranging from 15.62 to 62.5 µL/well, corresponding approximately to 1.5 – 6.25% (v/v) and MBC values between 31.25 and 62.5 µL/well, approximately 3.1 – 6.25% v/v for the evaluated bacteria. Meanwhile, the honey from *S. tubiba* presented an MIC of 62.5 µL/well, approximately 6.25% v/v for both strains and MBC values ranging from 31.25 to 62.5 µL/well, approximately 3.1 – 6.25% v/v. These findings highlight the influence of bee species on the biological properties of stingless bee honey and reinforce the potential of these products as functional foods with antimicrobial properties. The results also demonstrate the importance of a more in-depth characterization of honey produced to support product valorization and the scientific understanding of its bioactive potential. Considering the increasing demand for natural therapeutic alternatives due to the side effects of synthetic drugs and the growing problem of antibiotic resistance, stingless bee honey represents a natural resource with potential applications in health.

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P 39 RAPID TESTS AS SCREENING TOOLS FOR DETECTING ADULTERATION IN STINGLESS BEE HONEY

Mayara Faleiros-Quevedo, Tiago M. Francoy

USP, São Paulo, Brazil

Abstract

Honey adulteration represents a challenge for the honey market. Although analytical techniques are available to detect adulteration in honey from *Apis mellifera*, honey produced by stingless bees (Meliponini), which presents distinct physicochemical characteristics such as higher moisture content and different sugar profiles, still lacks specific and well-established techniques. Thus, this study evaluated the effectiveness of classical rapid reactions used for *A. mellifera* honey to detect adulterants in stingless bee honey. Honey samples from three species, *Tetragonisca angustula*, *Melipona quadrifasciata*, and *Tetragona elongata*, were experimentally adulterated with corn syrup, inverted sugar, water, and *A. mellifera* honey, and the Lugol, Fiehe, and Lund reaction tests were applied to detect these adulterants. The Lugol reaction successfully detected adulteration with corn syrup and inverted sugar at all tested dilutions. The Fiehe reaction was able to detect only adulteration with corn syrup. In contrast, the Lund test showed inconsistent results, probably due to the higher moisture content of stingless bee honey. None of the evaluated tests was able to detect adulteration by dilution with water or mixing with *A. mellifera* honey. The results suggest that the Lugol reaction may serve as a simple, rapid, and low-cost screening method to detect the most common adulterations. Considering the increasing commercial valorization of stingless bee honey, this approach may be useful for small producers in quality control, contributing to the monitoring of product authenticity, product valorization, and the strengthening of more sustainable production systems that support the conservation of bees and associated ecosystem services.

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OCCURRENCE OF PESTICIDE RESIDUES IN HONEY SAMPLES ANALYZED WITHIN THE OFFICIAL CONTROL PROGRAMME IN POLAND IN 2025

P 40

Milena Goliszek, Tomasz Kiljanek, Olga Burek

Department of Chemical Research of Food and Feed, National Veterinary Research Institute (PIWET), Pulawy, Poland

Abstract

Each year honey samples are analyzed within the national control programme for pesticide residues to be established by EU Member States according to Commission Implementing Regulation (EU) 2021/1355. As part of the Polish National Control Programme in 2025, a total of 26 honey samples were analyzed for 112 pesticide residues using a multi-residue method, and an additional 26 samples were tested for glyphosate, AMPA and glufosinate. Honey samples were analyzed by method validated and accredited according to ISO 17025 standard.

At least one pesticide residue was detected in 19 of the analyzed samples. The most frequently identified compounds were acetamiprid (neonicotinoid insecticide), thiacloprid, DMF (a metabolite of amitraz), and azoxystrobin. Acetamiprid was detected in 18 samples at concentrations ranging from 0.010 to 0.093 mg/kg. The highest concentration was recorded for DMF at 0.097 mg/kg, while the sum of amitraz reached 0.19 mg/kg and thiacloprid 0.15 mg/kg. All analyzed samples complied with the maximum residue limits established under Regulation (EC) No 396/2005.

Acetamiprid was the most prevalent pesticide, detected in the majority of positive samples; however, its concentrations remained well below the current maximum residue level. Acetamiprid can be applied to flowering plants and weeds, thereby contaminating the nectar. It is necessary to review the conditions of use of plant protection products containing acetamiprid, especially in the context of their use during crop flowering. The best example in that matter is glyphosate, one of the most widely used pesticides in the world. The frequency of glyphosate residues in honey has been shown to be very low. Recently, the European Commission approved an extension of its use in the EU, but with restrictions on its conditions of use. Glyphosate should not be used during crop flowering, control of flowering weeds and during high pollinator insect activity.

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PAVING THE WAY FOR THE DEVELOPMENT OF QUALITY LABELS FOR HONEY PRODUCED IN JORDAN

Amelia V. González-Porto[1], Gian Luigi Marcazzan[2], Banan Al-Shagour[3], Soledad Sagastume[1], Jose-Antonio López-Pérez[1], Mariano Higes[1], Carlos García-Villarrubia[1], Raquel Martin-Hernández[1]

[1]IRIAF-CIAPA, Marchamalo, Guadalajara, Spain
[2]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[3]NARC, Amman, Jordan

Abstract

Given the surge in demand for natural products, ensuring authenticity and preventing fraud through rigorous characterisation is imperative. Evaluating geographical, botanical, and physicochemical properties provides a robust framework for traceability and the implementation of official controls, thereby ensuring the highest standards of food safety and quality. MEDIBEES seeks to address these vulnerabilities by enhancing the industry's strengths and establishing a recognized seal of quality in Mediterranean Basin countries.

This study evaluated honey samples from Jordan using physicochemical, sensory, and pollen analyses. Findings revealed a low pollen count despite high botanical diversity, a characteristic that distinguishes Mediterranean honey and likely reflects how plants adapt nectar and pollen production to extreme climates. The majority of the analyzed samples were floral in nature. While predominantly multifloral, the specific combination of taxa identified includes pollen types that, under different seasonal conditions, could define monofloral honeys. Common plants identified by their pollen include Acacia, Orange Blossom, Eucalyptus, Jujube, Carob, and Prosopis. In Jordan, species from the Lamiaceae family are characteristic of local honeys, as is the case in other Mediterranean countries. However, they can be distinguished from those of neighbouring countries, as these plants are often accompanied by pollen from jujube, pomegranate or prosopis, and contain lower levels of olive and almond pollen than in other countries. This preliminary study requires further research and a larger sample size.

Scientific knowledge supports quality labels that ensure product authenticity, prevent unfair competition, and provide a competitive edge.

These results are under the project 2011-MEDIBEES, which is part of the PRIMA programme supported by the European Union.

FIRST STEPS TOWARDS THE IMPLEMENTATION OF QUALITY LABELS FOR LEBANESE HONEYS

P 42

Amelia V. González-Porto[1], Gian Luigi Marcazzan[2], Chadi Hosri[3], Rita Yaacoub[3], Dalida Darazy[3], Eduard Tabet[3], Raquel Martin-Hernández[1]

[1]IRIAF-CIAPA, Marchamalo, Guadalajara, Spain
[2]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[3]Lebanese University, Deknawek, Lebanon

Abstract

The honey production sector is of great importance, as it is an activity of significant ecological and commercial importance in Mediterranean Basin Countries. However, there are still some gaps in the system that leave the sector vulnerable in the fight against fraud. MEDIBEES aims to highlight the sector's strengths and support it on its journey towards achieving a distinctive mark of quality and added value.

In this study, ten honey samples from different Lebanese regions along north–south transects of this country were collected and characterized. The honey samples were subjected to physico-chemical, pollen and sensory analyses in order to obtain data that define their quality and characteristics within their specific geographical context. The samples included a diverse representation of herbaceous and shrubby plants typical of spring and summer in the Mediterranean Basin (*Cirsium, Scabiosa, Echinops, Lavandula, Origanum, Salvia officinalis, Thymus*), as well as fruit trees such as *Citrus* and *Persea*, and honeydew derived from *Cedrus* and *Quercus* species.

This knowledge, which underpins efforts to promote the authenticity of these regions' original products, represents a scientific basis as first step towards establishing quality labels as a tool in the fight against unfair competition and as a means of adding value in the face of fierce market competition.

These results are under the project 2011-MEDIBEES, which is part of the PRIMA programme supported by the European Union.

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ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT IN POLISH BEE POLLEN, BEE BREAD AND PROPOLIS

Katarzyna Jaśkiewicz, Marcin Gruba

The National Institute of Horticultural Research, Skierniewice, Poland

Abstract

Bee products, such as bee pollen, bee bread and propolis, play an important role in the human diet due to their nutritional and health-promoting properties. Phenolic compounds present in these products exhibit strong antioxidant effects, which can contribute to protecting the body from oxidative stress. As the health value of bee products is determined by their unique chemical compositions, the aim of the presented study was to investigate and compare the antioxidant properties and total phenolic content of Polish bee pollen, bee bread and propolis.

The bee pollen and bee bread samples were collected in 2024 beekeeping seasons in an ecological apiary of the National Research Institute of Horticulture, Apiculture Division in Puławy, located in the Poleski National Park. Propolis samples were obtained from the same Division of the National Research Institute of Horticulture and also from private beekeepers. The radical scavenging activity and total polyphenol content of the analyzed samples were determined using DPPH and Folin–Ciocalteu methods, respectively. The results showed that bee bread samples contained a higher total phenolic content (mean 13.8 mg GAE/g) compared to bee pollen (mean 3.2 mg GAE/g) collected from the same apiary. Antioxidant activity expressed as the percentage of DPPH reduction was also higher in bee bread samples (mean reduction 94.1%) compared to samples of bee pollen (mean reduction 88.0%). Nevertheless, propolis was characterized by the highest content of phenolic compounds (mean 195.3 mg GAE /g), while the mean antioxidant activity was 94.9%. The obtained results encourage further research into the possibility of using bee products as health-promoting products.

ASSESSING THE EFFECTS OF COUMAPHOS IN BEESWAX ON HONEY BEE BROOD

P 44

Lukas Jeker[1], Daniela Grossar[1], Verena Kilchenmann[1], Lars Straub[2], Christina Kast[1]

[1]Swiss Bee Research Centre, Agroscope, Bern, Switzerland
[2]Institute of Bee Health, University of Bern, Bern, Switzerland

Abstract

Coumaphos, a lipophilic acaricide widely used for *Varroa destructor* control, accumulates in beeswax and may expose developing honey bee (*Apis mellifera*) brood to residues. However, its effects under realistic field conditions remain insufficiently understood. Here, we experimentally assessed the impact of coumaphos-contaminated wax on worker brood development, emergence success, and adult body mass. Beeswax foundation sheets were spiked with coumaphos at nominal concentrations of 25, 50, and 100 mg kg^{-1}, reflecting residue levels reported from treated colonies, alongside untreated controls. After comb construction, experimental frames were introduced into four colonies, and queens were confined for 48 h to synchronize oviposition. Brood development was monitored across developmental stages, emergence success quantified, and newly emerged workers weighed individually. Chemical analyses confirmed a homogeneous distribution of coumaphos in wax, followed by redistribution into cell walls, resulting in approximately 2.4-fold reduction in effective brood exposure. Mortality during early brood stages (i.e., egg to larva) remained within natural variation across all treatments (18-20%). In contrast, brood reared in wax containing 100 mg kg^{-1} coumaphos exhibited increased mortality during pupation, leading to reduced emergence rates (64%) compared with controls and lower concentrations (70–75%). No treatment-related effects were detected on adult body mass. Coumaphos residues levels above 50 mg kg^{-1} in beeswax significantly reduced brood development, while the emergence rate of bees exposed to lower coumaphos levels was comparable to the control. These findings underscore the importance of accounting for residue redistribution and reduced effective exposure when assessing the risks of lipophilic varroacides. Future risk assessment should also consider potential interactions with co-occuring pesticide residues.

P 45 EVALUATION OF QUALITY PARAMETERS IN *PHACELIA* HONEYS FROM HUNGARY

Tünde Kiss, Judit Horel, Helga Déri, Lídia Lennert, Tiborné Szonda

Department of Apiculture and Bee Biology, Institute for Farm Animal Gene Conservation, National Centre for Biodiversity and Gene Conservation, Gödöllő, Hungary

Abstract

Phacelia tanacetifolia is regarded as one of the most significant bee pasture plants in Hungary. Its pollinator-attracting, nectar-rich flowers open sequentially, resulting in a prolonged flowering period. However, the knowledge concerning unifloral *Phacelia* honey remains extremely limited. The objective of the present study was to evaluate certain quality indicators, including diastase activity and proline content, in *Phacelia* honey samples from Hungary, in order to obtain more information about this unifloral honey. In the assessment of honey quality, diastase activity is widely accepted as a reliable indicator of freshness, while proline content is typically used as a marker of honey maturity and authenticity. In 2020 and 2022, a total of 28 honey samples were collected directly from beekeepers, predominantly from the north-western region of Hungary, and analysed. The botanical origin of the honey samples was identified by melissopalynological analysis. The diastase activity and the proline content were determined in accordance with the methods established by the International Honey Commission. Based on the measured parameters, one of the honey samples could not be classified as *Phacelia* honey. This finding was further confirmed by cluster analysis. It was established that all honey samples complied with the requirements of the EU Honey Directive, with the exception of four samples (two due to insufficient *Phacelia* pollen content and two showed signs of heat treatment). The diastase number of the honey samples ranged from 7.0 to 31.0, with an average value of 19.6 (SD: 4.8). In both years, the proline content varied within a wide range, between 103.0 and 543.3 mg/kg, with an average value of 364.5 mg/kg. The analysis of the data revealed no significant differences between the two years. It is noteworthy that Spearman's correlation analysis indicates a statistically significant positive relationship ($p < 0.05$) between diastase activity and proline content.

PHYSICOCHEMICAL TRAITS OF HONEY: A PRELIMINARY INVESTIGATION ON ITALIAN MONOFLORAL HONEYS

P 46

Filippo Lazzari[1], Federico Manganello[1], Gennaro Di Prisco[2], Paolo Viola[1], Clelia Covino[1], Aziza Ez-zaaraoui[1], Pier Paolo Danieli[1]

[1]Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy
[2]IPSP-CNR - Institute for Sustainable Plant Protection, National Research Council, Portici, Italy

Abstract

Monofloral honeys are valuable hive products that must meet regulatory standards for quality, safety and origin. This study, conducted within the MIEL.IT (MASAF n.J89I25002560007), evaluated honey quality by analysing physico-chemical traits in Italian monofloral honeys. Thirty-six samples from the 2025 beekeeping season were collected directly from Italian beekeepers and classified as follows: 9 *Castanea sativa,* 7 *Robinia pseudoacacia*, 5 *Coriandrum sativum*, 5 *Sulla* spp., 4 *Citrus* spp., 2 *Trifolium* spp., 2 honeydew samples, 1 *Erica* spp. and 1 *Eucalyptus* spp.

The variables measured were free acidity (FA; meq/kg), colour (mm Pfund), pH, electrical conductivity (EC; mS/cm) and moisture (%). A bibliographic review of Italian honeys was used for comparison, and Principal Component Analysis (PCA) explored multivariate relationships. FA (meq/kg) differed for *Castanea sativa* (9.52±3.37 vs. 11.96±4.06), while *Robinia pseudoacacia* showed higher values (13.45±3.48 vs. 6.28±1.76). Colour (mm Pfund) differences were observed for *Citrus* spp. (35.09±8.42 vs. 23.48±8.21) and *Castanea sativa* (95.63±11.92 vs. 59.74±19.78). Higher pH values compared to literature were found for *Robinia pseudoacacia* (3.83±0.37 vs. 3.08±0.88) and *Coriandrum sativum* (4.28±0.18 vs. 3.42±1.09). EC (mS/cm) values for *Citrus spp*. and *Sulla* spp. were also higher than reported (0.31±0.02 vs. 0.22±0.03; 0.33±0.05 vs. 0.22±0.03). Moisture (%) values were generally lower, particularly for *Citrus* spp. (18.00±0.12 vs. 16.23±0.97) and *Sulla* spp. (18.00±0.20 vs. 16.07±0.89), while *Coriandrum sativum* and *Castanea sativa* were comparable. PCA explained 79.3% of variance: PC1 (50.0%) was associated with pH, colour and moisture, separating dark/high-pH honeys from lighter ones, while PC2 (29.3%) was influenced by EC and colour.

In conclusion, PCA confirmed the discriminant value of the analysed traits. Further analyses are ongoing to include enzymatic activity for a more comprehensive quality profile of Italian monofloral honeys.

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OCCURRENCE, ORIGIN, AND ANALYTICAL DETERMINATION OF PYRROLIZIDINE ALKALOIDS AND PFAS IN HONEY: A SYSTEMATIC REVIEW

Marta Leite[1,2], Helena Rodrigues[1,2], Maria Beatriz Prior Pinto Oliveira[2], Andreia Freitas[1,2]

[1]National Institute for Agricultural and Veterinary Research (INIAV), Vila do Conde, Portugal
[2]LAQV-REQUIMTE, Faculty of Pharmacy, University of Porto, Porto, Portugal

Abstract

Honey's susceptibility to contamination from natural and anthropogenic sources emphasizes the need to preserve the quality and safety of this animal-derived food, a natural product of great nutritional, nutraceutical, and economic value. To support public health protection, promote appropriate and sustainable beekeeping practices, and strengthen environmental monitoring, it is essential to understand the occurrence, origin, and analytical determination of contaminants in honey, particularly pyrrolizidine alkaloids (PAs), pyrrolizidine alkaloid N-oxides (PANOs), and per- and polyfluoroalkyl substances (PFAS). PFAS have been reported in honey because of contaminated water, soil, or atmospheric deposition, although this remains an understudied area of research. Pyrrolizidine alkaloids can enter honey through contaminated floral resources or hive materials. Although contamination levels are generally low, localized risks may still occur.

A systematic review of the literature was performed to synthesize the available information on the occurrence, origin, and quantification of PFAs and alkaloids in honey originating from different geographical regions. Studies were selected for data extraction according to several criteria related to analytical robustness, geographical diversity, and relevance. The reviewed studies show that PAs and PANOs are the most extensively investigated contaminants in honey, with lycopsamine, intermedine, echimidine, senecionine, seneciphylline, retrorsine, and their N-oxides being the most frequently studied and detected compounds, whereas PFAS remain comparatively underexplored.

The review highlights an evolution in analytical approaches, from earlier studies targeting a limited number of PAs to more recent multi-analyte methods that increasingly include PANOs. Sample preparation relies mainly on solid phase extraction, while detection is predominantly based on LC- MS/MS and GC-MS/MS and related high-resolution mass spectrometry techniques.

The occurrence of PFAS and alkaloids in honey underscores the need for improved monitoring and stronger regulation to safeguard honey quality and safety, particularly for sensitive consumers such as children and high-level consumers.

POTENTIAL USE OF BEEKEEPING RESOURCES FOR APPLICATION IN AGRICULTURAL SOILS

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Jose-Antonio López-Pérez[1], Rosa Carrasco-Sáiz[1], Carlos García-Villarrubia[1], Amelia V. González-Porto[1], Antonio Nanetti[2], Giovanni Cilia[2], Marion Zammit Mangion[3], Mustafa Necati Muz[4], Thomas Galea[5], Soledad Sagastume[1], Mariano Higes[1], Raquel Martín-Hernández[1]

[1]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha, Centro de Investigación Apícola y Agroambiental de Marchamalo (IRIAF-CIAPA), Marchamalo, Guadalajara, Spain
[2]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[3]Department of Physiology and Biochemistry, University of Malta, Msida, Malta
[4]University of Namik Kemal (UNK), Faculty of Veterinary Medicine, Tekirdağ, Turkey
[5]Breeds of Origin 63, Triq Nerik Sant, Mosta, Malta

Abstract

Beekeeping is an important activity in many Mediterranean countries. Its integration into agricultural systems contributes to the functional balance of the environment and promotes the economic development of rural areas facing severe depopulation. As a contribution to the circular economy, the use of hive residues generated during beekeeping has been proposed as an agricultural resource due to its high organic matter content. Its application is particularly suitable for horticultural systems characterized by soils low in organic matter and moisture, which are typical of Mediterranean climate zones. Material from four Mediterranean countries, preserved by freezing and drying as a biosafety protocol, was analyzed, and its physicochemical characterization was carried out to assess its variability. In addition, phytotoxicity bioassays were performed with three batches of Spanish samples collected from the CIAPA apiaries to establish the maximum tolerable dose of this waste for various horticultural plants. These batches consisted of material obtained during two seasons (winter and spring) and material obtained from a hive infested with *Ascosphaera*. The tested doses included in the phytotoxicity trials were 0.5, 1.0, 2.5, and 5.0%. The analyzed plants included garden cress as a standard phytotoxicity reference, along with three major horticultural crops: broccoli, cucumber, and tomato. The physicochemical parameters showing the greatest variability among the different Mediterranean samples were electrical conductivity, phosphorus, and lipid content. Regarding phytotoxicity, broccoli was the most susceptible crop, and the maximum dose tolerated by all plants was below 2.5%. According to this preliminary analysis, this residue would be applicable primarily in alkaline soils that are deficient in organic matter and phosphorus.

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EFFECT OF BEEHIVE RESIDUES ON THE PHYTOPARASITIC NEMATODE *MELOIDOGYNE INCOGNITA* AND OTHER NEMATODES PRESENT IN AGRICULTURAL SOILS

Jose-Antonio López-Pérez, Rosa Carrasco-Sáiz, Carlos García-Villarrubia, Amelia V. González-Porto, Mariano Higes, Soledad Sagastume, Raquel Martín-Hernández

Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha, Centro de Investigación Apícola y Agroambiental de Marchamalo (IRIAF-CIAPA), Marchamalo, Guadalajara, Spain

Abstract

In areas where beekeeping is a common practice, hive management generates residues that could be useful for various purposes, contributing to a circular economy. This resource, rich in organic matter, is a mixture of materials obtained after wax extraction and from sanitary trays. Its potential as an organic amendment capable of reducing the presence of plant-parasitic nematodes in the soil has been studied. The effect of this waste was tested on *Meloidogyne incognita*, a plant-parasitic nematode of great economic importance. The infective stage of this pathogen was placed in each well of 24-well plates with different doses of the hive material. After three replicates, the appropriate and recommended doses for its intended application as a nematicide were characterized. The lethal concentration (LD_{50}) for *M. incognita* was established at 0.4% for the tested material. A second bioassay was conducted using nematodes extracted from the soil around an infested cucumber plant grown in a greenhouse. This extract contained *M. incognita* J2 and free-living nematodes present in that soil sample. The soil nematodes were classified according to their trophic group (bacterivores, fungivores, herbivores/phytoparasites, omnivores, and predators) in the soil food web. Five dilutions were tested to calculate the effective lethal concentration (LD_{50}) using probit analysis based on the number of dead nematodes after 24–48 h. Its effect on other soil nematodes varied depending on the trophic group; bacterivores and fungivores tolerated the highest concentrations and survived for 48 h. There was a differential response according to the trophic group, indicating that the effect of hive material was not generically nematicidal, but rather affected endoparasitic nematodes selectively.

APICULTURAL RESIDUES AS POTENTIAL AMENDMENTS FOR SOIL BIODISINFESTATION IN VEGETABLE CROPS AND ITS EFFECT ON SOIL BIODIVERSITY

P 50

Jose-Antonio López-Pérez, Rosa Carrasco-Sáiz, Carlos García-Villarrubia, Amelia V. González-Porto, Mariano Higes, Soledad Sagastume, Raquel Martín-Hernández

Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha, Centro de Investigación Apícola y Agroambiental de Marchamalo (IRIAF-CIAPA), Marchamalo, Guadalajara, Spain

Abstract

The use of apicultural residues as a complement for agricultural purposes has been evaluated by several authors as a crop substrate, seedling production medium, or organic fertilizer. These applications highlight its potential as valuable resource that can contribute to circular economy. The aim of this study was to use this material as an organic matter source for soil biodisinfestation, thereby reducing soil-borne phytopathogens. This technique relies on the microbiological stimulation that occurs when organic matter with an appropriate C:N ratio is incorporated into the soil. The experimental design included four treatments: composted cow manure, chicken manure, beehive residues and an unamended control. Four plots (4 m^2) per treatment were randomly distributed inside a greenhouse located at the CIAPA facilities. All organic residues were amended into de soil and watered to field capacity. The biodisinfestation process was carried out over 45 days, after which a Swiss chard crop was cultivated for four months. The effects on soil biodiversity - including annelids, nematodes, bacteria and fungi - were assessed. Annelids were present across all treatments. An increase in bacterivorous nematodes was observed, likely as a consequence of the enhanced soil microbial activity; the highest number of bacterivores occurred in plots treated with chicken manure, while the lowest was found in the control plots. In plots where cow manure was applied, the presence of fungivorous presence increased. In contrast, plots treated with beehive residues, no other trophic group increased significantly aside from the bacterivores. The population of *Meloidogyne incognita* (plant-parasitic nematode) juveniles in the soil increased in all treatments after crop growth, though to a greater extent in the control plots. Consequently, the use of beehive residues is recommended, following specific guidelines, as its efficacy depends on the characteristics of the soil where it is applied.

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HEAT TREATMENT AND ENZYMATIC ACTIVITY IN HONEY: A MINI-REVIEW

Federico Manganello[1], Clelia Covino[2], Filippo Lazzari[1], Paolo Viola[1], Aziza Ez-zaaraoui[1], Gennaro Di Prisco[2], Pier Paolo Danieli[1]

[1]University of Tuscia, Viterbo, Italy
[2]Institute for Sustainable Plant Protection, CNR, Portici, Italy

Abstract

Honey quality is increasingly addressed within the European regulatory framework, as highlighted by Directive (EU) 2024/1438, which emphasizes the effects of thermal processing. In this context, enzymatic activity is a key indicator of honey quality and technological history, as endogenous enzymes are sensitive to heat and storage conditions (White, 1978; Bogdanov et al., 2004). Based on the available literature, this contribution reviews the effects of thermal treatments on diastase, invertase, and glucose oxidase as process indicators. Studies describe a gradient of thermal sensitivity in which invertase and glucose oxidase are generally more sensitive than diastase (Oddo et al., 1999; Tosi et al., 2008; Karabournioti and Zervalaki, 2001; da Silva et al., 2016). At moderate temperatures (40-50 °C), diastase shows limited variation, whereas invertase declines more rapidly. Above 60 °C, both enzymes progressively decrease, with diastase potentially approaching near complete inactivation at temperatures close to 100 °C (Tosi et al., 2002; Tosi et al., 2008). Glucose oxidase is particularly sensitive and may decrease significantly already between 55 and 70 °C, resulting in reduced hydrogen peroxide production and antimicrobial activity (Kretavičius et al., 2010; Brudzynski, 2006; Bang et al., 2003; Sahin et al., 2020). Enzymatic activity also shows wide natural variability (invertase ranging from <0.5 to >30 IN), influenced by botanical origin, honey type (monofloral vs multifloral), and storage conditions (Oddo et al., 1999). Enzyme degradation further depends on time-temperature interactions, with longer exposure leading to greater losses even at moderate temperatures (Tosi et al., 2002; Karabournioti and Zervalaki, 2001; Sahin et al., 2020). Enzyme inactivation kinetics vary with honey matrix and experimental conditions (Bogdanov et al., 2004; da Silva et al., 2016). This variability limits the definition of universal thermal thresholds, highlighting the need for standardized studies and integrated use of multiple enzymatic markers to better assess honey quality.

MULTIDIRECTIONAL CHARACTERISATION OF HONEYBEE (*APIS MELLIFERA*) LARVAE AS A NOVEL FOOD SOURCE: NUTRITIONAL COMPOSITION, BIOACTIVITY, AND SAFETY ASSESSMENT

P 52

Eliza Matuszewska-Mach[1], Martyna Pajewska-Szmyt[1], Jacek Jachuła[2], Jan Matysiak[1]

[1]Poznan University of Medical Sciences, Poznan, Poland
[2]The National Institute of Horticultural Research, Skierniewice, Poland

Abstract

The growing demand for sustainable protein sources has intensified interest in edible insects, including honeybee (*Apis mellifera*) larvae. Although the European Commission is reviewing honeybee brood as a novel food, comprehensive larvae-specific data remain scarce, creating a critical knowledge gap for regulatory decision-making. Most existing studies analyse whole bee brood (eggs, larvae, and pupae combined) rather than larvae specifically, limiting our understanding of this developmental stage.

This study presents a multidimensional characterisation of honeybee worker and drone larvae collected separately at the final developmental stage from three geographically distinct Polish apiaries, accounting for natural product heterogeneity. Proteomic profiling using nanoLC-MALDI-TOF/TOF MS/MS identified over 100 proteins in larvae, with enzymes comprising the largest functional group, reflecting intensive metabolic activity during larval development. Additional functional classes included ribosomal proteins, regulatory proteins, binding proteins, and storage proteins. Notably, hypothetical proteins previously undescribed were identified for the first time. Importantly, several proteins with known allergenic potential were detected, including arginine kinase, glyceraldehyde-3-phosphate dehydrogenase, and major royal jelly protein isoforms, highlighting the need for thorough safety assessment.

Carbohydrate analysis via HPLC targets energy-providing sugars, including glucose, fructose, sucrose, and trehalose. Vitamin profiling using UHPLC-MS/MS focuses on B-complex vitamins, vitamin C, and fat-soluble vitamins. Additionally, amino acid, lipidomic, and multi-elemental analyses are performed, along with pesticide screening, allergenicity testing, microbiological evaluation, and bioactivity assessment via antioxidant and enzyme inhibition assays.

The results demonstrate that honeybee larvae represent a nutrient-rich food source with diverse functional compounds. However, the possible presence of allergenic proteins necessitates a comprehensive safety evaluation. This ongoing research will generate the most complete dataset on honeybee larvae composition to date, supporting EU novel food authorisation while advancing sustainable nutrition strategies and honeybee conservation efforts.

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P 53 EFFECT OF HONEYBEE CROSS - POLLINATION ON QUANTITATIVE AND QUALITATIVE TRAITS OF ORANGE FRUITS

Chrysanthi Mimikou, Chrysoula Kontogianni, Melina Georgitsi, Romina Karyda, Petros Roussos, Antonios Tsagkarakis

Agricultural University of Athens, Athens, Greece

Abstract

The honeybee, *Apis mellifera* L. (Hymenoptera: Apidae), is the most important pollinator, contributing to the fertilization of more than 300 plant species, both wild and cultivated. Given its key role in agricultural production, this study investigated the effect of honeybee colony placement in an orange orchard, focusing on both quantitative and qualitative fruit characteristics. The experiment was conducted in orange orchards Argos and Athens, Greece, where beehives were installed. Three treatments were applied: (1) open pollination, (2) flowers covered with tulle bags impermeable to bees, and (3) flowers covered with paper bags. After flowering, all coverings were removed. At fruit maturity, fruits were harvested and evaluated for weight and diameter. The results showed that fruits produced in the orchard with honeybee presence had higher weight and larger diameter compared to those from the control orchard. Differences were also observed in qualitative fruit characteristics. In addition, reduced fruit set was recorded in covered flowers, particularly in those enclosed in paper bags. In conclusion, the placement of honeybee colonies in citrus orchards enhances pollination efficiency and improves both yield and fruit quality, highlighting the crucial role of honeybees in modern sustainable agriculture.

MULTIPLEX PCR DETECTION OF *VAIRIMORPHA* (= *NOSEMA*) SPP. IN HONEY: COMPARISON OF COMMERCIAL AND BEEKEEPER SAMPLES

P 54

Jana Morochovičová[1], Imrich Szabó[1], Ivona Kožárová[2], Monika Sučik[1]

[1]Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia
[2]Department of Food Hygiene and Technology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

Abstract

Microsporidia of the genus *Vairimorpha* (= *Nosema*) are important pathogens of *Apis mellifera*, contributing to reduced colony health and productivity. Honey-derived environmental DNA (eDNA) provides a non-invasive source for pathogen surveillance, although DNA degradation during honey processing may limit diagnostic sensitivity.

This study evaluated the applicability of multiplex PCR for detecting *Vairimorpha* spp. in honey. A total of 51 samples were analysed, including 20 commercially processed honeys and 31 raw honeys supplied by beekeepers in Slovakia. DNA was extracted using a modified CTAB protocol optimised to reduce PCR inhibition. Species-specific multiplex PCR targeting *V. apis* (321 bp) and *V. ceranae* (218 bp) was performed, and amplicons were visualised by agarose gel electrophoresis.

No *Vairimorpha* DNA was detected in commercial honey samples, whereas 4 of the 31 beekeeper-derived samples tested positive. These findings suggest that technological processing substantially reduces detectable eDNA, thereby limiting the sensitivity of pathogen detection. Additional beekeeper samples are currently under analysis.

Honey-derived eDNA appears suitable for qualitative detection of *Vairimorpha* spp., particularly in minimally processed samples. Multiplex PCR represents a practical and efficient screening tool for monitoring nosemosis in honey bee populations.

Keywords

Vairimorpha (= *Nosema*), honey, eDNA, multiplex PCR, *Apis mellifera*, nosemosis.

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A PRELIMINARY ASSESSMENT OF ECONOMIC LOSSES, COSTS AND FEASIBILITY OF DIFFERENT POST WINTER HONEY BEE COLONY REPLENISHMENT PRACTICES

Tome Nestorovski[1], Aleksandar Cvetkovikj[1], Blagica Sekovska[1], Aleksandar Uzunov[2,3,4], Lazo Dimitrov[5]

[1]Faculty of Veterinary Medicine – Skopje, Ss. Cyril and Methodius University in Skopje, Skopje, North Macedonia
[2]Faculty of Agricultural Sciences and Food – Skopje, Ss. Cyril and Methodius University in Skopje, Skopje, North Macedonia
[3]Macedonian Academy of Sciences and Arts, Research Centre for Environment and Materials, Skopje, North Macedonia
[4]Chinese Academy of Agricultural Sciences, Institute of Apicultural Research, State Key Laboratory of Resource Insects, Beijing, China
[5]Department of Agricultural Business, Economy and Rural Development, Institute of Agriculture, University Ss. Cyril and Methodius in Skopje, Skopje, North Macedonia

Abstract

Overwintering is the primary physiological bottleneck for *Apis mellifera* colonies in temperate climates, with winter mortality, among other factors, largely driven by the parasitic mite *Varroa destructor* and its associated viral complexes. Across Europe and North America, increasingly high winter loss rates have been documented over the past two decades. N. Macedonia, meanwhile, has seen a sustained expansion of its beekeeping sector over the same period, with the number of registered colonies growing rapidly. This study evaluates the economic impact and feasibility of various colony replenishment practices following the 2024/2025 winter season. Using an adapted COLOSS questionnaire supplemented with cost-analysis components, we surveyed 112 beekeepers managing 10,080 colonies nationwide. The recorded colony mortality rate from the surveyed beekeepers was 10.3% (1,039 colonies), out of which 4.8% were replenished by purchasing new colonies and 3.1% by nuclei, while the vast majority (80.6%) were replenished through colony splitting of their own colonies. The economic losses are 251.2 EUR/colony, accounting for colony value and unrealized honey production. The total national economic losses are estimated over 7 million EUR. This is more than twice than total national subsidies provided to the sector. Colony splitting thus represents the dominant capital-sparing but labor-intensive practice in the Macedonian beekeeping context. Given that total losses exceed state subsidies by a factor of two, these data underscore the need for more targeted support mechanisms aligned with actual sector vulnerability. These preliminary results represent a baseline assessment of the losses by productive colonies, excluding the replenishment practices as well as weak and non-productive ones from the initial cost calculations.

AI-BASED AUTOMATED POLLEN ANALYSIS AND QR-BASED TRANSPARENCY FOR HONEY ORIGIN SCREENING (BOTANICAL/GEOGRAPHICAL)

P 56

Iratxe Perales, Naoufal Amrani, Asma Ghorab

MICROFY SYSTEMS SL, Barcelona, Spain

Abstract

Ensuring transparency regarding the origin of honey is increasingly important for beekeeping industry, consumer trust and EU regulatory compliance. Melissopalynology is one of the main methods used to characterize botanical and geographical origin; however, its manual nature, high cost, and reliance on specialized expertise limit its routine application.

HoneyAI is an affordable automated microscopy system combined with artificial intelligence designed to perform on-site pollen analysis and related honey origin screenings. Funded by European TITAN project, HoneyAI was further developed and validated as a screening tool to support the assessment of declared botanical and geographical origin in the label. Importantly, HoneyAI does not claim fraud detection, but rather alerts users when a sample presents suspicious or inconsistent results with the declared geographical/botanical origins, enabling follow-up actions within expert laboratories with sophisticated (and expensive) techniques.

During the project, region-specific AI models were developed using representative pollen datasets to improve screening precision. The system was evaluated for its ability to identify deviations in botanical/geographical declaration, and also to distinguish between naturally occurring pollen in honey and exogenous pollen potentially added as fraudulent practices. HoneyAI also detects and quantifies yeasts and starch, indicators often associated with non-compliance beehives feeding using syrups, which may negatively affect honey quality with residues.

A key innovation is the integration of a QR-code feature linked to each analyzed batch. The QR provides consumers with easy-to-understand data-based information on botanical and geographical origins, while also explaining what pollen is, and its importance, the characteristics of monofloral honeys, and the importance of supporting national honey consumption.

This QR application is already in commercial use in Spain. By connecting automated AI-based honey analysis with consumer-facing transparency, HoneyAI offers an affordable scalable tool to support producer responsibility, consumer education, and alignment with upcoming EU regulatory frameworks, reinforcing transparency in honey labeling.

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MULTILOCUS DNA METABARCODING OF POLLEN MOCK MIXTURES TO IMPROVE BOTANICAL AUTHENTICATION OF HONEY

Andreia Quaresma[1], Monica Honrado[1,2], Maria Alice Pinto[1], Joana S. Amaral[1]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal
[2]LAQV-REQUIMTE & Department of Chemistry, University of Aveiro, Aveiro, Portugal

Abstract

Honey is a valuable food product, and its botanical origin plays a key role in determining its flavour and colour. Due to high market value and consumer preference for monofloral honeys, mislabelling of botanical origin has become a widespread form of adulteration. Traditionally, the floral source of honey has been verified by microscopy-based melissopalynology, but this method is time-consuming, labour-intensive, and requires specialised expertise, while often providing limited taxonomic resolution. DNA metabarcoding has recently emerged as a promising alternative for assessing the botanical authenticity of honey, although reliable estimation of pollen species proportions remains unclear. In this study, four barcodes were tested, namely two chloroplast rbcL regions (~200bp and ~350bp), trnL (~150bp), and the nuclear ITS2 (~350bp), with the aim of identifying the barcode combination that provides the highest resolution for botanical identification. The performance of these barcodes was assessed using pollen mock mixtures composed of five and 13 commercial pollen species. ITS2 showed the best overall performance, identifying all species in the mock mixtures except *Zea mays*. In contrast, the longer rbcL amplicon and trnL successfully detected *Z. mays*, improving the botanical resolution of the samples. However, the estimated pollen percentages were not consistent with the DNA metabarcoding read proportions, and *Quercus* species was clearly overestimated by all markers except ITS2. These results show that combining another barcode with ITS2 can improve botanical identification in honey, although challenges remain in the detection of low-abundance species. Future work will focus on increasing sequencing depth to improve detection and quantification of minor taxa in honey matrices.

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HONEY CONTAMINATION BY ANTIBIOTICS AND MYCOTOXINS: STATE OF THE ART AND PRELIMINARY RESULTS USING A SINGLE EXTRACTION AND DETECTION METHOD

P 58

Helena Rodrigues[1,2], Marta Leite[1,2], Maria Beatriz Prior Pinto Oliveira[2], Andreia Freitas[1,2]

[1]National Institute for Agricultural and Veterinary Research (INIAV), Vila do Conde, Portugal
[2]LAQV-REQUIMTE, Faculty of Pharmacy, University of Porto, Porto, Portugal

Abstract

Honey is a valued natural food, appreciated not only for its nutritional properties but also for its bioactive compound content. Despite this, the occurrence of antibiotics and mycotoxins may raise apprehension regarding their safety. Furthermore, the lack of established maximum residue levels for honey and other bee products highlights the need for developing robust and broadly applicable methodologies for monitoring these chemical contaminant classes.

This work aimed, first, to provide an overview of the available literature on the analytical determination of antibiotics and mycotoxins in honey, and second, to perform a preliminary assessment of the applicability of a single experimental approach for their simultaneous extraction and detection.

The systematic review data demonstrated that antibiotic residues in honey are widely documented, with sulfonamides being the most studied compounds, followed by quinolones, tetracyclines, macrolides, aminoglycosides, and prohibited compounds such as chloramphenicol. Although mycotoxins have been less extensively studied, they have increasingly been reported in honey in recent years, particularly aflatoxins, deoxynivalenol, zearalenone, ochratoxin A, and T-2/HT-2 toxins. After an initial extraction step, liquid and gas chromatography remain the main separation techniques, with mass spectrometry becoming the dominant detection method in recent years.

Based on these findings, several target compounds were selected for analysis, including the antibiotic classes β-lactams, lincosamides, macrolides, quinolones, sulfonamides, tetracyclines, and diaminopyrimidines, as well as regulated and emerging mycotoxins, namely aflatoxins B1, B2, G1, G2, and M1; beauvericin; citrinin; deoxynivalenol; fumonisins; HT-2 and T-2 toxins; penicillic and tenuazonic acids; ochratoxin A; and zearalenone. A single QuEChERS extraction approach, followed by UHPLC-TOF-MS analysis, demonstrated applicability for the determination of both contaminant groups.

Honey quality, along with consumer safety, may be threatened by the presence of both contaminant classes. Their possible co-occurrence highlights the need for improved monitoring and preventive strategies.

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POLLEN DIVERSITY AND NUTRITIONAL QUALITY IN APIARIES LOCATED IN EUCALYPTUS FOREST ECOSYSTEMS OF NW SPAIN

María Carmen Seijo Coello, Sonia Harbane, Manuela Chouza-Carou, Yasmine Saker, Olga Escuredo, María Shantal Rodríguez-Flores, Ana Diéguez-Antón

Universidade de Vigo, Ourense, Spain

Abstract

Plant pollen grains are essential for honey bee nutrition, and previous research has shown that the temporal overlap and diversity of flowering periods enhance dietary quality. However, pollen diversity varies across ecosystems depending on the composition of plant species within different vegetation strata. This study aims to identify pollen resources in eucalyptus-dominated forest areas of the northwestern Iberian Peninsula and to assess their contribution to honey bee nutrition. Bee pollen samples were collected from three apiaries between March and June 2025, with biweekly sampling. Analyses focused on determining botanical origin and nutritional composition, particularly amino acid and fatty acid profiles, were performed. Pollen collection showed an irregular pattern, with a marked decline from June onwards. This reduction coincided with increased pressure from the Asian hornet (*Vespa velutina*), which negatively affected foraging activity and the decrease of flowering periods. The principal taxa identified included *Cytisus*, *Echium*, *Taraxacum officinale*, *Papaver*, *Plantago*, *Castanea*, *Simethis planifolia*, and *Trifolium*, along with shrub species such as *Erica* and *Rubus*. Mean values for water, sugar, and protein content were 16%, 36%, and 21%, respectively. The maximum total amino acid content reached 152.5 mg/g, with proline as the dominant amino acid (mean: 25 mg/g), followed by asparagine, arginine, lysine, glutamic acid, alanine, and leucine + isoleucine. Among fatty acids, β-linolenic acid predominated, followed by palmitic, β-linoleic, oleic, and myristic acids. Significant relationships were identified between nutritional composition and botanical origin, highlighting the relevance of plant diversity for honey bee nutrition.

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MOLECULAR AND MICROBIOLOGICAL APPROACHES TO HIVE HEALTH AND BEE PRODUCTS

P 60

Živilė Strazdaitė-Žielienė[1], Urtė Busilaitė[1,2], Elena Servienė[1]

[1]State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania
[2]Life Sciences Center of Vilnius University, Vilnius, Lithuania

Abstract

Beekeeping environments are prone to microbial imbalance and infection as honeybee-associated microorganisms influence hive health and the biological composition of bee products. Microbial, plant, and insect DNA may serve as markers for assessing the quality and authenticity of honey. Plant-derived essential oils (EOs) modulate microbial growth, and their effects depend on the type, concentration, and target organism. The aim of this study was to identify honeycomb-associated microorganisms and assess the effects of essential oil (EO) vapours on their diversity, as well as explore honey quality using microbial, plant, and insect molecular markers.

Cultivable bacteria (*Pantoea*, *Staphylococcus*, *Enterobacter*, *Bacillus*, *Pseudomonas*, and *Pristia*) and yeasts (*Starmerella*, *Rhodotorula*, *Aureobasidium*, *Debaryomyces*, and *Zygosaccharomyces*) were identified in honeycombs using molecular methods. To evaluate the impact of mandarin EO on microbial diversity, EO was applied by diffusion in hives for one month, and honeycomb samples were collected. Following EO exposure, microbial communities were altered, with an increase in Gram-negative bacteria (*Klebsiella*, *Pseudomonas*, *Serratia*, and *Enterobacter*) and a reduction in yeast diversity, with only *Aureobasidium* and *Cystobasidium* detected. The presence of microbial, plant, and insect DNA was evaluated in honey samples from retail and beekeepers' sources. While bacterial and plant DNA were detected in all samples tested, yeast and insect markers were consistently present in natural honey but absent or reduced in many commercial samples. Authentic honey contained multiple plant DNA fragments at higher concentrations, whereas several commercial samples showed only a single, low-abundance fragment, indicating possible dilution. Overall, honeycomb microbial profiling, along with the application of plant secondary metabolites in beekeeping, offers valuable insights into the management of hive health. The bacterial and yeast genetic signatures, along with other biological markers, expand the possibilities for assessing the quality of bee products.

This study was funded by the Research Council of Lithuania (project no. S-MIP-24-55).

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COFFEE BREAKS ON A BALCONY: SEASON-LONG POLLINATOR DYNAMICS

Viktor Szigeti

Lendület Ecosystem Services Research Group, HUN-REN Centre for Ecological Research, Vácrátót, Hungary

Abstract

Pollinators are widely acknowledged for their outstanding ecological, cultural, and economic value. Beyond their regulatory and provisioning ecosystem services, pollinators hold largely untapped potential for citizen-science engagement, education, and recreation. Flowers and pollinators are relatively easy to observe, offering accessible nature experiences. Such outdoor activities may also contribute to reducing the risk of depression and anxiety. Despite these benefits, the recreational and aesthetic dimensions remain surprisingly underestimated compared with other ecosystem services. Only a limited well-developed examples and accessible opportunities are available that demonstrate how engaging pollinator observation can be, particularly in revealing exciting fine-scale temporal patterns that can be documented by almost anyone interested in pollinators.

Here, I aimed to inspire citizen-oriented approaches in pollinator ecology by simple observations. Thinking beyond conventional sampling methods, I conducted a full-season, fine-scale sampling on a single urban balcony in a mid-sized Eastern European city. I recorded the day-to-day pollinator activity during coffee breaks in home-office or on weekends, regardless of weather conditions. From March to October, across 124 sampled days, I completed 505 five-minute surveys between 06:41 and 20:04, from 1.4 to 36.9 °C. In total, 4,219 pollinators were recorded: 25% hoverflies, 20% cavity-nesting bees, 19% other solitary bees, 19% wasps, 13% other Diptera, 3% honeybees, <1% bumblebees and Lepidoptera. Dataset reveals clear seasonal turnover among pollinator groups and a pronounced daily rhythm, highlighting substantial temporal dynamics even at the scale of a single balcony.

This balcony-scale example demonstrates that exciting, analyzable citizen-science datasets can emerge even from very small urban spaces. When coupled with simple environmental variables (e.g., flowering phenology, vegetation structure, and local weather conditions), such efforts could be scaled up into meaningful monitoring systems, while simultaneously providing restorative offline experiences for a broader audience.

FROM PRACTICE TO PRINCIPLES: GOOD PRACTICES FOR HONEY BEE VENOM PRODUCTION ENSURING QUALITY, STANDARDIZATION AND BEE WELFARE

P 62

Ekin Varol, Banu Yücel

Ege University, İzmir, Turkey

Abstract

Honey bee venom is a valuable apicultural product that is widely used in apitherapy, cosmetics, and pharmaceutical research due to its bioactive components including melittin, apamin, and phospholipase A_2. Increasing global demand for bee venom has led to the expansion of venom harvesting practices. However, the lack of standardized production protocols and the use of inappropriate collection methods may negatively affect both venom quality and honey bee welfare. While bee venom is a highly valuable product, its production is even more critical for ensuring both product quality and colony well-being. In recent years, there has been growing interest in developing good production practices that enable efficient venom harvesting while minimizing stress and potential harm to honey bee colonies. Bee venom is typically collected using electrical stimulation devices that allow venom extraction without killing the bees. Nevertheless, several factors can significantly influence both venom yield and quality, including electrical stimulation parameters, harvesting frequency, colony strength, seasonal conditions, environmental factors, and colony management practices. Quality-oriented and welfare-friendly harvesting methods are therefore essential for sustainable honey bee venom production. It is important to standardize venom collection procedures, optimizing harvesting conditions, and reducing colony stress during venom extraction. Implementing well-defined production practices can improve venom quality and consistency while supporting sustainable beekeeping and the protection of honey bee health. The development of internationally accepted guidelines for honey bee venom production is therefore important for both scientific research and commercial applications to ensure the long-term sustainability of this valuable apicultural product.

Climate change

ADAPTATION AND RESILIENCE OF LOCAL HONEY BEE SUBSPECIES IN ALGERIA

P 63

Noureddine Adjlane, Soumeya Kaouche

Department of Agronomy, University of Boumerdes, Boumerdes, Algeria

Abstract

Local honey bee subspecies in Algeria, particularly *Apis mellifera intermissa* and *Apis mellifera sahariensis*, display remarkable adaptations to diverse environmental conditions, from the Mediterranean north to arid and Saharan south. They provide a valuable genetic resource for studying resilience to environmental stress and climate change.

During 2024 and 2025, a field-based study was conducted across multiple agro-ecological zones to assess the adaptive traits and survival of these subspecies. Observations in selected apiaries included colony performance, behavioral traits, and health status. Key parameters measured were hygienic behavior (removal of diseased brood), grooming activity against parasites, colony strength, and seasonal survival. Laboratory analyses evaluated infestation by *Varroa destructor* and the presence of common pathogens.

Results showed that both subspecies possess distinct adaptive traits. *Apis mellifera intermissa* exhibited strong hygienic behavior and moderate resistance to brood diseases, while *Apis mellifera sahariensis* tolerated extreme temperatures and drought, maintaining activity under limited resources. Grooming behavior also contributed to reducing *Varroa destructor* infestations.

Despite these advantages, local populations are increasingly threatened by uncontrolled importation of non-native bees, habitat degradation, climate change, and pathogen spread, which may lead to genetic erosion and reduced resilience.

The conservation and valorization of these subspecies, combined with selective breeding programs based on local adaptive traits, represent a key strategy for sustainable and climate-resilient apiculture in arid and semi-arid regions. Promoting these measures is essential to safeguard Algeria's unique bee biodiversity and maintain productive and resilient colonies under changing environmental conditions.

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CLIMATE IMPACTS OF HONEY PRODUCTION ACROSS EUROPE AND BEYOND: INSIGHTS FROM LIFE CYCLE ASSESSMENT

Louise Bartek[1], Annely Brandt[2], Robert Brodschneider[3], Giles E. Budge[4], Raffaele Dall'Olio[5], Mattias Eriksson[6], Marin Kovačić[7], Victoria Soroker[8], Magdalena Wilde[9], Cecilia Costa[10]

[1]KTH Royal Institute of Technology, Stockholm, Sweden
[2]LLH-Bee Institute, Kirchhain, Germany
[3]University of Graz, Graz, Austria
[4]Newcastle University, Newcastle upon Tyne, United Kingdom
[5]BeeSources, Bologna, Italy
[6]Swedish University of Agricultural Sciences, Uppsala, Sweden
[7]J.J.Strossmayer University of Osijek, Osijek, Croatia
[8]Agricultural Research Organization of Israel, Rishon LeZion, Israel
[9]Pasieka Magdalena Wilde, Olsztyn, Poland
[10]CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Honey bees are essential to agricultural production and ecosystem functioning, yet the environmental impacts associated with beekeeping practices operating under different geographical conditions remain poorly quantified. This pilot study, as part of BEE-GUARDS project, evaluates the climate impact of honey production using life cycle assessment (LCA) across three climatic regions, including northern (cold humid), central (warm humid), and southern (warm dry) conditions. The system boundary considers all stages from hive construction to supplementary feeding, management, and packaging. To account for the multifunctional nature of beekeeping, economic allocation was applied to distribute environmental impacts between honey production and pollination services.

Results show substantial regional variation in climate impact. The northern region exhibited the highest impact per kg of honey (2.6 kg CO_2eq), more than twice the impact observed in the southern region (1.0 kg CO_2eq), while the central region showed intermediate values (1.4 kg CO_2eq). These differences were primarily driven by variations in hive productivity, the need for supplementary feeding, packaging materials, and transport distances. Sensitivity analysis further indicated that management practices can considerably influence the climate impact of honey bee production, with choices related to input use, resource management, packaging systems, and feeding strategies representing important leverage points for reducing emissions.

Overall, the results show that the climate impact of honey production is strongly shaped by both regional conditions and management practices. The findings highlight clear opportunities to improve the environmental performance of beekeeping through targeted management strategies and regionally adapted beekeeping practices. By providing one of the first comparative LCA assessments of honey production across different climatic regions, this study contributes to the growing evidence base needed to support more sustainable and resilient beekeeping systems in Europe and beyond.

ENHANCING HONEY BEE COLONY PERFORMANCE THROUGH HIVE THERMODYNAMIC OPTIMIZATION: PRACTICAL MODIFICATIONS TESTED WITHIN THE BETTER-B PROJECT

P 65

Nuno Capela[1], Anna Dupleix-Marchal[2,3], Emmanuel Ruffio[3], Anne Lavalette[2,3], Dirk C. de Graaf[4], José P. Sousa[1]

[1]Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[2]CRISES, EA 4424, Université Paul-Valéry, Montpellier, France
[3]AltRD, Co-actions Company, Begles, France
[4]Laboratory of Molecular Entomology and Bee Pathology (LMEB), Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium

Abstract

Honey bee colonies depend on stable in-hive temperature conditions to support brood development, metabolic efficiency, and overall colony resilience. However, increasing climatic variability challenges the bees' capacity to maintain optimal thermoregulation, often resulting in elevated energetic costs and reduced colony performance. Within the framework of the BETTER-B project, which aims to strengthen honey bee health, resilience, and sustainability across European beekeeping systems, we investigated whether simple, low-cost hive modifications can improve hive thermodynamics and support colony development.

In this experiment, reflective insulation materials were strategically incorporated into different hive components (i.e., inner cover, hive walls, and a dividing frame) to assess their impact on internal temperature stability and colony performance metrics. These modifications were selected to ensure compatibility with existing hive infrastructures, enabling beekeepers to adopt them without replacing equipment or altering management practices.

Preliminary results indicate that colonies housed in modified hives exhibited earlier brood expansion, and improved early-season development compared to control hives. These findings suggest that targeted thermal adjustments can enhance colony efficiency and may contribute to increased resilience under environmental stress.

These simple colony adaptations have a low-cost implementation, are compatible with common hive types, reduce colony energetic burden, and the improvements in brood viability lead to an increase in overall colony strength and field productivity. By focusing on simple hive modifications, this work contributes to scalable solutions that may help mitigate climate-related stressors and support more resilient European honey bee populations.

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INTEGRATING TRANSCRIPTOMICS AND TARGETED GENE EXPRESSION TO INVESTIGATE THERMAL STRESS IN MEDITERRANEAN *APIS MELLIFERA* SUBSPECIES

Giovanni Cilia[1], Soledad Sagastume[2], Fernando Doblado[2], Banan Al-Shagour[3], Marion Zammit Mangion[4], Mustafa Necati Muz[5], Dilek Muz[5], Mariano Higes[2], Antonio Nanetti[1], Raquel Martín-Hernández[2]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]Centro de Investigación apícola y agroambiental (CIAPA), Marchamalo, Spain
[3]Bee Research Directorate, National Agricultural Research Center (NARC), Amman, Jordan
[4]Department of Physiology and Biochemistry, University of Malta, Msida, Malta
[5]UNK- University of Namik Kemal, Faculty of Veterinary Medicine, Tekirdag, Turkey

Abstract

Climate change poses a threat to the welfare of *Apis mellifera*, exacerbating nutritional stress, increasing pathogen susceptibility and altering bee physiology. One of the most concerning abiotic stressors associated with climate change is rising temperatures, which induce heat stress and trigger molecular responses, including changes in gene expression and enzyme activity. The project MEDIBEES aims to investigate the impact of climate change on different *A. mellifera* subspecies across the Mediterranean region. In this study, the effects of temperature and relative humidity (RH) on survival and adaptability were evaluated in five subspecies: *A. m. ligustica* (Italy), *A. m. iberiensis* (Spain), *A. m. syriaca* (Jordan), *A. m. ruttneri* (Malta), and *A. m. anatoliaca* (Turkey). Newly emerged workers were used in survival assays under six environmental conditions: 35°C, 40°C, 45°C at 75% RH, and 35°C at 15%, 30%, 50% RH. Bees were sampled after 2 hours and 2 days to evaluate acute and chronic stress. In parallel, dehydration capacity was assessed by measuring body water loss (BWL) over 2 hours under nine combinations of temperature (35°C, 40°C, 45°C) and RH (10%, 25%, 50%). Overall, twenty-one samples were obtained for each subspecies (12 from survival trials and 9 from BWL assays). For each sample, pools of nine bees were used for total RNA extraction. Whole transcriptome analysis using RNA-seq enabled the identification of genes associated with thermal stress responses. On the same RNA samples, gene expression was further quantified by targeted qPCR assays evaluating 31 stress-related and 4 housekeeping genes. These results provide valuable insights into the molecular mechanisms underlying thermal stress resilience in Mediterranean honey bee subspecies and contribute to identifying potential biomarkers relevant for conservation strategies and sustainable beekeeping under climate change.
This research is part of the 2011-MEDIBEES project under the EU-supported PRIMA programme.

DAILY CHANGES IN HIVE WEIGHT AGAINST THE MEAN DAILY AIR TEMPERATURE

P 67

Krystyna Czekońska, Sylwia Łopuch

University of Agriculture in Kraków, Kraków, Poland

Abstract

Among meteorological variables, air temperature strongly affects plant growth and the behaviour of honey bees related to the collection of food in the nest. In the context of progressing climate changes, rising temperatures may affect the availability and quantity of collected food. Therefore, the study aimed to analyse daily changes in hive weight against the mean daily air temperature. Hive weight and external air temperature data were obtained from 53 honey bee colonies placed on electronic hive scales distributed in predominantly forested habitats across the country. Data were collected in seasons in 2018-2019 and 2022-2024.

It was found that 2018 and 2024 were the warmest, while 2022 was the coolest. In the two warmest seasons, the mean daily air temperature ranged from 15 to 21°C, and in the others, from 12 to 21°C. In four seasons, the increases in hive weight ranged from 0.14 to 0.19 kg/day, while in 2019 they were 0.06 kg/day. An increase in daily hive weight of over 0.1 kg/day was observed at the mean air temperature exceeding 12°C, more frequently in April, May and June than July, August and September, when hive weight losses predominated.

Daily changes in hive weight are strongly dependent on the mean daily air temperature. In a temperate climate, the honey bees store food mainly in spring, when it is abundant in the environment. In summer, when the mean daily temperatures are higher, the quantities of food stored in the nest are insufficient to meet the colonies' needs. The results indicate the need to introduce plant species that produce nectar at higher temperatures and tolerate drought into the environment.

P 68 INFLUENCE OF LANDSCAPE NUTRITION QUALITY ON HONEY BEE IMMUNITY

Gennaro Di Prisco[1], Brandt Annely[2], Marin Kovačić[3], Melanie Parejo[4], Iratxe Zarraonaindia[4], Lena Ladewig[2], Josipa Štavalj[3], Aitor Albaina Vivanco[5], Jorge Eliseo Langa Arranz[6], Viviana Valenzano[7,8], Roberta Santopietro[7], Flavia V. Chianese[7], Cecilia Costa[9]

[1]CNR-IPSP, The Italian National Research Council Institute for Sustainable Plant Protection, Portici, Napoli, Italy
[2]LLH-Bee Institute Kirchhain, Erlenstr, Kirchhain, Germany
[3]University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia
[4]Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country (UPV/EHU), Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country (UPV/EHU), Spain
[5]Department of Zoology and Animal Cell Biology, University of the Basque Country (UPV/EHU), Leioa, Bilbao, Spain
[6]Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country (UPV/EHU), Leioa, Bilbao, Spain
[7]NR-IPSP, The Italian National Research Council Institute for Sustainable Plant Protection, Portici, Napoli, Italy
[8]Department of Biology, University of Napoli Federico II, Napoli, Italy
[9]CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Honey bee immunity is a complex system shaped by a wide range of responses to both biotic and abiotic environmental stressors. Immune mechanisms operate across several levels of organization, from the colony, where social immunity plays a critical role in protecting against external threats, to the individual bee. At the individual level, immunity is expressed by cellular, humoral, and molecular responses, all of which are integral to the innate immune system's ability to defend the organism against pathogens and environmental stressors. A suggested relationship exists between immune function and the colony's nutritional state, where the parasitic *Varroa destructor* mites and associated viruses disrupt this balance by suppressing individual immune responses and facilitating pathogen transmission. These interactions may be further exacerbated by climate change and the presence of xenobiotics, particularly pesticides and chemicals used in beekeeping management. This study, part of the BEE-GUARDS project, investigates honey bee immune functions, with a particular focus on how different nutritional values of the landscape affect both individual and colony-level immune responses, pathogen resilience and the holobiont. A vegetation analysis is performed to characterize the environment within the foraging range of honey bees, providing further insights into the surrounding landscape and its potential influence on colony health. In addition, artificial intelligence approaches are incorporated to improve understanding of these complex dynamics.

HOW DOES AN INDUCED SUMMER BROOD INTERRUPTION AFFECT THE LIFESPAN AND HEALTH OF WINTER BEES IN *APIS MELLIFERA*?

P 69

Lena Frank[1], Sandra Barth[2], Benjamin Lamp[2], Annely Brandt[1]

[1]LLH - Bee Institute Kirchhain, Kirchhain, Germany
[2]Institute of Virology, Justus-Liebig-Universität Gießen, Gießen, Germany

Abstract

Beekeeping faces increasing challenges due to climate change. Particularly higher temperatures in summer compromise the effectiveness of established *Varroa destructor* management practices. In detail, established treatments for controlling the parasitic mite *V. destructor* are increasingly questioned, as commonly used treatments - such as formic acid evaporation - can be harmful to bees at high temperatures or lose effectiveness under cold and wet conditions. Developing resilient and sustainable beekeeping practices therefore require *V. destructor* management strategies that are effective and save for bees under variable environmental conditions.

Induced brood interruption achieved through temporary queen caging in summer for 25 days followed by an oxalic acid treatment is an effective and temperature-independent treatment against *V. destructor*. Here, we investigate how an induced summer brood interruption affects the lifespan and health of worker bees emerging after the broodless period (n = 6 colonies with caged queens, 6 control colonies).

In order to examine whether workers produced in the period from July to October contribute to the formation of the winter bee cohort, we regularly introduced age-defined marked workers into the colonies and observed their individual lifespan. In addition, we examined their viral pathogen loads and susceptibility through experimental infections with the Deformed Wing Virus (DWV) under controlled laboratory conditions. Moreover, colony development was monitored through population estimations based on the Liebefeld method every 21 days in order to calculate the average life expectancy of worker bees.

By linking *V. destructor* management practices with physiological and health parameters, this study contributes to the development of sustainable, climate-adapted management strategies for resilient beekeeping.

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COMPARATIVE STUDY OF POLLINATING INSECT PRESENCE IN TWO COTTON SPECIES (*GOSSYPIUM HIRSUTUM* AND *G. BARBADENSE*) UNDER NORMAL AND REDUCED IRRIGATION

Katerina Karinou[1], Evaggelia Arapostathi[1], Stavroula Kallergi[1], Ioanna Kakabouki[1], Dimitrios Bilalis[1], Myrto Barda[2], Antonios Tsagkarakis[1]

[1]Agricultural University of Athens, Athens, Greece
[2]Benaki Phytopathological Institute, Kifissia, Greece

Abstract

Cotton is a key crop in Greece, covering approximately 250,000 hectares and contributing around 80% of European cotton production. *Gossypium hirsutum* is the dominant species, followed by *G. barbadense*, which is valued for its high-quality fibers. Climate crisis is already negatively affecting crop productivity, pollinator populations, and biodiversity. Given that cotton is a highly irrigation-demanding crop and that Greece is increasingly facing drought conditions, it is important to assess how water availability may influence pollinator communities. This study examined pollinator diversity and abundance in *G. hirsutum* and *G. barbadense*, under two irrigation regimes (normal and reduced). The experimental design followed a two-factorial scheme, with three replicate plots for each combination of cotton species and irrigation treatment. Pollinator data were collected during blossom using both optical observations and pan traps. In each plot, one set of pan traps (blue, white, and yellow) was deployed at dawn remained on-site until the afternoon. Additionally, two (morning and afternoon) 10-minute observation sessions were conducted per plot, to record insect visits to floral and extrafloral nectaries. Collected specimens were identified in the laboratory using taxonomic keys. Statistical analyses were performed as GLMM ($\alpha < 0.05$). Results revealed a significant preference of pollinators for *G. barbadense* compared with *G. hirsutum*, consistently observed across both sampling methods. In contrast, no significant differences were detected in overall pollinator abundance between normal and reduced irrigation treatments. The pollinator community included key taxa such as *Apis mellifera* (Hymenoptera: Apidae), *Xylocopa pubescens* (Hymenoptera: Apidae), *Lasioglossum* spp. (Hymenoptera: Halictidae), *Pemphredonidae* spp. (Hymenoptera), and *Ischiodon* spp. (Diptera: Syrphidae). The results highlight the role of cotton species in shaping pollinator visitation patterns. At the same time, pollinator activity remained relatively unaffected by reduced water availability, suggesting a certain level of resilience. This is encouraging for the management of cotton systems under growing climatic stress.

HOW TO INSULATE A DADANT HIVE FROM EXTREME WEATHER CONDITIONS

P 71

Anne Lavalette[1], Anna Dupleix-Marchal[1,2], Emmanuel Ruffio[1]

[1]Co-actions, Alt-RD, Bègles, France
[2]CRISES, Montpellier, France

Abstract

The temperature in the hives is a factor influencing bees' behavior, health, and the beekeeper's productivity. The hive temperatures result from a thermal balance between the colony activity, the hive environment, and the weather variations. Beehives receive energy via (1) outside air convection, (2) solar radiation, and (3) infrared radiation with surrounding objects. Depending on the season, weather conditions may be strong enough to alter the colony activities, the honey quality, and could even soften the wax, leading to a collapse of the honeycombs.

To help beekeepers mitigate the effect of weather conditions, this study investigates the effect of various Dadant hive modifications on the temperature and on the thermal response time of the hive. Among other modifications, we investigated the use of white paint/cover on the roof, insulated roof (aluminum bubble sheet, polystyrene...), insulated feeder, insulated frames, open/closed floor... Some tests are performed on inhabited hives, but to provide more reliable results, most of them are performed using two empty hives. The first one is not changed and serves as a reference. The second one undergoes the modifications mentioned above. Temperature, wind, and luminosity sensors monitor the hive temperatures and the local weather. For some tests, a controlled electric heater is installed to generate a temperature gradient, which allows us to compare the insulation efficiency of different modifications.

The results indicate that minor changes can have a significant impact on the temperatures of the hive (-27°C at peak for the white roof in summer). This knowledge should help beekeepers to identify and reduce any thermal stresses before they occur - while minimizing the number of interventions in the apiaries - and move towards more sustainable beekeeping.

This work is funded by the European Better-B project (101081444-Better-B) in the frame of European Programme HORIZON-CL6-2022-BIODIV-02-two stage on resilient beekeeping (2023-2027).

P 72 HIGH TEMPERATURE EFFECTS ON HONEYBEES LEARNING AND MEMORY

Davide Liga, Albrecht Haase, Elisa Frasnelli

CIMeC - University of Trento, Rovereto, Italy

Abstract

Climate change is increasingly exposing pollinators to extreme temperature events, with potentially severe consequences for their cognitive performance and survival. This project investigates the effects of acute thermal stress on learning and memory in honeybees (*Apis mellifera*), a species of fundamental importance for both ecosystem functioning and agriculture.

Learning and memory are assessed using the Proboscis Extension Reflex (PER) conditioning paradigm, a well-established method in which bees learn to associate an olfactory stimulus (*e.g., 2-Hexanol*) with a sucrose reward. This approach enables precise quantification of cognitive performance. To ensure high experimental control and reproducibility, a temperature- and humidity-controlled incubator was specifically adapted, and an automated system was developed to regulate odor delivery, reward administration, and subject rotation. Bees are tested in groups of twelve using a circular "revolver" apparatus, which minimizes experimenter interference. Behavioral responses are automatically classified through a machine-learning algorithm capable of detecting proboscis extension during odor presentation. The primary aim of the study is to determine whether acute exposure to elevated temperature (40 °C) during conditioning impairs learning rate. A secondary objective is to evaluate the effects of thermal stress on memory retention, assessed at 30 minutes and 12 hours post-training, corresponding to short- and long-term memory phases. Here we will present behavioral data collected during the spring and summer seasons. Future work will involve manipulating temperature at different stages of the experimental protocol and integrating behavioral assays with *in vivo* neuroimaging to examine how thermal stress affects neural responses to olfactory stimuli. Overall, this research aims to provide new insights into the cognitive vulnerability of honeybees in the context of climate change.

CLIMATIC GRADIENT SHAPE WING LENGTH VARIATION IN EUROPEAN HONEY BEES

P 73

Ana R. Lopes[1], Pedro J. Rodrigues[2], Aicha Rodriguez[1], Ellen Danneels[3], Anna Dupleix-Marchal[4], Linn F. Groeneveld[5], Robert J. Paxton[6], Marco Pietropaoli[7], Dirk C. de Graaf[3], Matthew T. Webster[8], Maria Alice Pinto[1]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal
[2]CeDRI, Instituto Politécnico de Bragança, Bragança, Portugal
[3]Ghent University, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium
[4]Laboratoire CRISES EA 4424, Université Paul Valéry, Montpellier, France
[5]Norges Birøkterlag (Norwegian Beekeepers Association), Lillestrøm, Norway
[6]Martin Luther University Halle-Wittenberg, Halle, Germany
[7]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Viterbo, Italy
[8]Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden

Abstract

Morphological traits in insects, including body size and wing length, often reflect adaptive responses to environmental gradients. In honey bees, wing length has been proposed as a trait shaped by climatic pressures, with potential relevance for understanding responses to ongoing climate change. In this context, we examined variation in wing length across a broad latitudinal gradient in Europe. A total of 1,325 colonies from 35 European countries were included in the analysis, each colony contributing five right forewings from worker bees, resulting in more than 6,000 wings. Forewings were dissected and imaged under a stereomicroscope equipped with a digital camera, and their lengths were measured automatically using a newly developed feature of the DeepWings© software. Wing length increased with latitude, with northern European populations showing higher mean values (e.g., Norway: 6.68 mm; Ireland: 6.67 mm) than southern populations (e.g., Portugal: 6.42 mm; Montenegro: 6.35 mm; Cyprus: 6.28 mm), consistent with Bergmann's rule. Larger body dimensions, including wings, may confer thermoregulatory or aerodynamic advantages in colder climates, whereas smaller wings may reflect adaptations to the energetic constraints of warmer environments. Overall, these results support the hypothesis that wing length in honey bees varies predictably with latitude, reflecting adaptive responses to climatic gradients across Europe.

P 74 UNVEILING THE CLIMATOLOGICAL INFLUENCE ON HONEY PRODUCTION IN SPAIN

María J. Ramos-Román[1], Olga Escuredo[2], Javier Pérez-Hernández[1], María Carmen Seijo Coello[2], María Shantal Rodríguez-Flores[2], Jose-Antonio López-Pérez[1], Carlos García-Villarrubia[1], Amelia V. González-Porto[1]

[1]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha, Centro de Investigación Apícola y Agroambiental – CIAPA, Marchamalo (Guadalajara), Spain
[2]Universidad de Vigo, Campus Auga, Instituto de Agroecología e Alimentación, Facultad de Ciencias, Grupo de Investigación en Sistemas Agroambientales, Ourense, Spain

Abstract

The honey production sector faces multiple challenges that may influence the long-term sustainability of beekeeping, an activity of major ecological importance. Adulteration, fraud, and misleading labelling practices continue to undermine consumer trust. Although honey has been widely studied, determining its geographical and botanical origin remains essential for verifying authenticity, supporting local production, and protecting the future of beekeeping. Environmental and climatic conditions may also influence honey production, as they influence floral resource availability and honeybee colony responses.

Understanding how spatial and climatic factors influence honey properties is therefore crucial for improving authenticity assessment and supporting informed decision-making. This study evaluates the spatial variability of honey characteristics across different Spanish regions, while accounting for interannual variability and long-term climatic context.

A total of 750 honey samples from different Spanish locations were analysed over three consecutive years. The samples were characterized using physicochemical parameters, sensory evaluation, melissopalynological analysis, enzymatic activity, volatile compound profiling, polyphenol, and flavonoid content, antioxidant and antimicrobial capacity. Climatic conditions were incorporated at two complementary scales: long-term climatic series over several decades were used to characterize the climatic background of each region and to assess their influence on honey variability, while year-specific climatic variables described the conditions during each sampling period. Climatic data were aggregated at the regional level, supporting the development of a model to estimate future variability in honey production across regions.

The results show significant differences in honey composition associated with geographical origin and climatic conditions. Parameters such as moisture content, electrical conductivity, enzyme activity, polyphenol concentration, and biological activity exhibited notable differences. Additionally, distinct regional patterns were identified, with northern and western samples differing from those of central, eastern, and southern areas, reflecting the influence of contrasting climatic conditions and landscapes across Spain.

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A THOUSAND-GENOME PANEL REVEALS CLIMATE-ADAPTIVE VARIATION OF THE WESTERN HONEY BEE IN EUROPE

P 75

Demetris Taliadoros[1], Ana R. Lopes[2], Fernanda Li[2], Giselle Martin Hernandez[3], Dora Henriques[2], Daniela Barbosa[2], Dirk C. de Graaf[4], Maria Alice Pinto[2], Matthew T. Webster[1]

[1]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden
[2]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[3]Uppsala University, Uppsala, Sweden
[4]Laboratory of Molecular Entomology and Bee Pathology, Faculty of Sciences, Ghent University, Ghent, Belgium

Abstract

Climate change is a threat to the viability of honeybee (*Apis mellifera*) populations in Europe. Here, we have analyzed a thousand-genome panel of honey bees sampled across Europe to understand how they are adapted to their local environments and to predict the impact of future climate change. Our analyses confirm the deep divergence between the M and C evolutionary lineages and reveal extensive human-mediated introgression of C-lineage bees into central and northern European bees. Despite this widespread admixture, we detected strong signatures of local adaptation to different climates in Europe, and identified genes underlying these adaptations. Specifically, we identified 74 high-confidence candidate genes in the C lineage and 35 in the M lineage linked to climatic adaptation, including genes involved in stress responses and metabolism. We also identified genomic regions where locally adapted M-lineage ancestry persists despite admixture, indicating ancestry-specific selection. Together, these results show that climatic adaptation in European honey bees is highly polygenic and that the interaction between admixture and selection shapes the adaptive potential of honey bees in Europe to ongoing climate change.

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BETTERB, BEEPLANTS.EU, A CITIZEN SCIENTIST FOOD SOURCE SHARING STUDY AMONGST POLLINATING INSECTS

Jozef van der Steen[1], Dirk C. de Graaf[2]

[1]AlveusAB consultancy, Oisterwijk, Netherlands
[2]Ghent University, Department of Biochemistry and Microbiology, Ghent, Belgium

Abstract

To address concerns about food available for pollinating insects, the beeplants.eu (app: www.beeplants.eu) baseline study is being conducted with beekeeper citizen scientists. We started in 2024. The app records the number of honey bees, bumblebees, solitary bees, hoverflies, and "other insects". The study set-up enables the collection of a large amount of data from various locations. The participants choose a plant in the bee plant catalogue in the app to make the recordings. They do the same observations at least three times a week at the same location of the same flowers/parts of trees during the entire flowering period of those plants. Location and time are recorded by default. The data were analysed for the relative presence of specific flowers in winter, spring, and summer, the impact of temperature, and combinations of insects per observation. No plant is exclusively visited by honey bees, bumblebees, hoverflies, solitary bees, or other insects. The number of insects depends on the location, temperature, number of attractive flowers, and season. The study will be continued in 2025 and 2026, in Finland, Estonia, Latvia, Sweden, Norway, Denmark, Ireland, the Netherlands, Belgium, France, Spain, Portugal, Italy, Greece, Cyprus, Poland, and Germany. Medio March 2026, 508 citizen scientists from 21 countries participated and have submitted 19932 observations.

PLANT-BUMBLEBEE NETWORKS ALONG AN ELEVATIONAL GRADIENT: EVIDENCE OF POTENTIAL EFFECTS OF CLIMATE CHANGE ON MONTANE GRASSLANDS

P 77

Virginia Zanni[1,2], Edoardo Asquini[1,2,3], Filippo M. Buian[1], Davide Frizzera[1], Simone Ghassempour[1], Silvia Parenzan[1], Elisa Seffin[1], Tatiana Trifonova[1], Giacomo Trotta[1,4], Paolo Cingano[1,2], Marco Vuerich[1,2], Pietro Zandigiacomo[1], Desiderato Annoscia[1], Francesco Nazzi[1,2], Francesco Boscutti[1,2]

[1]Department of Agricultural, Food, Environmental and Animal Sciences (Di4A), University of Udine, Udine, Italy
[2]National Biodiversity Future Center (NBFC), Palermo, Italy
[3]Department of Sciences of the Earth and the Sea, University of Palermo, Palermo, Italy
[4]Department of Life Sciences, University of Trieste, Trieste, Italy

Abstract

Climate change is dramatically altering the world's ecosystems through increasing temperatures and escalation of extreme weather events. Under climate change pressure, fundamental plant-animal interactions may be affected generating unpredictable consequences associated with range shifts and phenological mismatches altering the timing of key life-history events. To evaluate changes in biodiversity patterns and pollination networks structure is therefore essential to predict future dynamics and guide conservation attempts. The biotic responses to climate changes can be predicted based on the results of "space-for-time" studies that consider elevation gradients as natural analogues of climate variation. In fact, elevation gradients impose strong biotic filtering on both plants and pollinators representing the main drivers in species turnover and shaping plants and animals' communities' assemblages.

This research aimed at analyzing plants and bumblebees' interactions in 25 secondary grasslands along an elevation gradient, from the lowlands to the tree line, in a biodiversity rich Italian region in the south-eastern Alps. Bumblebees were sampled while visiting flowers, along a 100 m transect at four points during the flight season (May, June-July, August, and October) for two years. Plant species were recorded in the same areas in 10 x 10 m plots at the growing peak.

This framework allowed us to disentangle the contribution of climatic and vegetational drivers that shape bumblebees' diversity and the interaction pattern along the elevational gradient.

Our results highlighted an increase of plant species richness according to the elevation. Bumblebees' species richness and the structure of plant-bumblebee interaction network are affected both by the elevation and plants' evenness. The effects of the elevational gradient on plants and bumblebees' communities make it possible to predict how increasing temperatures may affect pollination networks enabling us to implement strategies to reduce the effects of climate changes on this key mutualistic interaction.

Decline of bee populations and policies

FACTORS CONTRIBUTING TO HONEY BEE COLONY LOSSES IN ALGERIA: A MULTIFACTORIAL ANALYSIS

P 78

Noureddine Adjlane, Kaouche Soumeya

Department of Agronomy, University of Boumerdes, Boumerdes, Algeria

Abstract

Honey bee colony losses in Algeria represent a growing concern, threatening both apicultural productivity and pollination services. In 2025, a comprehensive study was conducted to investigate the main factors associated with colony mortality across different agro-ecological regions of the country.

The methodological approach combined field surveys and laboratory analyses. A structured questionnaire was administered to beekeepers from coastal, Tellian, and steppe regions to collect data on management practices, seasonal colony losses, environmental conditions, and disease control strategies. In parallel, biological samples (adult bees and brood) were collected from selected apiaries and analyzed using standard parasitological and microbiological methods. These analyses focused on the detection and quantification of *Varroa destructor*, as well as the identification of bacterial and fungal pathogens and microsporidia of the genus *Nosema*.

The results indicated a marked decline in honey production and elevated colony mortality rates during 2025. Colony losses were found to be driven by multiple, interacting factors. Infestation by *Varroa destructor* was identified as a key determinant, frequently associated with secondary infections and colony weakening. In addition, exposure to agricultural pesticides and the degradation of natural habitats contributed significantly to colony stress.

Environmental factors played a critical role, particularly prolonged drought, high temperatures, and reduced availability of floral resources. Seasonal patterns showed that the highest losses occurred during summer, due to thermal and water stress, and in winter, due to insufficient food reserves.

Overall, this study highlights the multifactorial nature of colony losses in Algeria and underscores the urgent need for integrated approaches combining improved disease management, sustainable beekeeping practices, restoration of floral resources, and adaptation to climate change.

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CHRONIC FEEDING TESTS WITH BUMBLEBEES UNDER NATURAL CONDITIONS: KEY INSIGHTS TO DATE

Annika Alscher[1], Johannes Lückmann[1], Nadja Staffel[1], Mareike Roeder[2], Julian Fricke[2], Olaf Klein[2]

[1]Rifcon GmbH, Hirschberg, Germany.
[2]Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany

Abstract

According to the revised EFSA Bee Guidance (EFSA 2023), bumblebee risk assessment should include semi-field and field studies with bumblebee colonies following the application of plant protection products to flowering crops. If the lower-tier risk assessment indicates a clear predominance of dietary exposure, feeding trials with a two-week exposure phase under field conditions should be conducted despite the absence of standardized test protocols. Annex C of the revised guidance describes the method only in broad terms and does not provide sufficient information on practical feasibility or on the generation of comparable and reproducible data. A particularly critical issue is that, although a positive control is envisaged, neither a specific substance nor suitable feeding concentrations for the test organisms are defined.

Members of the ICPPR Bee Protection Group conducted exploratory studies to evaluate the suitability of the proposed experimental design and potential positive controls. To this end, we performed feeding studies in 2023 and 2024 with free-flying bumblebee colonies under natural environmental conditions at two sites in Germany. Colonies were offered a spiked sugar solution via a feeder inside the nest box for a two-week exposure period followed by a six-week monitoring period.

In this poster we will present the obtained results, discuss methodological challenges arising from the EFSA specifications and outline further steps required to develop a valid and robust test system.

WHEN MITE CONTROL REQUIRES CAUTION: EXPLORING THE POSSIBLE SIDE EFFECTS OF OXALIC ACID

P 80

Desiderato Annoscia, Elisa Seffin, Sofia Mondello, Mattia Tesolin, Davide Frizzera, Virginia Zanni, Francesco Nazzi

Department of Agricultural, Food, Environmental and Animal Sciences (Di4A), University of Udine, Udine, Italy

Abstract

Oxalic acid (OA) is an active substance that is widely used to control *Varroa destructor* in honey bee colonies, where a single application is highly effective to reduce mite population under broodless conditions. However, repeated administrations in presence of brood are common among beekeepers to target the mites emerging from the cells as adult bees eclose.

This study investigates the potential side effects of OA treatment on honey bees under laboratory conditions.

To this aim, we monitored the survival of newly emerged bees maintained in cages at 34.5 °C and 75% RH, after 0, 1, 2, or 3 weekly topical applications of OA at a field realistic dose (175 µg/bee). We found that OA treatment significantly reduced the honey bees' survival, which progressively declined as the number of OA administrations increased, indicating that the substance under study induces permanent effects on the bees.

To corroborate our results and to gain insight into the observed phenomenon, we investigated the mode of action of OA in honey bees. In particular, to test the hypothesis that OA can damage the honey bees' cuticle impairing hygroregulation, we treated or not a convenient number of worker bees with a topical field realistic OA dose, and reared them at 34.5 °C under standard humidity conditions (i.e. 75% RH) or under stressful conditions (i.e. 37.5% RH). We found that OA treatment reduced the survival of bees at 75% RH, and longevity declined even further at 37.5% RH. Consequently, water consumption increased after OA exposure, and the effect was more marked at lower humidity, supporting the hypothesis that the substance compromises the cuticular integrity of treated individuals, accelerating dehydration.

Although OA treatment is essential for controlling *Varroa* mite infestation, our findings underscore the need for a responsible use in the context of a sustainable colony management.

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PRELIMINARY INSIGHTS ON THE BEHAVIOURAL AND BIOLOGICAL RESPONSE OF WORKERS AND DRONE OF APIS MELLIFERA TO PFAS EXPOSURE

Michela Bertola[1], Arrigo Moro[1], Laura Zulian[1], Karen Power[2], Anna Granato[1], Franco Mutinelli[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, NRL for honey bee health, Legnaro, Italy
[2]Department of Biology, University of Naples Federico II, Naples, Italy

Abstract

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants, often referred to as "forever chemicals" given their chemical stability. Due to their widespread industrial use and improper disposal, now PFAS are ubiquitously detected with concerning concentrations in air, soil, water, and even in plant matrices such as pollen and nectar. Honey bees are well-established bioindicators and have long been used to monitor environmental quality. However, bee populations are increasingly threatened by multiple stressors, including chemical contaminants. To date, studies investigating PFAS effects on bees are limited, and no data are currently available for drones.

In this preliminary study, we evaluated the behavioural responses and mortality of worker bees and drones following PFAS exposure (PFOA, PFOS and PFBA). Acute oral toxicity tests were conducted at the IZSVe insectary in August 2025. Groups of ten specimens were maintained in PFAS-free cages and exposed to sugar syrup contaminated with the different PFAS. The dose levels was selected among those evaluated in acute oral toxicity tests were conducted at the IZSVe insectary in August 2025 (Zulian et al., in preparation). Both a positive control and a negative control were included. Histopathological analysis was performed on surviving individuals (results presented in other contribution).

PFAS exposure resulted in increased and earlier mortality, particularly in drones. Neurological symptoms, including temporary immobilization, were observed, although partial recovery suggested possible detoxification mechanisms. Effects varied depending on both PFAS compound and bee caste, with drones appearing more susceptible, potentially due to their lower baseline exposure to environmental contaminants.

These findings highlight the need for further investigation into their accumulation and biomagnification in acute, subacute, and chronic effects of PFAS on pollinators, including potential impacts on male fertility. Given the persistence of PFAS in the environment, strengthening regulatory measures and improving management strategies are essential to mitigate long-term ecological risks.

DARK EUROPEAN HONEY BEE (*APIS MELLIFERA MELLIFERA*) DISTRIBUTION AND GENETIC DIVERSITY IN LITHUANIA

P 82

Laima Blažytė-Čereškienė, Svetlana Orlovskytė, Eduardas Budrys,
Vesta Skrodenytė-Arbačiauskienė

State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania

Abstract

Across Europe, the population of dark European honey bee (*Apis mellifera mellifer*a) has been significantly affected by the introduction and propagation of non-native subspecies of honey bees. In the Baltic countries, including Lithuania, honey bees are also being imported from abroad. For a long time, the state of the surviving native Lithuanian *A. m. mellifera* bees was unknown. Therefore, the aim of our study was to determine the current distribution of this subspecies in Lithuania and the diversity of its mitotypes.

In total, honey bee samples were collected from more than 600 colonies in traditionally managed apiaries to investigate their maternal ancestry using the mtDNA COI–COII intergenic spacer sequence. The results revealed that 52% of the studied maternal lines belonged to A. m. mellifera. They were assigned 20 unique COI–COII intergenic spacer mitotypes. Of these, nine mitotypes (A4b, M4e, M4e', M4fa', M4g, M4na, M4sb, M4tb, M-LT3), representing 44% of the studied Lithuanian *A. m. mellifera* colonies, have been already known from other countries. The other 11 mitotypes were new and detected for the first time. The most common mitotype M4fa constituted 37% of the studied Lithuanian *A. m. mellifera* colonies. Other mitotypes were significantly scarce: four of them (M4g, M4na', M-LT4, M-LT8') were represented by a single colony.

In Lithuania, maternal lines of the dark European honey bee *A. m. mellifera* have survived and represented a significant part of the colonies in the isolated and traditionally managed apiaries. The native honey bee population has retained a relatively high genetic diversity.

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BEE DIVERSITY AND RESOURCE PARTITIONING IN THE CENTRAL-NORTHERN APENNINES NATIONAL PARKS: PRELIMINARY RESULTS OF THE ITALIAN NATIONAL BIODIVERSITY DIRECTIVES

Chiara B. Boni[1], Francesca Coppola[1], Simona Sagona[1], Gaia Di Francescantonio[1], Leo Sassano[1], Davide Alberti[2], Stefano Tempesti[2], Marco Bonifacino[3], Cristina Mantoni[4], Elena Curcetti[5], Roberta Latini[6], Anna M. Lazzeri[6], Marco Di Santo[7], Luciano Di Martino[7], Rossella Ferretti[7], Marino Quaranta[8], Antonio Felicioli[1], Giorgio Davini[5]

[1]University of Pisa, Pisa, Italy
[2]Ente Parco Nazionale delle Foreste Casentinesi, Monte Falterona e Campigna, Santa Sofia, Italy
[3]University of Florence, Florence, Italy
[4]Università degli studi dell'Aquila, L'Aquila, Italy
[5]Ente Parco Nazionale Gran Sasso e Monti della Laga, Assergi, Italy.
[6]Ente Parco Nazionale Abruzzo, Lazio e Molise, Pescasseroli, Italy
[7]Ente Parco Nazionale della Maiella, Guardiagrele, Italy
[8]CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Entomophilous pollination, particularly bee pollination, is essential for over 87% of wild plant species and 70% of crops. Since 2021, the Biodiversity Directives issued by the Italian Ministry of Environment and Energy Security have promoted and funded monitoring activities coordinated by ISPRA and the University of Turin (UNITO-DBIOS) within National Parks, aimed at assessing and conserving wild pollinators. Following the protocol annexed to the Directive and aligned with the European EUPoMS protocol, species at risk such as *Bombus konradini*, *Colletes wolfi*, and *Lasioglossum marginellum* were recorded in four parks of the Central-Northern Apennines.

Furthermore, pollen network analysis revealed a general partitioning in the use of available trophic resources between managed honey bees and wild bees. These preliminary data, published in the journal *Reticula*, provide an important basis for further research into plant–pollinator interactions, highlighting the crucial role of pollinators in National Parks.

COMPARATIVE LETHAL AND SUBLETHAL EFFECTS OF GLYPHOSATE AND IVERMECTIN ACROSS THREE POLLINATOR SPECIES

P 84

Lisa Cabiddu[1], Lucia Lenzi[1], Francesco Lami[1], Mariana P. Mazzei[2], Giovanni Cilia[3], Francesca Mapelli[4], Elena S. Crotti[4], Sara Basiglio[4], Silvia Casini[5], Ilaria Caliani[5], Tommaso Campani[5], Agata Di Noi[5], Laura Giovanetti[5], Fabio Sgolastra[1]

[1]Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy
[2]Instituto de Investigaciones en Ciencias Agrarias de Rosario, IICAR-CONICET- Universidad Nacional de Rosario, Rosario, Argentina
[3]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[4]Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy
[5]Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy

Abstract

Pollinating insects are increasingly exposed to a wide range of chemical contaminants in agricultural landscapes, including not only insecticides but also herbicides and veterinary drugs. Understanding how different pollinator species respond to these compounds is essential for improving environmental risk assessment strategies.

We investigated the lethal and sublethal effects of two widely used contaminants with different modes of action: glyphosate, a globally used herbicide, and ivermectin, a veterinary drug widely applied in livestock production. Laboratory experiments were conducted on three model pollinator species representing different ecological and taxonomic groups: the honey bee *Apis mellifera*, the solitary bee *Osmia bicornis*, and the hoverfly *Eristalis tenax*. Individuals were chronically exposed through oral administration to field-realistic concentrations of each compound, applied individually and in combination.

Survival analyses revealed clear interspecific differences in sensitivity. Ivermectin significantly reduced survival in both *A. mellifera* and *O. bicornis*, while *O. bicornis* also showed decreased survival under glyphosate exposure alone. In E. tenax, higher mortality was detected only in the mixture treatment.

In addition to lethal endpoints, we have assessed sublethal responses, including changes in gut microbiome abundance and diversity and a battery of biomarkers. Overall, a limited role of microbiome-mediated effects on insect survival in response to the tested pollutants is suggested by the microbiological results, which indicate the bacterial communities associated with *Apis mellifera* as the more sensitive ones. Integrating these physiological responses with survival data can provide a more comprehensive picture of species-specific vulnerability to chemical exposure. Our results highlight the importance of incorporating multiple pollinator taxa into environmental risk assessment frameworks to better inform policies aimed at mitigating pollinator decline in agricultural landscapes.

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RESEARCH ON HONEY BEE (*APIS MELLIFERA* AND *APIS CERANA*) IMMUNE PRIMING IN KOREA

Yongsoo Choi

National Institute Agricultural Science, Rural Development Administration, Jeonju, Korea, Republic of Korea

Abstract

South Korea has over 3 million of managed honey bee (*Apis mellifera* 2.7 milliom, *Apis cerana* 0.3million) colonies before 2009 years ago, which produce the highest quantity of honey in the Korea; however, almost Apis cerana colony (90%) were collapsed by Korean Sacbrood Virus (KSBV) in South Korea. As a global phenomenon, climate change such as abnormal weather can cause winter-loss reason of decrease in honeybees' immunity. In this study, we investigated the expression patterns of Vg in the fat body and MRJPs 1-7 in the hypopharyngeal glands of nurse bees after feeding them live or heat-killed *Paenibacillus larvae* and *Bacillus subtilus*. The expression levels of MRJPs and defensin-1 in the hypopharyngeal glands were up-regulated along with Vg in the fat body of nurse bees fed with live or heat-killed *P. larvae* over 12 h or 24 h. We observed that the expression patterns of MRJPs and defensin-1 in the hypopharyngeal glands and Vg in the fat body of nurse bees upon bacterial ingestion were differentially expressed depending on the bacterial status and the time since bacterial ingestion. Thus, our findings indicate that bacterial ingestion upregulates the transcriptional expression of MRJPs in the hypopharyngeal glands as well as Vg in the fat body of *A. mellifera* and *Apis cerana* nurse bees. The concentration of bacteria for immune priming differs from that of *A. melifera* and *A. cerana*, but the gene expression of immune-related peptides and vitellogenin was excellent in both species, and the immunity-enhancing effect was finally confirmed in the next generation of larvae. According to the results of this study, we are pursuing research to apply it to excellent epigenetic honeybee breeding using Immune priming technology, and we intend to establish a herd immunity system of honeybees by distributing them in the form of feed to beekeepers in Korea.

WITHDRAWN

THE HORIZON EUROPE BEE-GUARDS PROJECT: RESILIENT BEEKEEPING AND BREEDING TO SAFEGUARD NATURAL GENETIC RESOURCES AND POLLINATION SERVICES

P 86

Cecilia Costa[1], Marina D. Meixner[2], Marin Kovačić[3], Martin S. Gabel[2], Aleksandar Uzunov[4,5,6], Jesús Yániz[7], Janez Prešern[8], Dario De Nart[9], Melanie Parejo[10], Iratxe Zarraonaindia[10,11], Gennaro Di Prisco[12], Annely Brandt[2], Giles E. Budge[13], Louise Bartek[14], Mattias Eriksson[14], Gherardo Bogo[1], Flemming Vejsnæs[15], Robert Brodschneider[16], Raffaele Dall'Olio[17], Karin Scubart[18], Ursula Wirtz[18], Daniel Schubart[18]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy. [2]LLH-Bee Institute, Kirchhain, Germany. [3]J.J.Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences, Osijek, Croatia. [4]Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Skopje, North Macedonia. [5]Macedonian Academy of Sciences and Arts, Research Center for Environment and Materials, Skopje, North Macedonia. [6]Chinese Academy of Agricultural Sciences, Institute of Apicultural Research, State Key Laboratory of Resource Insects, Beijing, China. [7]BIOFITER Research Group, Environmental Sciences Institute (IUCA), Department of Animal Production and Food Sciences, University of Zaragoza, Huesca, Spain. [8]Agricultural Institute of Slovenia, Ljubljana, Slovenia. [9]CREA Research Centre for Engineering and Agro-Food Processing, Milano, Italy. [10]University of the Basque Country, Bilbao, Spain. [11]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain. [12]Laboratory of Apidology, Institute for Sustainable Plant Protection, National Research Council (IPSP-CNR), Naples, Italy. [13]School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom. [14]Swedish University of Agricultural Science, Department of Energy and Technology, Uppsala, Sweden. [15]Danish Beekeepers Association, Sorø, Denmark. [16]University of Graz, Graz, Austria. [17]BeeSources, Bologna, Italy. [18]ConsulTech GmbH, Berlin, Germany.

Abstract

BEE-GUARDS is a EU-funded project aiming to strengthen resilience of the European beekeeping sector by providing sustainable management practices, novel breeding strategies, and digital forecasting tools. At the core of BEE-GUARDS is a multi-site field study testing innovative threshold-based *Varroa* management strategies across 456 colonies at 19 apiaries spanning multiple climatic zones in Europe and beyond. The apiaries are digitally linked via IoT devices, yielding real-time results on colony development and meteorological conditions, and enabling the disentanglement of environmental effects. A complementary accelerated 12-month breeding programme integrates natural *Varroa* selection pressure, with advanced generations already established in four countries, supported by a growing network of cryobanks for long-term genetic conservation. Samples from the apiary network have been used for microbiome and virome research, revealing that agricultural landscapes significantly alter gut microbiota structure, with detectable associations between microbiota diversity, viral load, and colony strength. An in-depth study at three locations is investigating the interaction between management and nutritional landscape and the effects on honey bee immunity, both at the social and individual level, with pollen metabarcoding approaches being benchmarked to better characterize the nutritional landscape. Climate-driven modelling tools are predicting *Varroa* population dynamics and assessing how shifting foraging patterns may increase susceptibility to emerging viral diseases. Life cycle assessments of honey production across three European climatic regions are quantifying the environmental footprint of beekeeping, while pollinator interaction studies suggest that responsible hive management can support balanced coexistence between managed and wild bees. Science-derived best practices and beekeeping knowledge are available openly through WikiBEEdia, a multilingual community platform designed to deliver locally adapted solutions to the European apicultural sector. A lively Multi Actor Forum has promoted policy briefs on topics with high relevance for stakeholders, and updates from the project and beyond are regularly narrated through social media and dedicated podcasts.

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LIVING ON THE EDGE: OSMIA BICORNIS FITNESS PARAMETERS VS. LINEAR STRUCTURES

Aleksandra Cwajna, Dawid Moroń

Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, Poland

Abstract

Highways, railway embankments, and levees are widespread linear landscape structures (LLS) found across much of the world, accompanied by elements such as nature strips, sidewalk buffers, parking strips, tree lawns, or other embankments. Due to their elongated and continuous form, they traverse landscapes and regions, affecting connectivity between isolated habitat patches. Moreover, they can also serve as supplementary habitats for many species.

The global decline in pollinator abundance and diversity highlights the need for conservation actions and research on the effects of anthropogenic structures on wild bees. The aim of this study was to determine how the fitness parameters of the solitary bee *Osmia bicornis* change depending on the distance from three types of LLS: levees, railway embankments, and highways. In a field experiment, artificial nests containing 100 cocoons were placed at varying distances from LLS. GIS data were used to determine distances and to characterize land cover within a 500 m buffer around the study sites. The following fitness parameters were analyzed: number of brood cells, survival, emergence time, sex ratio, dry body mass, fat content, and wing asymmetry. The number of brood cells increased with distance from highways and decreased with distance from levees. Survival declined with increasing distance from levees but increased in the case of railway embankments; no significant relationship was found near highways. Emergence time was not affected by distance. The proportion of males increased with distance from highways. Female body mass was not related to LLS, whereas fat content decreased with distance from highways and increased with distance from levees and railway embankments. Wing traits showed no significant relationships.

These results highlight the important role of LLS in shaping pollinator fitness and provide insights for their conservation in human-modified landscapes.

GLOBAL INEQUITIES IN PESTICIDE LEGISLATION AND BEE HEALTH: NEARLY HALF OF LATIN AMERICA'S APPROVED PESTICIDES NOT ALLOWED IN THE EU

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Grecia S. de Groot[1,2], Carolina L. Morales[1,2], Patricia Aldea-Sánchez[2,3], Marcelo A. Aizen[1], Karina Antúnez[2,4], Natalia Arbulo[2,5], Marina Basualdo[2,6], Belén Branchiccela[2,7], Adriana Correa Benítez[2,8], Eva Y. Gutiérrez-Gamiño[2,8], Natalie Herrera Salazar[2,9], Pablo Juri[2,10], Silvia I. Martinez[1,2], Gonzalo A. Molina[2,11], Diana Pimentel Betancurt[2,12], Jaime I. Rodriguez[2,13,14], Sheena Salvarrey[2,15], Carlos M. Silva-Neto[2,16], Diego E. Vazquez[2,4], Gherardo Bogo[2,17]

[1]ECOPOL Grupo de Investigación en Ecología de la Polinización, Instituto de Investigaciones en Biodiversidad y Medio Ambiente (INIBIOMA, CONICET-Universidad Nacional del Comahue), San Carlos de Bariloche, Argentina. [2]Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay. [3]Facultad de Ciencias de La Salud, Universidad SEK, Instituto de Investigación Interdisciplinar en Ciencias Biomédicas SEK, Santiago de Chile, Chile. [4]Laboratorio de Microbiología y Salud de las Abejas. Departamento de Microbiología. Centro de Investigaciones en Ciencias Ambienales. Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay. [5]Centro Universitario Regional del Este, Universidad de la República. Sede Rocha., Rocha, Uruguay. [6]Universidad Nacional del Centro de la Provincia de Buenos Aires, Facultad de Ciencias Veterinarias, PROANVET, Tandil, Argentina. [7]Sección Apicultura. Instituto Nacional de Investigación Agropecuaria. Ruta 50 m 11,Colonia, Uruguay. [8]Departamento de Medicina y Zootecnia de Abejas, Conejos y Organismos Acuáticos (DMZ:ACyOA), Facultad de Medicina Veterinaria y Zootecnia (FMVZ), Universidad Nacional Autónoma de México (UNAM)., Ciudad de México, Mexico. [9]Museo Nacional de Historia Natural, La Paz, Bolivia. [10]Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay. [11]Instituto Nacional de Tecnología Agropecuaria (INTA), Instituto de Investigación y Desarrollo Tecnológico para la Agricultura Familiar (IPAF), Región Patagonia, Plottier, Argentina. [12]Teagasc Food Research Centre, Moorepark, County Cork, Ireland. [13]Laboratorio Boliviano de Biota y Desarrollo, La Paz, Bolivia. [14]Sociedad Boliviana de Entomología, La Paz, Bolivia. [15]Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay. [16]Federal Institute of Education, Science and Technology of Goiás, Goais, Brazil. [17]CREA Research Centre for Agriculture and Environment, Bologna, Italy.

Abstract

Pesticide use has become a core strategy to control agricultural pests; however, highly hazardous pesticides may threaten non-target organisms, like wild and managed bees. Although international treaties and health recommendations call for banning hazardous pesticides, globally harmonized pesticide governance remains elusive. Latin America (LATAM) is the main net food exporter worldwide, a major producer and exporter of honey and beehive products, and the home to thousands of native bee species. In LATAM, pesticide use has increased by ~500% since 1990, compared to only ~3% in Europe. To assess the environmental and health rigor of LATAM's pesticide legislation, we reviewed active ingredients (AIs) approved for ten major crops and AIs banned in eight LATAM countries, assessing their hazardousness according to international sources. We also registered the legal status of these AIs in the European Union (EU) and explored sources of variation in the number of AIs approved in LATAM. We identified 523 AIs approved and 236 banned in LATAM; ~50% of approved and ~85% of banned AIs in LATAM were either not approved or prohibited in the EU. Higher crop production and, to a lesser extent, export value were linked to an increase in AIs approved in LATAM. These results imply a weaker regulatory framework for hazardous pesticides in LATAM, suggesting more permissive environmental policies compared to the EU. This finding shows the urgency of addressing asymmetries in pesticide governance across regions, considering pesticide toxicological tests on honeybees as well as on native bees of the region of usage before approval, and the need to implement ecological, environmental and bee-friendly agricultural practices for pest management.

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ITALIAN POLLINATORS: TRENDS AND STEPS FORWARD TOWARD THE FUTURE ITALIAN RED LIST OF WILD BEES

Simone Flaminio[1,2], Laura Bortolotti[1], Denis Michez[2], Alessandro Fisogni[3], Natasha De Manincor[2,3]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Mons, Belgium
[3]CEFE, CNRS, Univ Montpellier, EPHE, IRD, Montpellier, France

Abstract

Wild bees (Hymenoptera: Anthophila) are key pollinators sustaining both natural ecosystems and agricultural production, yet their diversity and population trends remain insufficiently understood in many regions. In Italy, despite hosting over 1,000 species, knowledge of wild bee distribution and temporal dynamics is still fragmented, largely due to the lack of standardised historical data. In this study, we provide the first comprehensive assessment of the status and trends of Italian wild bee fauna, based on an extensive and standardised dataset, derived from the revision and digitisation of major museum collections alongside contemporary monitoring initiatives and European projects.

Our results reveal contrasting patterns between national and European-scale assessments. Notably, several species showing declining trends at the national level are currently classified as Least Concern in the recent European Red List, highlighting potential mismatches in conservation status. Conversely, bumblebees (genus *Bombus*) exhibit predominantly positive trends in Italy, largely driven by widespread and expanding species, while patterns in parasitic and cavity-nesting bees suggest complex interactions between host dynamics, body size, and ecological traits. Declines observed in certain taxa, including brood parasites and species within *Lasioglossum*, may reflect both ecological pressures and biases associated with historical data and sampling methods. Overall, our findings emphasise the importance of integrating historical collections with modern datasets to reconstruct reliable baselines and detect long-term changes in bee communities. They also demonstrate that national-level analyses are essential to uncover early signals of decline that may be masked at broader geographic scales. Strengthening taxonomic expertise, improving data quality, and promoting targeted sampling efforts are therefore critical steps to support effective conservation strategies for wild bees in the Mediterranean region.

EMERGING *TROPILAELAPS* SPP. IN THE EU: ARE WE READY? STRENGTHENING DIAGNOSTIC CAPACITY AND SURVEILLANCE FRAMEWORKS

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Stéphanie Franco[1], Marion Laurent[1], Aurélie Del Cont[1], Amandine Tison[1], Jérémy Desplanques[1], Marie-Pierre Chauzat[2], Véronique Duquesne[1]

[1]Honey Bee Pathology Unit, French Agency for Food, Environmental and Occupational Health and Safety (Anses), Sophia-Antipolis, France
[2]Paris-Est University, Laboratory for Animal Health, French Agency for Food, Environmental and Occupational Health and Safety (Anses), Maisons-Alfort, France

Abstract

Tropilaelaps mites are damaging ectoparasites of honey bees, originating from Southeast Asia. By reproducing within brood cells and feeding on developing bees, they cause colony weakening, increased mortality, and production losses. Although the European Union (EU) remains free from these pests, the risk of introduction is increasing. Intensifying global trade and climate change are accelerating their spread beyond their native range, making *Tropilaelaps* a significant emerging threat to EU apiculture. Given their impact, *Tropilaelaps* spp. are subject to EU regulation, requiring a high level of preparedness. Early detection is therefore critical and relies on robust, sensitive, and harmonised diagnostic capacities across Member States.

To address this challenge, the European Union Reference Laboratory (EURL) for Bee Health has implemented coordinated actions to ensure that official laboratories are fully prepared. These include the development and validation of reference methods for the reliable and accurate identification of *Tropilaelaps* spp., thereby supporting the harmonisation of diagnostic practices across the EU.

In parallel, the EURL delivers training sessions for EU National Reference Laboratories, provides reference materials, and organises interlaboratory comparison exercises to assess and strengthen diagnostic performance. Surveillance capacity is further reinforced through continuous epidemiological monitoring and proactive communication activities, including regular updates via the EURL website, the production of a leaflet for beekeepers, and the development of technical guidelines for *Tropilaelaps* surveillance.

Together, these initiatives enhance the EU's capacity for early detection and ensure a coordinated and effective response in the event of *Tropilaelaps* introduction.

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BEE COLONY LOSSES IN POLAND FOLLOWING THE 2024–2025 WINTER BASED ON DATA FROM THE "COLOSS" SURVEY

Anna M. Gajda, Ewa Mazur

Warsaw University of Life Sciences, Warsaw, Poland

Abstract

The team at the Laboratory of Bee Diseases conducted a survey among Polish beekeepers on the extent of winter colony losses (in collaboration with the COLOSS network). Data were collected electronically (including via the LimeSurvey platform) or on paper. Information about the survey was published in "Pszczelarz Polski," "Pszczelarstwo," and "Pasieka," as well as on the websites of beekeeping associations and agricultural advisory centers.

By the end of June 2025, 171 beekeepers had correctly completed the survey.

Overall winter colony losses in Poland amounted to 15.14%. In 7 provinces, overall losses in apiaries were at an "acceptable" level of up to 10%. As in previous years, there were significant differences in the magnitude of overall losses across individual provinces (ranging from 42.4% in the Podlaskie Province to 5.4% in the Świętokrzyskie Province).

The vast majority of respondents treated their bees against the V. destructor (99%), but only 77% monitored the level of infestation in their colonies. The most popular method of controlling varroa mites was amitraz fumigation (68%), followed by the removal of drone brood (54%). 97% of respondents reported replacing frames with foundation.

The sources of information that respondents trusted the most were beekeeping books and knowledge shared by scientists. Respondents expressed the least trust in information presented on social media.

THE EURL FOR BEE HEALTH REACHES OUT WITH EXPLICATIVE VIDEOS

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Kathleen Grignet[1], Marion Laurent[1], Véronique Duquesne[1], Stéphanie Franco[1], Karim Sidi-Boumedine[1], Magali Ribière[1], Marie-Pierre Rivière[1], Marie-Pierre Chauzat[2]

[1]ANSES, Sophia Antipolis, France
[2]ANSES, Maisons-Alfort, France

Abstract

Although European Union Reference Laboratories (EURLs) play a central role in strengthening animal disease prevention and diagnostics across the EU, their missions often lack visibility among the broader public they ultimately serve. To address this communication gap, the EURL for Bee Health has produced three short narrated animated photomontages that explain, in an accessible and engaging format, the EURL's missions, the European regulatory framework in which it operates, and - as a concrete illustration - the actions currently undertaken to prevent the introduction of *Tropilaelaps* spp. mites into the EU. To ensure broad reach across all Member States, these videos will be translated into the 24 official languages of the EU.

Tropilaelaps spp. mites have been reported in Russian provinces (in 2021 and 2024), Uzbekistan (2022), and Georgia (2024). This rapid spread across continents raises serious concerns. Early detection is therefore a key step, and beekeepers must be informed and official laboratories prepared to carry out analyses quickly.

In this purpose the EURL for Bee Health develops and validates diagnostic methods. The EURL team organises training and interlaboratory tests for the European National reference laboratories (NRLs). During these exercises, laboratories performances are assessed by the analyses of identical or comparable samples. By participating in such tests, NRLs demonstrate their ability to carry out reliable diagnostics and to implement harmonised diagnostic methods.

Although the EU currently remains free from *Tropilaelaps* spp., the risk of introduction is increasing, making beekeeper awareness a critical component of any effective surveillance system. The EURL's communication initiative complements the technical work carried out with NRLs across the EU, ensuring that scientific preparedness translates into actionable awareness at the apiary level. By making its missions visible and understandable to the beekeeping community, the EURL strengthens the human component of the EU's broader surveillance and early detection capacity.

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BEYOND BLOOM: IMPACTS OF AUTHORIZED INSECTICIDE APPLICATIONS DURING CROP PETAL FALL ON WILD POLLINATOR COMMUNITIES

Ameyalli Hernández-Márquez[1,2], Lidia del Arco[1], Laura M. Usuelli[3], Fabio Sgolastra[4], Anselm Rodrigo[1,2], Jordi Bosch[1], Sergio Albacete[4]

[1]Centre for Ecological Research and Forestry Applications (CREAF), Bellaterra, Spain
[2]Universitat Autònoma de Barcelona, Barcelona, Spain
[3]Universita di Milano Bicocca, Milano, Italy
[4]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy

Abstract

Pollinators provide essential pollination services for natural and agricultural ecosystems. However, intensive agriculture and widespread insecticide use have been identified as major drivers of pollinator decline. To mitigate these impacts, EU regulations restrict insecticide applications during crop flowering and promote the establishment of field margins and flower strips to support floral resources. Insecticides are nevertheless authorized during petal fall to control pests emerging at the end of flowering. At this time, beekeepers remove their hives from treated areas, but wild pollinators remain on site. Wildflowers in and around fields may become contaminated by these authorized insecticide applications, creating an often overlooked scenario of insecticide exposure at a time when non-crop flowers are the main pollen-nectar resources available wild pollinators. Here, we present an IUCN-funded project that develops a standardized and replicable methodology to assess the effects of insecticide applications on wild pollinators and their pollination services in agricultural landscapes. The study is conducted in integrated pest management orchards in Catalonia (Spain) using paired treated–control sites. We combine sentinel plants placed at multiple distances from orchard edges (0, 5, and 10 m) to quantify spatial patterns of pesticide exposure, with synchronized pollinator observations, pollination service assessment (via stigma pollen deposition), and chemical analyses of pesticide residues in flowers. This design enables simultaneous evaluation of exposure pathways and ecological responses before and after insecticide application.

A key innovation of the project is the co-development of monitoring protocols involving youth participation, through collaborations with universities and existing pollinator monitoring networks. We provide the methodological framework together with preliminary results indicating shifts in pollinator community composition and visitation patterns following insecticide applications. We expect that our findings will guide decisions by producers, farm advisors and regulatory agencies on the timing of late-bloom insecticide applications, thus providing and improved framework for wild pollinator conservation.

PRACTITIONER CLUSTERING REVEALS DIVERSIFICATION IN BRAZILIAN MELIPONICULTURE OVER TIME

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Ana C. Isepan-Motta[1], Celso Barbiéri[1], Sheina Koffler[2], Yuri Ribeiro Diogo[3], Clarice De Paula Silva[1], Mayara Faleiros-Quevedo[1], Michelle Manfrini Moraes[4], Tiago M. Francoy[1]

[1]Escola de Artes, Ciências e Humanidades - Universidade de São Paulo, São Paulo, Brazil
[2]Centro de Ciências Naturais e Humanas - Universidade Federal do ABC, São Bernardo do Campo, Brazil
[3]Universidade Federal de São Paulo, Diadema, Brazil
[4]Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Paulo, Diadema, Brazil

Abstract

Brazilian meliponiculture has expanded in recent years due to its ecological, economic, and social relevance. In São Paulo state, this expansion has been driven by knowledge-sharing networks, training programs, specialized events, and adjustments in environmental legislation since the mid-2010s. Examining the organizational patterns linked to these transformations in meliponiculture is essential for understanding communication and knowledge flows between science, regulation, and practitioners as the activity develops. This study compares meliponiculture in São Paulo between 2016 and 2022, focusing on management practices, learning pathways, and practitioner profiles. Data were collected through online questionnaires (2016: n= 255; 2022: n= 330). Variables were grouped into 11 categories and analyzed using Multiple Factor Analysis followed by hierarchical clustering. In 2016, three clusters were identified: (1) novice urban practitioners with small stingless bee stocks and low species diversity; (2) technified practitioners, often multiplying colonies and keeping allochthonous species; and (3) experienced rural meliponists, with large stocks, profitability, also practicing apiculture. In 2022, four clusters emerged: (1) small-scale practitioners with limited diversity and supervised learning pathways; (2) urban hobby-oriented meliponists, often self-taught; (3) technically intensive practitioners combining meliponiculture and apiculture; and (4) a small skilled commercial group managing large and diverse stingless bee stocks. The results indicate a sector in transition. The practitioner base has expanded from hobbyists to commercial producers, including an emerging commercial segment. In parallel, species diversity and the adoption of technical practices have increased across the sector. These findings suggest that research-based knowledge, technological innovations, and regulatory changes are incorporated into meliponiculture through practitioners, as reflected in the clusters identified. The study highlights the need for regulamental proposals and adequate training that acknowledge the diversity of practitioner profiles while promoting sustainable and economically viable meliponiculture.

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MELIPONICULTURE'S RESURGENCE AND EMERGING WAYS OF 'MELIPONICULTURING': A DISCURSIVE ANALYSIS FROM BRAZIL

Ana C. Isepan-Motta, Celso Barbiéri, Cayo M. de A. Scot, Tiago M. Francoy

Escola de Artes, Ciências e Humanidades - Universidade de São Paulo, São Paulo, Brazil

Abstract

Historically practiced by traditional communities, meliponiculture in Brazil was marginalized with the predominance of apiculture (mainly with Africanized honey bees), which became central to scientific and productive discourses in the field. In recent decades, however, meliponiculture has reentered public debates and undergone transformations, shaped by both present and preexisting discourses. Therefore, this study aimed to characterize the historical and discursive conditions that have enabled this contemporary reemergence. The analysis examined how practitioners position themselves as meliponists; how the memory of apiculture is reactivated in current statements; the tensions and convergences among subjects positions and regimes of meaning, and how these may inform public policies for the sector. Grounded in the French tradition of Discourse Analysis, the analysis draws on 38 series of discursive sequences derived from 9 interviews with meliponists, alongside 25 materials used as complementary analytical corpus. Contemporary meliponiculture is understood as a discursive event enabled by the consolidation of environmental agendas, the pollinator crisis, and the widespread circulation of discourses on sustainability and sustainable development. Findings indicate the emergence of a technical–scientific–regulatory discursive formation that reorganizes power-knowledge relations, produces new subject positions, such as "the modern meliponist", and differentiates ways of practicing, classifying and speaking about meliponiculture. Although the memory of apiculture permeates the formations, sometimes as incompatibility and at others as a reference for techniques, regulation, and product commercialization, meliponiculture points toward a distinct developmental trajectory, increasingly aligned with socio-environmental concerns. Ultimately, despite ongoing discursive disputes, contemporary meliponiculture reveals the potential to bring experiential and technical–scientific–regulatory formations into dialogue, as meliponists increasingly claim participation in knowledge production and in decision-making processes governing the practice, thereby opening possibilities for participatory policies and institutional arrangements capable of ensuring its continuity.
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ICPPR – BEE PROTECTION GROUP: NON-*APIS* WORKING GROUP

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Tobias Jütte[1], Silvio Knäbe[2], Ivo Roessink[3], Nina Exeler[4], Roberta Nocelli[5], Olaf Klein[2]

[1]Julius Kühn-Institute - Federal Research Centre for Cultivated Plants, Institute for Bee Protection, Braunschweig, German
[2]Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany
[3]Wageningen Environmental Research, Wageningen, Netherlands
[4]Bayer AG Crop Science, Monheim, Germany
[5]Center for Agricultural Science - Federal University of São Carlos, São Carlos, Brazil

Abstract

Risk assessment of plant protection products (PPPs) for pollinators has historically relied on the honey bee (*Apis mellifera*) as model organism. However, substantial differences in behavior, life-history, ecology, physiology, and therefore exposure pathways among bee taxa meant *Apis mellifera* cannot adequately represent pollinator diversity. To address this, a non-*Apis* working group (WG) was established in 2014 within the International Commission for Plant-Pollinator Relationships (ICP-PR). This to advance and refine methods for assessing the potential risks that PPPs may pose to a wider range of bee species.

The WG focuses on bumble bees, solitary bees and stingless bees, key pollinators that differ considerably from honey bees in their distinct life cycle and behavior. Early research concentrated on *Bombus terrestris* and *Osmia bicornis*, as these species were already incorporated into existing regulatory risk assessment frameworks of the European Food Safety Authority (EFSA 2013). Driven by updated guidance from EFSA in 2023 and meanwhile also by other authorities around the world (e.g. EPA, MAFF, IBAMA) there is a global regulatory need for standardized, lower- and higher-tier tests for non-*Apis* bees. To facilitate the development of standardized testing approaches, the WG is organized into several subgroups focusing on specific experimental setups, including laboratory assays and semi-field studies. The primary goal is to establish internationally harmonized protocols that capture acute and chronic effects across relevant exposure routes and support the development of a threshold-based approach for defining specific protection goals for non-*Apis* bees. Method reliability and consistency are validated through interlaboratory ring tests. Several methods have already been published, and additional work has contributed to the establishment of new OECD test guidelines.

The ICP-PR non-*Apis* WG activities strengthen the scientific foundation of pollinator risk assessment by incorporating a broader range of bee species into testing frameworks and thereby improving the protection of pollinator diversity.

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SIMULTANEOUSLY APPLYING DARWINIAN SELECTION FOR HONEY BEE SURVIVAL IN 8 EUROPEAN COUNTRIES: INSIGHTS FROM TWO YEARS OF SELECTION

Séverine D. Kotrschal[1], Delphine Panziera[1], Maria Alice Pinto[2], Matthew T. Webster[3], Martin Bencsik[4], Chloe Davis[4], Dirk C. de Graaf[5], Trudy van den Bosch[1], Nuno Capela[6], Bjørn Dahle[7], Ellen Danneels[5], Lina De Smet[5], Daniel S. Dezmirean[8], Jeroen Eerens[5], Klaus Felgenträger[9], Alexandru-Ioan Giurgiu[10], Linn F. Groeneveld[7], Michael Hardt[11], Mariano Higes[12], Paul O. Koehler[13], Robert J. Paxton[13], Ana R. Lopes[14], Raquel Martín-Hernández[15], Antonio Pérez-Pérez[15], Camilla Sundby[7], Demetris Taliadoros[3], Jolanda Tom[1], Emma Bossuyt[5]

[1]Wageningen University and Research, Wageningen, Netherlands. [2]Instituto Politécnico de Bragança, Bragança, Portugal. [3]Science for Life Laboratory, Uppsala, Sweden. [4]Nottingham Trent University, Nottingham, United Kingdom. [5]Ghent University, Ghent, Belgium. [6]University of Coimbra, Coimbra, Portugal. [7]Norwegian Beekeepers Association, Kløfta, Norway. [8]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania. [9]Teslastraße [10], Leipzig, Germany. [10]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania. [11]Imkerei Michael Dr. Hardt, Leipzig, Germany. [12]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla, Marchamalo, Spain. [13]Martin-Luther-Universität Halle-Wittenberg, Halle, Germany. [14]LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal. [15]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha, Marchamalo, Spain.

Abstract

The ectoparasitic mite *Varroa destructor* remains the biggest biotic threat to European honey bees (*Apis mellifera* L.). Most beekeepers rely on varroa treatments to maintain their colonies, but their effectiveness is limited for several reasons, including miticide resistance and continued brood production throughout winter, which reduces miticide efficacy and contributes to high colony losses. A potentially more sustainable solution focuses on breeding honey bee lineages with natural resistance to *V. destructor*. Observations of European populations surviving without treatment highlight the beneficial role of adaptation in addressing the varroa problem. In response, a method has been developed to establish sustainable populations of untreated European honey bees by splitting the fittest colonies: the Darwinian Black Box (DBB) protocol. A notable case demonstrates 17 years of survival without varroa treatment, likely due to naturally selected resistance traits as well as beekeeping practices incorporated into the DBB protocol. However, the robustness of this approach across diverse climatic conditions and honey bee populations remains uncertain.

In this study, we apply the DBB protocol across eight European countries using locally adapted *A. mellifera* subspecies, monitoring colony performance and survival to identify early signatures of surviving DBB colonies. Data collected include genetic markers, colony development patterns captured through automated hive weight monitoring, and colony responsiveness to vibrational stimuli.

Here we present insights from the first two years of selection across participating countries, highlighting emerging patterns associated with colony survival and performance. By assessing the broader application of this method and identifying early indicators of success, we evaluate the effectiveness and limitations of the DBB protocol as a practical tool for sustainable beekeeping. This approach may provide a pesticide-free strategy supporting resilient honey bee populations.

THE ICPPR BEE MODELLING GROUP: DRIVING ADVANCES IN BEE EFFECT MODELLING FOR IMPROVED RISK ASSESSMENT

P 98

Johannes Lückmann[1], Amelie Schmolke[1], Jeffrey Minucci[2], Vanessa Roeben[3]

[1]Rifcon GmbH, Hirschberg, Germany
[2]US Environmental Protection Agency, Washington DC, United States
[3]Bayer AG, Monheim, Germany

Abstract

The Bee Modelling Interest Group was established within the International Commission for Plant-Pollinator Relationships (ICPPR) with the aim of improving the usability and acceptability of exposure and effect models in bee risk assessments. Mechanistic pesticide exposure and effect models have the potential to improve the efficiency and accuracy of risk assessments for bees and other pollinators. However, these models are not yet well integrated into the risk assessment process, and a number of challenges remain. The interest group provides a platform for exchange and discussion between stakeholders from different groups including regulators, industry, contract research organizations and academia. The interest group organised an online symposium earlier this year to inform a broad audience about available bee models developed for environmental risk assessment. In addition, three currently active work groups within the interest group are focused on specific topics that were deemed important to improve the usability and acceptability of bee models: (1) improving characterization of exposure pathways, (2) incorporation of sublethal effects in bee models, and (3) establishing a bee model inventory. The work groups aim to review available models and data, address options for modelling, and point out important data gaps. The outcomes will be made publicly available. We present these collaborative efforts to pave the way for the adoption of mechanistic models within regulatory frameworks, ultimately enabling more accurate and efficient pollinator risk assessments that better protect bee populations while supporting sustainable agriculture.

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OCEANIC ISLANDS AS RESERVOIRS OF CRYPTIC BEE DIVERSITY: IMPLICATIONS FOR CONSERVATION ASSESSMENT

David Lugo[1], Pilar De la Rúa[2], Carlos Ruiz[1]

[1]Universidad de La Laguna, San Cristobál de La Laguna, Spain
[2]Universidad de Murcia, Murcia, Spain

Abstract

Wild bees are vital pollinators, particularly in island ecosystems where high levels of endemism are often met with highly vulnerability to anthropogenic pressures. The Canary Islands, a hotspot where 39% of the bee species are endemic, exemplify this situation. While recent efforts have increased conservation assessment coverage from one-third to 87% of the bee fauna, significant knowledge gaps remain. Specifically, cryptic diversity, species that look identical but are genetically distinct, may be masking the true extinction risk.

To uncover this hidden diversity, we collected over 1,000 specimens across the archipelago and generated a DNA barcode library of 800 sequences from 122 species (covering 83% of local bee diversity). While results were largely congruent with traditional taxonomy, evidence of several putative cryptic taxa and distinct evolutionary lineages was found in 21 species (representing 26.5% of known diversity). These findings suggest the archipelago's true richness may exceed 140 taxa.

The presence of such overlooked diversity suggests that current assessments underestimate the number of species at risk. Our results show that considering this putative cryptic diversity leads to a significant variation in the assessment category under IUCN criteria, with the majority of this putative cryptic species (98%) meeting the thresholds for threatened categories (CR, EN, or VU). This major change is mainly due to a strong reduction in the Extent of Occurrence (EOO), which dropped to a much smaller fraction of its original value across the archipelago. These findings underscore the urgent need to integrate molecular tools and fine-scale spatial analysis in insular conservation strategies. The omission of cryptic taxa leads to an underestimation of total extinction rates, as a significant portion of biodiversity loss remains unreported.

THE FREE-B PROJECT: UNDERSTANDING AND CONSERVING EUROPE'S FREE-LIVING HONEY BEES

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Aicha Meliani-Rodríguez[1], Maria Alice Pinto[1], Léon Koenig[2], Joachim R. de Miranda[3], Andrzej Oleksa[4], Fabrice Requier[2], Steve Rogenstein[5], Grace P. McCormack[6]

[1]CIMO – Centro de Investigação de Montanha, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[2]Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, Gif-sur-Yvette, France
[3]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[4]Faculty of Biological Sciences, Kazimierz Wielki University, Bydgoszcz, Poland
[5]Honey Bee Watch, Galway, Ireland
[6]Zoology, School of Natural Sciences and Ryan Institute, University of Galway, Galway, Ireland

Abstract

Europe is home to several endemic subspecies of the Western honey bee (*Apis mellifera*), yet the status of wild colonies, known as free-living colonies (FLCs), remains poorly understood and has even been presumed extinct in many regions, following the spread of *Varroa destructor* across the continent between the 1960s and 1990s. Consequently, in its most recent assessment of bee conservation status in Europe, the IUCN reclassified wild honey bee populations as Endangered in the European Union's 27 Member States.

FLCs are important for Europe's biodiversity, as they act as reservoirs of the locally adapted gene complexes that have been shaped by natural selection. Preliminary research has shown that FLCs can survive over the winter, maintain a high degree of genomic integrity, and display pest and pathogen loads comparable to those in managed colonies. This suggests that FLCs may harbour key adaptive traits that confer tolerance or resistance to *Varroa destructor* and other emerging pathogens. Such traits, refined under strong natural selection in unmanaged conditions, represent a potentially invaluable resource for the long-term conservation of honey bee genetic diversity.

FREE-B is a multidisciplinary research consortium with partners from Ireland, Portugal, France, Poland and Sweden whose aim is to identify, document and monitor FLCs across Europe and assess the climatic, biogeographic, genetic, pathological and anthropogenic factors influencing their distribution and survival. By integrating citizen-science monitoring with genomic, phenotypic and pathological analyses of these colonies, the project aims to enhance public awareness, involve beekeepers and citizen scientists, and disseminate findings. Ultimately, it aims to develop nature-based solutions to lower mortality rates among managed bee colonies, improve beekeeping's sustainability, preserve biodiversity, and protect threatened wild honey bee populations.

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FROM WHOM DO BEEKEEPERS REALLY TAKE ADVICE? A LARGE-SCALE COLOSS SURVEY FROM 2020 AND 2025

Linde Morawetz[1], Lotta Fabricius Kristiansen[2], Alison Gray[3], Ondrej Vencalek[4], B-RAP / Monitoring Consortium[5], Robert Brodschneider[6]

[1]AGES - Austrian Agency for Health and Food Safety, Vienna, Austria
[2]Swedish University of Agricultural Sciences, Skara, Sweden
[3]University of Strathclyde, Glasgow, United Kingdom
[4]Palacky University Olomouc, Olomouc, Czechia
[5]COLOSS, Bern, Switzerland
[6]University of Graz, Graz, Austria

Abstract

Professional education and up-to-date knowledge of beekeepers are essential for healthy honey bee colonies and sustainable beekeeping. Several studies show that a higher level of beekeeping experience correlates positively with bee health and colony survival. However, with acute pest/pathogen spread and climate change, beekeepers need to react to new challenges by adapting beekeeping practices and participating in pest /pathogen monitoring and control. This requires trust in key stakeholders (e.g. government, associations, advisors, researchers) to ensure adoption of necessary actions and cooperation in developing solutions. Our aim was to describe beekeepers' trust in selected stakeholders across countries worldwide. The B-RAP/Monitoring consortium consists of two core projects of the COLOSS association. It conducted surveys in 2020 and 2025 asking beekeepers how trustworthy they considered a range of sources of information for beekeeping (2020:10 sources; 2025: 16 sources). Sources included stakeholders (e.g. advisors, researchers) and information channels (e.g. books, social media), rated on a four-level scale. Answers from 38 countries (N per country: between 20 and 8,866) revealed significant differences in beekeepers' trust between countries, categories and years. We will present selected categories and discuss the implications for knowledge transfer and cooperation.

B-RAP/Monitoring consortium: P Aldea-Sanchez, A Arab, A Ballis, L Blažytė-Čereškienė, K Bolmanis, J Božič, V Brusbardis, L Cadahıa, NL Carreck, P Chantawannakul, JD Charriere, R Chlebo, MF Coffrey, B Cornelissen, CA Costa, J Danihlik, EL Danneels, M Fedoriak, J Filipi, A Dieguez-Antono, T Dittman, C Dobrescu, M Fedoriak, J Fischer, T Gebretinsae, A Gregorc, LF Groeneveld, B Gustavsson, S Hašimbegović, F Hatjina, J Johannesen, E Kaliali, JM Kirbas, M Kirby, P Kristiansen, M Martikkala, ED Mazur, C McGettigan, F Mutinelli, HM Oo, A Ozkirim, M Parejo, A Raudmets, R Scarlett, A Schiesser, J Stevanovic, A Uzunov, F Vejsnæs, A Williams, GR Williams, M-C Wu.

UNDERSTANDING LOCAL HABITAT AND LANDSCAPE DRIVERS OF URBAN POLLINATOR COMMUNITIES

P 102

Agata Morelli, Francesco Lami, Lucia Lenzi, Serena Magagnoli, Laura Zavatta, Giovanni G. Bazzocchi, Giovanni Burgio

Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

Abstract

Urbanisation, through increasing impervious cover and habitat fragmentation, is driving pollinator declines globally. Nevertheless, urban green spaces (UGSs) are increasingly recognised as important refuges for pollinator conservation, potentially mitigating some of the negative effects of urban development. However, the effectiveness of UGSs in supporting pollinator communities depends on the interaction between local habitat quality, landscape structure, and floral resource composition. Moreover, despite growing interest in urban pollinator conservation, limited evidence exists on how different UGS land uses, particularly parks and urban farms, differentially support pollinator communities and plant-pollinator interactions. Here, we aimed to assess how local- and landscape-level factors influence urban wild bee and hoverfly communities across Bologna, Italy, while also examining plant-pollinator networks to identify plant species and habitat features that best support pollinators. We sampled 15 UGSs, comprising parks and urban farms, using transects and pan traps, alongside network analyses, including centrality and modularity. Across pollinator groups, local floral richness, bloom cover, UGS type, and landscape composition and configuration influenced abundance and richness, with responses varying among taxa and sampling methods. In general, urban farms supported higher pollinator activity and diversity than parks, while urban fabric and connectivity influenced community patterns at broader spatial scales. Network analyses further highlighted distinct but complementary roles of plant species across habitat types, revealing a subset of structurally important and highly connected species that contribute to interaction redundancy and network stability. Urban farms were characterised by intentionally planted ornamentals, herbs, and crop species that supported high visitation rates, whereas parks were dominated by spontaneous herbaceous species providing more stable and continuous resources. Together, these findings emphasise that both habitat design and landscape context shape urban pollinator communities, and that integrating diverse floral resources with well-distributed, connected green spaces can strengthen pollinator conservation in cities.

P 103 EU REGULATIONS AND THE PENDING MENACE OF *TROPILAELAPS MERCEDESAE*

Franco Mutinelli, Arrigo Moro, Michela Bertola

Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

Abstract

The ectoparasitic mite *Tropilaelaps mercedesae* represents an emerging transboundary threat to European apiculture, with potential implications for colony health, honey production, and ecosystem services essential to both agriculture and biodiversity. Native to Asia and originally associated with the giant honey bee, Apis dorsata, this parasite has increasingly adapted to the Western honey bee (*Apis mellifera*). Although not yet established within the European Union (EU), the recent westward spread of *T. mercedeseae* into regions such as Russia and Georgia has significantly increased the risk of introduction.

In *A. mellifera* colonies, *Tropilaelaps mercedesae* parasitizes and reproduces rapidly within capped brood cells, causing severe brood damage, deformities, and increased larval mortality. Due to its fast reproductive cycle and limited phoretic phase on adult bees, infestations can escalate quickly, potentially leading to swift colony collapse. Its impact is considered comparable to that of *Varroa destructor*, exacerbating existing pressures on European honey bee populations.

To mitigate this risk, the EU has established a comprehensive regulatory framework under Regulation (EU) 2016/429 (Animal Health Law) and related implementing Regulation (EU) 2018/1629 and 2018/1882, which classify infestation with *Tropilaelaps* spp. as a listed disease and establish harmonized requirements for surveillance, prevention, notification, and control measures across Member States. In addition, Member States may apply further restrictive national measures pursuant to Article 257 of Regulation (EU) 2016/429 where justified by epidemiological circumstances, enabling reinforced surveillance, movement controls, and targeted preventive actions.

Nevertheless, introduction pathways persist through global trade, migratory beekeeping, and accidental or illegal movement of bees and contaminated materials.

Given that eradication is feasible only at early stages of incursion, strengthening surveillance systems, improving diagnostic capacity, and enhancing beekeeper awareness are critical. Coordinated implementation of preventive measures across Member States is essential to prevent the establishment and ensure the long-term resilience and sustainability of the European beekeeping sector.

MULTISENSORY EDUCATIONAL TOOLS ON STINGLESS BEES: CONTRIBUTIONS TO INCLUSIVE SCIENCE EDUCATION

P 104

Maria Cecília Ortis, Ana C. Isepan-Motta, Celi Rodrigues Chaves Dominguez, Tiago M. Francoy, Verónica M. Guridi

Escola de Artes, Ciências e Humanidades - Universidade de São Paulo, São Paulo, Brazil

Abstract

This study explores the Natural Sciences teaching from the perspective of Inclusive Education in Early Childhood Education. The aim was to develop and implement a didactic sequence using multisensory teaching resources in order to promote the participation and learning of children with and without disabilities,integrating inquiry-based practices with accessible pedagogical materials. The project was conducted in a public school located in the city of São Paulo, Brazil, involving 80 children aged 4 to 5 years old. The didactic sequence addressed the pollination process and the biodiversity of native stingless bees. Following an inquiry-based approach, the sessions began with drawings and guided discussions to identify, beginning with drawings and guided discussions to identify the children's prior knowledge about bees.Multisensory teaching materials were developed, including tactile models representing a stingless bee nest and its developmental stages (life cycles). Melted beeswax was used to represent the nest structure along with small pots with pollen to simulate the storage structures. Propolis provided an olfactory sensory stimulus and a honey tasting activity was also carried out, exploring the sense of taste. Results showed that children initially associated bees mainly with honey and hives. After interacting with the materials, an expansion of their understanding about bees was observed, including aspects related to their social behavior, life cycle and habitat. The multisensory materials promoted curiosity, engagement, and active participation. They also supported inclusive participation by allowing children to learn also through visual and tactile exploration. This study highlights how multisensory materials can connect Science learning and environmental awareness in early childhood inclusive education, providing meaningful opportunities for all children to engage with scientific knowledge.

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BEYOND *BOMBUS TERRESTRIS*: A SENSITIVITY ASSESSMENT OF *BOMBUS PASCUORUM* AND *BOMBUS SYLVARUM* TO A COMMONLY USED INSECTICIDE

Johanna Ott, Abdulrahim Alkassab, Ricarda Scheiner, Ingolf Steffan-Dewenter

Julius-Maximilians-University, Würzburg, Germany

Abstract

Agrochemicals are among the most important drivers of pollinator declines. Both lethal and sublethal effects can severely impact individuals and for social bees, colony performance. A harsh decline in numbers has also been observed for bumblebees, an important group of pollinators in European landscapes.

Most ecotoxicological studies on bumblebees focus on the buff-tailed bumblebee *Bombus terrestris* due to easy access to commercial colonies and convenient colony rearing. However, studies comparing different species show that *Bombus terrestris* is not representative of other species of bumblebees. To assess the impact of agrochemicals on wild bumblebees in our landscape, a wider range of species needs to be tested.

For this reason, we conducted the first ecotoxicological assay on two common, yet understudied bumblebee species, *Bombus pascuorum* and *Bombus sylvarum*. We raised colonies from wild caught queens. In a first tier assessment, we assessed the sensitivity of both species to the commonly used insecticide Coragen (Chlorantraniliprole 200g/l) in a chronic exposure scenario. To allow comparisons to previous studies, we also included *Bombus terrestris* in the experiment. We recorded both lethal effects of the insecticide and sublethal behavioral changes.

This study is the first to conduct ecotoxicological assays on those two bumblebee species and represents the first step towards a more holistic understanding of agrochemical impacts on bumblebees.

THE ICPPR BEE PROTECTION GROUP

P 106

Jens Pistorius[1], Anne Alix[2]

[1]Institute for Bee Protection, JKI, Braunschweig, Germany
[2]Corteva Agriscience, Abingdon, United Kingdom

Abstract

The International Commission of Plant-Pollinator Relationship (ICP-PR) was founded in 1950 and is one of the 82 scientific commissions of the International Union for Biological Sciences (IUBS). The ICPPR Bee Protection Group (BPG) established in 1980, falls under the umbrella of the ICP-PR. The BPG is open to all interested parties and serves as a scientific forum for addressing challenges and uncertainties associated with protecting and enhancing the health of honey bees (*Apis mellifera*) and non-*Apis* bees. The forum aims to provide a means of fostering collaboration and coordinating international research efforts within academia, government, and industry to develop suitably vetted testing and evaluation methods for assessing exposure to and effects of factors affecting bee health. This group also ensures that the proposed testing methods are fit-for-purpose in terms of providing consistent, reproducible and reliable data to inform decision-making. The underlying methods resulting from the collaborative efforts of researchers within the ICP-PR BPG have served as a foundation for developing and ring-testing formal regulatory test guidelines and guidance documents of the Organization for Economic Cooperation and Development (OECD) and have contributed to global harmonization of testing and assessment methods.

The ICP-PR BPG consists of multiple subgroups that are tailored to address topics that need advancement based on input from international regulatory authorities. Working groups within the ICP-PR BPG were recently reorganized to more efficiently address uncertainties and testing needs including non-*Apis* bee testing, honey bee higher-tier (e.g., semi- and full-field) testing and monitoring, modelling (*Apis* and non-*Apis* social and solitary), and microbial pesticides. This poster will provide an overview of the on-going research and various products of the ICP-PR Bee Protection Group and will outline future workstreams.

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LINKING *APIS MELLIFERA* COLONY CONDITIONS TO LARVAL TOXICOLOGICAL RESPONSES: IMPLICATIONS FOR OECD 239 LABORATORY VALIDITY

Benedetta Ponti[1], Erica Holzer[2], Claudia Ferrario[1], Matteo Venturi[2], Daniela Lupi[2]

[1]LabAnalysis Life Science, Origgio, Italy
[2]Department of Food, Environmental and Nutritional Science (DeFENS), Università degli Studi di Milano, Milano, Italy

Abstract

Colony strength is a fundamental parameter for assessing the health status and suitability of *Apis mellifera* colonies as a source of biological material for ecotoxicological studies. Reliable larval testing requires colonies with stable brood production, adequate food reserves, and low levels of parasitic infestation, as these factors directly influence the quality and standardization of sampled larvae. This study aimed to evaluate how colony conditions affect the reliability of laboratory ecotoxicological assays, and to investigate the effects of a fungicide (silthiofam) and a plant growth regulator (NAA and NAD) on honeybee larvae using OECD Guideline 239.

Field monitoring was conducted on experimental colonies at the University of Milan apiary to assess colony strength through the quarter-frame method, quantifying brood area, food reserves, and empty space, alongside queen oviposition dynamics. *Varroa destructor* infestation was checked throughout the season and also the absence of spores of *Vairimorpha* spp. These parameters were used to ensure the selection of homogeneous and suitable colonies for repeated larval sampling.

Larval toxicity tests were performed under controlled laboratory conditions following OECD 239. While both substances showed low acute toxicity, sublethal and lethal effects emerged under repeated exposure. The plant growth regulator evidenced alterations in larval development, with effects extending to pupation and pre-emergence stages, indicating interference with metamorphosis and suggesting growth regulator-like activity not predictable from the known modes of action.

Field data demonstrated that optimal colony conditions occurred during May–June, when brood availability and colony strength supported reliable larval sampling. Conversely, seasonal declines in oviposition from July onwards limited test feasibility, highlighting colony condition as a critical factor for experimental success.

These findings demonstrate that colony strength is a key determinant of the validity and reproducibility of OECD 239 assays, and that integrating field-based colony assessment with laboratory testing enhances the ecological relevance of pollinator toxicology.

A LIVING DATABASE OF WILD HONEYBEE COLONY SURVIVAL AND POPULATION DEMOGRAPHY IN EUROPE

P 108

Benjamin Rutschmann[1], Patrick L. Kohl[2]

[1]Agroscope, Zürich, Switzerland
[2]University of Hohenheim, Stuttgart, Germany

Abstract

The population trends of the western honeybee (*Apis mellifera*) in the wild have been neglected by conservationists because the species has been considered to consist of managed colonies only. New data suggest that wild honeybee colonies (still) make up one sixth to one fifth of the overall European honeybee population. Like other native wild taxa, these wild cohorts can be assessed demographically, but require methodological adjustments to account for immigration from managed colonies via swarming. We used data on wild colony survival rates from seven European countries to model their autonomous population changes over ten-year periods, the time frame considered for population evaluation by the International Union for the Conservation of Nature (IUCN). Populations of wild honeybee colonies currently represent demographic sinks in six out of seven countries with an estimated population decline of 56% per decade. Building on these results, we introduce a curated and continuously updateable database of comparable survival estimates for wild-living honeybee colonies across Europe, designed as an open resource for future demographic assessments and IUCN reassessments. We further highlight the central role of citizen science for generating continent-scale demographic data on a species that is otherwise difficult to monitor. At the same time, citizen-science datasets can be affected by several biases (e.g. variable reporting effort, urban detection bias). We outline practical quality-control steps to address these limitations. Strengthening and harmonizing such citizen-science monitoring protocols - complementary to expert-led monitoring - will be critical for robust future IUCN reassessments and for evidence-based conservation of wild honeybee populations in Europe.

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CONTRIBUTIONS OF LATIN AMERICAN EPISTEMOLOGY TO MORE PARTICIPATORY PRACTICES IN MELIPONICULTURE SCIENCE: THE EDUCOMMUNICATIVE PARADIGM

Cayo M. de A. Scot, Celso Barbiéri, Ana C. Isepan-Motta, Tiago M. Francoy

School of Arts, Sciences and Humanities, University of São Paulo, São Paulo, Brazil

Abstract

From the Latin American struggles of the twentieth century against dictatorships and for the right to communication, educational and communicative praxis emerged, grounded in subjects' emancipation. Educommunication, an emerging epistemological and interdisciplinary field, engages with the production and circulation of knowledge. This study aimed to investigate possible contributions of educommunication to more participatory practices in scientific production in meliponiculture. A case study approach was adopted, taking the project *Meliponiculture* and *Citizen Science* as the object of investigation. Among the multiple domains of sustainability encompassed by meliponiculture, it represents an expression of the cultural domain. Developed in Brazil, the project articulates theoretical content on the biology and management of stingless bees with a theoretical–practical approach to citizen science. However, the participation of volunteers is still largely restricted to data collection and analysis. Although citizen science is essentially participatory, the educommunicative paradigm points toward more disruptive horizons. Beyond enabling joint design and implementation of projects with meliponists, educommunication challenges the primacy of science by recognizing that innovations from everyday management practices are also part of the ecology of knowledges in meliponiculture, favoring more symmetrical flows among different realities, knowledges, and cultures within scientific projects. Educommunicative citizen science interventions also manifest in the collective production of actions and outputs, integrating instructors and participants. The *BeeKeep* platform, used for data collection and analysis, could become more interactive by incorporating records of meliponists' experiences, expanding communication between participants and instructors, and functioning as a public repository for collectively produced materials. Overall, educommunication can bring academia and meliponists closer through more reciprocal relationships. As an epistemology of the South, it shifts the focus from media to mediations, guiding the creation of horizontal, dialogical, critical, intercultural, and democratic communicative ecosystems.

We thank FAPESP for funding this work through the project 2025/07156-3 and the grant 2026/11858-6.

OPTIMIZING *VESPA VELUTINA* CONTROL: THE CRITICAL ROLE OF INSECTICIDE SELECTION AND EXPOSURE PATHWAY

P 110

Soraia Sousa Santos, Paula Malaquias Souto, Artur Sarmento, José P. Sousa, Nuno Capela

Centre for Functional Ecology, Department of Life Sciences, Associated Laboratory TERRA, University of Coimbra, Coimbra, Portugal

Abstract

The invasive yellow-legged hornet, *Vespa velutina nigrithorax*, is a rapidly expanding predator of honey bees in Europe, contributing to colony losses, increased management costs, and broader risks to pollination services. Although chemical insecticides remain the primary tool for control, mainly through nest injection and baited traps, their performance is still poorly optimized.

Here, we provide the first integrative evaluation of both contact and oral toxicity for four widely used insecticide, acetamiprid (formulation Starpride MAX), spinosad (formulation Spintor), cypermethrin (formulation Cythrin), and natural pyrethrins (formulation Pirecris), on *V. velutina* workers under controlled laboratory conditions. By directly comparing exposure routes, we reveal substantial differences in toxicological profiles that have immediate consequences for field application strategies.

Across compounds, oral exposure was generally more toxic than contact exposure, yielding lower LC and LD values for all formulations except pyrethrins. Acetamiprid caused rapid mortality at low doses, whereas spinosad produced delayed but persistent effects, including sublethal behavioural impairments. In contrast, hornets actively avoided ingesting cypermethrin at higher concentrations, even after starvation, indicating behavioural avoidance that may severely limit its suitability for bait-based control.

Importantly, under realistic conditions, such as nest injection, where multiple exposure routes co-occur, effective control may be achieved at lower concentrations than predicted from single-route assays. However, the optimal compound depends strongly on the intended application: while acetamiprid and spinosad perform well orally, cypermethrin remains effective primarily through contact.

Overall, this study demonstrates that the choice of insecticide critically influences control success against *V. velutina*. By providing a more ecologically relevant assessment of toxicity, we support the development of targeted, effective, and sustainable management strategies that minimize risks to non-target organisms and pollinators.

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CASTE DIFFERENTIATION IN YELLOW-LEGGED HORNETS (*VESPA VELUTINA NIGRITHORAX*)

Elina Turapova[1], Ben Jones[1], Giles E. Budge[2]

[1]Fera Science Ltd., York, United Kingdom
[2]Newcastle University, Newcastle, United Kingdom

Abstract

Native to Southeast Asia, the invasive Yellow-legged hornet, *Vespa velutina nigrithorax*, has the capacity to cause economic and environmental damage in the United Kingdom due to its predation on both managed honey bee colonies and native insect species. Since its introduction to the UK in 2016, the National Bee Unit has performed eradication efforts by locating and destroying nests, ideally before reproductive individuals emerge and produce the next generation of hornets. Although identification of drones is straightforward due to their lack of sting, distinguishing between the female sterile and reproductive castes presents a more significant challenge. Abdominal dissections reveal a developed reproductive system and high levels of fat stores in gynes; however, dissections are time-consuming and difficult with degraded samples. Morphometric analyses, primarily weight and maximum mesoscutal width (the maximum distance between tegulae), have proven to be an effective alternative, with maximum mesoscutal width and dry weight exhibiting clear bimodal distributions in existing datasets. No such work has yet been done on the UK population.

Here, we measure the wet weight, dry weight and maximum mesoscutal width of Yellow-legged hornet females from UK nests between 2024 and 2025, with the aim of establishing reliable parameters for discriminating between sterile and reproductive castes. We also attempt to quantify exact levels of fat bodies present within the castes by measuring the dry mass of hornets before and after lipid extraction, to see if such a method can be used as an alternative to traditional dissections. Finally, we explore how supplementary metadata (such as nest size, population size and time of year) can aid in predicting gyne production in discovered nests. Our findings develop on existing research in Europe and will be beneficial in informing future outbreaks by providing further insight into differences between the female castes and the timing of gyne production.

ALTITUDE-INDUCED HYPOXIA SUPPRESSES *VARROA* MITE REPRODUCTION IN HONEYBEES

P 112

Ruike Wei[1,2], Shangning Yang[1], Xinlan Chen[3], Yicheng Lu[1], Yao Liu[1], Fuliang Hu[1], Stephen J. Martin[4], Luo Zha[3], Huoqing Zheng[1]

[1]Zhejiang University, Hangzhou, China
[2]Wageningen University and Research, Wageningen, Netherlands
[3]Xizang Academy of Agriculture and Animal Husbandry Sciences, Lhasa, China
[4]The University of Salford, Manchester, United Kingdom

Abstract

Due to increasing anthropogenic effects on the environment our reliance on honeybees (*Apis mellifera*) for pollination services and honey production continues to increase. The ectoparasitic *Varroa destructor* mite remains the biggest pest of honeybees forcing beekeepers into continual miticide use. Beekeepers on the Tibetan Plateau however, report rarely seeing varroa in their colonies, unlike all other areas of China. We conducted a series of translocation experiments to investigate this phenomenon. Here we show that hypoxic conditions on the Tibetan Plateau reduces the mite fecundity and arrests offspring development. This discovery of varroa's reproductive physiological limit could unlock new innovative mite-control methods that will benefit both the beekeeping industry and broader global population.

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STATUS OF ORGANIC BEEKEEPING IN EUROPE: SUMMARY OF EXPERTS' OPINIONS

Aleksejs Zacepins[1], Lauriane Mariame[2], Veronica Manara[2], Claudia Mortellaro[2]

[1]Latvia University of Life Sciences and Technologies, Jelgava, Latvia
[2]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy

Abstract

Within the B-THENET project, we investigated the current state, challenges, and prospects of organic beekeeping across the Europe through two complementary approaches: an electronic survey of 775 active beekeepers in 16 EU countries, and a structured expert consultation covering 25 European countries. Organic honey production in Europe is influenced by national regulations, certification frameworks, landscape conditions, and beekeeping practices; thus, reliable information is often fragmented and inconsistent and sometimes unavailable. Even the interpretation of the rules of organic beekeeping is different from country to country. The results reveal a striking paradox: while 86.5% of respondents are aware of organic beekeeping possibilities and 50.3% express interest in transitioning, only 7.9% report currently holding organic certification. Based on available EU-wide hive statistics and our survey, a plausible approximate share of organic hives is on the order of low single-digit percent (e.g. ~2%). The information collected can contribute to building the first Europe-wide overview of organic beekeeping practices. The findings will support better policymaking, improved certification procedures, and a clearer understanding of the opportunities and barriers for organic honey production within the EU. Nearly 80% of beekeeper respondents lack adequate awareness of EU Regulation 2018/848, whose full entry into force in January 2025 marks a major shift from an equivalence to a compliance system for organic imports. Pesticide contamination from surrounding agricultural areas is identified as the primary barrier to organic transition by 69.6% of respondents. Wide national variation in regulatory interpretation, foraging area availability, and market conditions further constrain adoption across member states.

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Ethics in bee science

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A CAPABILITIES-BASED FRAMEWORK TO HONEY BEE WELFARE AND INSIGHTS FROM BEEKEEPER PERSPECTIVES

Lisa Dickel[1], Heather Browning[2], Andrew Crump[3], Helena Röcklinsberg[4], Anette Wichmann[4], Barbara Locke[1]

[1]Honey Bee Research Centre, Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[2]Department of Philosophy, University of Southampton, Southampton, United Kingdom
[3]Department of Pathobiology and Population Sciences, Royal Veterinary College, London, United Kingdom
[4]Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Uppsala, Sweden

Abstract

Honey bee (*Apis mellifera*) welfare is an emerging concern within animal welfare science and bee biology, driven by increasing disease pressure and human management of colonies alongside growing evidence of insect sentience. Traditionally, animal welfare research has focused on vertebrates and primarily focused on health and productivity metrics. However, recent advances in welfare science led to the expansion to more taxa, and emphasize the importance of positive welfare states, including opportunities for agency, choice, and the expression of species-specific behaviors.

Resulting from an interdisciplinary literature review, we propose a conceptual framework to honey bee welfare based on the capabilities approach, which focuses on the opportunities animals have for species-relevant behaviors. Applying welfare frameworks to honey bees raises key questions about how welfare should be defined and measured across both individual and colony levels, given their complex social organization and their specific relationship to humans as partially captive and dependent livestock.

To complement this conceptual framework, we explored beekeepers' perceptions of honey bee welfare and the values shaping them through focus group discussions. Our findings indicate that bee welfare is predominantly viewed through a health-centered or nature-centered lens, with limited consideration of bees' mental states. While negative states such as stress were recognized, beekeepers did not report positive mental states of honey bees. A central divide exists between stakeholders who perceive bees as "wild and free" versus those viewing them as "captive and domesticated", which strongly influenced how they viewed responsibility for their welfare.

Building on these insights, we identify key knowledge gaps and formulate hypotheses regarding honey bee welfare. We outline future empirical approaches to assess how common beekeeping may influence honey bees' mental states. Advancing honey bee welfare through both conceptual and empirical work will contribute to improved management practices and support the broader development of insect welfare science.

Microbiome and nutrition

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INFLUENCE OF *VAIRIMORPHA (NOSEMA) CERANAE* INFECTION ON THE SURVIVAL OF HONEYBEE FAMILIES EXPOSED TO FUNGICIDES

Rida Ali, Erica Holzer, Gabriele Tarzia, Sara Basiglio, Francesca Mapelli, Elena S. Crotti, Daniela Lupi

University of Milan, Milan, Italy

Abstract

Apis mellifera is frequently exposed to multiple stressors. In this study, we investigated the survival over time of honeybees from three different families after chronic oral exposure to boscalid, copper(II) chloride dihydrate, and their mixture under laboratory conditions, compared to a control. During the experiment, variability in mortality among families suggested the presence of additional biological factors. Microscopic analysis confirmed the presence of spores of *Vairimorpha* (*Nosema*) sp. in all families. To better clarify the species and the level of infection, a molecular analysis was performed on bees collected from each treatment 4 days after the first exposure. Following surface sterilization, the gut was dissected, and DNA was extracted. First, using a specific PCR targeting a fragment of the SSU rRNA gene of *V. ceranae*, the presence of the microsporidian in the gut from the control treatment was assessed. No amplification of *V. apis* was detected, confirming the absence of co-infection. Subsequently, 5 bee samples for treatment and for family were subjected to a quantitative PCR (qPCR) specific for *V. ceranae*. The results showed that, in all families, the fungicides, either individual or in combination, did not alter the abundance of *V. ceranae* gene copies/gut compared with the control. However, the highest levels of *V. ceranae* gene copies/gut were found in the L1 family. Response to fungicide exposure was strictly related to infection. Families with the lowest load of *V. ceranae* (F5 and F3) exhibited the highest mortality caused by boscalid; in contrast, the family L1 with the highest infection of *V. ceranae* showed an opposite pattern, with bees exposed to boscalid surviving significantly more than the control. These findings demonstrate that pathogen load can fundamentally reshape honeybee responses to chemicals, underscoring the need to integrate infection status into ecotoxicological risk assessment frameworks.

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LACTOBACILLUS DORSATAE SP.NOV. FROM *APIS DORSATA*, *LACTOBACILLUS PSEUDOPHONI* SP.NOV. FROM *PSEUDOPHONUS RUFIPES* AND *LACTOBACILLUS XYLOCOPAE* SP.NOV., FROM *XYLOCOPA VIOLACEA*

Loredana Baffoni, Chiara Braglia, Daniele Alberoni, Gregorio Fernandez de Landa, Giacomo Braschi, Lorenzo Siroli, Diana Di Gioia

Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

Abstract

Solitary insects harbour a diverse and unique community of gut mutualistic bacteria, including lactic acid bacteria from the *Lactobacillus genus*. The lack of in-depth studies on the gut microbiome of wild insects represents a current gap in microbial knowledge that should be filled to better understand the environmental dynamics affecting both wild and managed insects. Most research focused on identifying novel Lactobacillaceae species targeting social insects like *Bombus* and *Apis* genera; however, insects are the widest animal class representing a promising source of novel microbial symbionts. This study focuses on the isolation and characterization of novel Lactobacillaceae strains from the carpenter bee (*Xylocopa violacea*), the strawberry seed beetle (*Pseudophonus rufipes*) and from *Apis dorsata*. Four novel *Lactobacillus* species were isolated and characterized - XV13L (=DSM 104957=LMG 30141T) and XV31L (=DSM 120036=MSCL 1776T) from *X. violacea*, CA30L (=DSM 119968=MSCL 1777T) from *P. rufipes* and C449L (=NCIMB 15632 =MSCL 1775T) from *A. dorsata*. Full genome sequencing and annotation, as well as G+C content determination, were performed. Phylogenetic relationships were determined using partial and complete 16S rRNA sequences, which confirmed the classification of the new strains within the *Lactobacillus* genus. Also, phenotypic and enzymatic analyses highlighted the ability of the strains to metabolize complex carbohydrates such as arbutin, esculin, salicin and cellobiose, with broader substrate utilization observed in XV13L and C449L, supported by - and β-glucosidase activities. Fatty acid (FA) profiles showed distinct lipid signatures, with oleic acid (C18:1 cis-9) as the main component (34.05–73.53%) and variable saturated fractions (SFA 18.32–53.63%), including palmitic acid (C16:0 up to 19.25%) and cyclopropane fatty acids (C19 cyc9 up to 25.31%). These traits suggest adaptive metabolic strategies and highlight their potential as beneficial symbionts with biotechnological relevance for nutrient utilization and pollinator resilience.

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IMPACT OF A COMPLETE POLLEN REPLACING FEED ON THE FITNESS AND MICROBIOME OF HONEYBEE COLONIES IN ENCLOSED AND FIELD CONDITIONS

Thierry Bogaert[1], Kelly Kulhanek[2], Diego Castillo Franco[1], Ella Viaene[1], Taylor Reams[3], Brandon Hopkins[2], Jan Bogaert[1]

[1]APIX Biosciences, Wingene, Belgium
[2]Washinton State University, Washington, United States
[3]APIX Biosciences, Arkansas, United States

Abstract

Honeybees are our only livestock that still depends on access to natural forage (pollen) for growth and survival. We developed a pollen-free nutritionally complete artificial feed that sustains colonies in tented enclosures for >120 days (May to September; > 9 capped brood cycles, several replacements of all bees in the colony). The pollen replacing feed has a Feed Conversion Ratio that is 30% better than pollen and similar to that of commercial shrimp and chicken livestock feed (1.7 kg diet/kg honey bee produced). Colonies fed a commercial protein supplements severely declined in brood production after 36 days and died out.

By omitting individual micronutrients from the diet, we identified isofucosterol, a pollen phytosterol with which no honeybee experiments were reported to date, as a critical micronutrient in the honeybee diet, that cannot be compensated for by the other major honeybee phytosterols (Bogaert et al. https://doi.org/10.1098/rspb.2024.3078; unpublished).

We will present data from large field trials. In these trials we fed colonies that in stressed conditions with our balanced diets or a commercial supplementary diet or no supplementary diet. We will report data from multi-year trials in >1500 colonies in commercial beekeeper operations in >25 locations across the USA assessing the effect of supplementary feeding in colonies experiencing summer and fall pollen dearth stresses as well as in queen breeding. Results demonstrate measurable benefits to colonies and beekeepers in colony size, survival, brood and queen production compared to current feeding practices (Kulhanek et al. https://doi.org/10.3390/insects17030243;unpublished).

Finally, we will report on the impact on the microbiome of colonies maintained from spring to fall in the tented enclosures and fed exclusively on the pollen replacing feed and sugar syrup and how this compares to the impact on the microbiome of feeding the pollen replacing feed versus controls in the large scale field trials (unpublished).

FROM DIET TO PROTEOME: BOTANICAL POLLEN SPECIES DRIVE VITELLOGENIN DYNAMICS IN HONEY BEES

P 118

Chiara Braglia[1], Daniele Alberoni[1], Cecilia Rudelli[1], Diana Di Gioia[1], Alessandra Giacomelli[2], Michel Bocquet[3], Philippe Bulet[4,5]

[1]University of Bologna, Bologna, Italy
[2]UNAAPI- Unione Nazionale Associazioni Apicoltori Italiani, Firenze, Italy
[3]Apimedia, Annecy, France
[4]Institut Pluridisciplinaire Hubert Curien (IPHC), Strasbourg, France
[5]Plateforme BioPark d'Archamps, Archamps, France

Abstract

Botanical pollen diversity strongly influences honeybee physiological resilience, but rapid tools to assess nutritional status in field conditions remain limited. We integrated SDS-PAGE and MALDI-TOF mass spectrometry (MALDI BeeTyping®) to investigate how pollen origin and seasonal context shape haemolymph protein profiles and vitellogenin dynamics in *Apis mellifera*. Cage experiments using mono-botanical pollens collected from multiple Italian regions in different periods were combined with field supplementation tests. Correlation analyses revealed distinct molecular ion–protein structures between experimental contexts. Cage datasets showed tightly coupled associations, whereas field samples displayed broader but robust correlations, reflecting environmental complexity. A cluster-based integration approach accounting for minor *m/z* shifts identified a restricted subset of reproducible molecular ion–protein associations across datasets. Multivariate analyses indicated that pollen origin modulated selected haemolymph components without globally restructuring the proteome. Instead, principal component analysis revealed a continuous physiological gradient aligned with vitellogenin concentration. Linear models adjusting for region and period identified a typical MALDI profile associated with vitellogenin levels. Molecular ions at *m/z* 1196, *m/z* 8947, and *m/z* 13,512 were consistently positively associated with vitellogenin, whereas *m/z* 3371 showed a negative association. Our research proposes for the first time a proteomic fingerprint of honeybee physiological condition and supports MALDI-TOF profiling as a rapid tool for nutritional and health monitoring in apiculture.

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APIS MELLIFERA UNICOLOR CORE MICROBIOTA IS STABLE OVER TIME IN THE TROPICAL ISLAND OF LA RÉUNION WITH POTENTIAL PATHOGENS-RELATED VARIATIONS

Hugo Charbonnier[1], Ivan Wawrzyniak[1], Benoît Jobart[2], Benjamin Pélissié[3], Hélène Delatte[3], Nicolas Blot[1]

[1]LMGE, UCA-CNRS, Clermont-Ferrand, France
[2]GDS, La Plaine Des Cafres, France
[3]CIRAD, UMR PVBMT, Saint-Pierre, France

Abstract

As a holobiont, *Apis mellifera* and its microbiota has been well studied in temperate areas. In winter, when resources are scare or absent, it exhibits obvious seasonal dynamics with shifts in colony activity (*e.g.* absence of brood), honeybee physiology (*e.g.* workers with long lifespan) and bacterial symbionts (*e.g.* higher abundance of lactic acid bacteria). Under tropical climate, where resources are available and where brood is maintained throughout the year, one wonders if such reduced seasonal dynamics is accompanied with a greater stability of the honeybee symbionts. To test this hypothesis, we studied the microbiota of *Apis mellifera unicolor*, a subspecies belonging to the African A lineage that is indigenous in the tropical island of La Réunion. The abundance of bacterial symbionts and of the microsporidian parasite *Vairimorpha* (*Nosema*) *ceranae* was monitored from January 2020 to June 2022 in two apiaries, located at high (H-A) and low altitude (L-A), by sequencing the 16S rDNA V3-V4 region and/or by QPCR. *A. m. unicolor* microbiota was dominated by *Gilliamella* spp., with high abundances of *Frischella perrara*, *Bartonella apis* and the potentially pathogenic *Spiroplasma melliferum*. Total bacterial and core members contents were stable over time, with a diversity shift in H-A from February linked to a higher abundance of *Spiroplasma*, suggestive of an infection event. *V. ceranae* loads decreased from August to December in H-A and from August to March in L-A before recovering to initial state. Both *S. melliferum* and *V. ceranae* infections may be seasonal. As a conclusion, the reduced seasonal dynamics of colonies in tropical climate is indeed reflected by a reduced dynamic of the honeybee microbiota, emphasizing the importance of the unity of the holobiont unit that globally responds to environmental cues including the presence of pathogens.

HONEY BEE MICROBIOTA AND VIRAL DYNAMICS UNDER CONVENTIONAL AND BROOD-INTERRUPTION MANAGEMENT ACROSS CONTRASTING LANDSCAPES CONTRASTING LANDSCAPES

P 120

Irene de Carlos[1], Melanie Parejo[1], Roberta Santopietro[2], Josipa Štavalj[3], Viviana Valenzano[2,4], Marin Kovačić[3], Gennaro Di Prisco[2], Iratxe Zarraonaindia[1,5]

[1]Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country (UPV/EHU), Leioa-Bilbao, Spain
[2]CNR-IPSP, The Italian National Research Council Institute for Sustainable Plant Protection, Portici, Naples, Italy
[3]University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia
[4]University of Napoli Federico II, Department of Biology, Naples, Italy
[5]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Abstract

Honeybee (*Apis mellifera*) health is influenced by environmental conditions, pathogen pressure and beekeeping practices. Honeybee gut microbiota contributes to nutrient metabolism, detoxification and immune regulation. Consequently, microbiota composition may influence how colonies survive and cope with parasites such as *Varroa destructor*, and understanding how it changes, may help explain differences in colony resilience.

Here we present a longitudinal field study conducted in Croatia and Italy within the Horizon Europe BEE-GUARDS project to monitor honeybee colonies bacterial composition and viral loads. In 2024, two apiaries were established in each country in contrasting landscapes, including semi-natural and agricultural environments. Colonies within each apiary were maintained under two *varroa* management regimes: conventional beekeeping practices or an innovative brood interruption strategy without chemical treatments over two seasons. Colonies were monitored for varroa infestation and colony strength. Honeybee hindgut microbiota diversity and composition of summer (pretreatment) and autumn samples were characterised using 16S rRNA sequencing, and Deformed Wing Virus (DWV) loads were quantified by qPCR.

Preliminary analyses show significant differences in gut microbiota composition between countries, apiaries and sampling timepoints. In the Croatian experiment, colonies belonged to two genetic lines, one from a breeding program selected for *Varroa* resistance and one non-selected line, and significant differences in microbiota composition between lines were observed. In terms of alpha diversity, management effects were detected in the semi-natural apiary, where colonies under innovative management showed higher diversity.

Our results demonstrate that the environment plays a primary role in shaping the honeybee gut microbiota. Moreover, host genetics contributes to microbiota variation supporting the existence of host–microbiota specificity within the holobiont. In contrast, management effects were small and environment-dependent. Ongoing analyses from the second monitoring year (2025-2026) will clarify the long-term effects of different management practices, and reveal how honeybee genetics, microbiota, and *Varroa* interact to influence colony resilience.

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SEASON-DEPENDENT EFFECTS OF ASCORBIC ACID SUPPLEMENTATION IN HONEY BEES

Pavel Dobeš, Jana Hurychová, Pavel Hyršl, Jacek Marciniak, Silvana B. Poiani

Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czechia

Abstract

Ascorbic acid (vitamin C) is widely recognized for its antioxidant and immunomodulatory properties and has been proposed as a dietary supplement in honey bee management. However, its actual effects on bee physiology and health remain insufficiently understood and may strongly depend on seasonal context.

In this study, we investigated the dynamics of ascorbic acid levels in honey bee hemolymph throughout the year and experimentally tested the effects of its dietary supplementation. Measurements revealed pronounced seasonal variation, with naturally low levels in winter and peaks during the active foraging season. To assess the impact of supplementation, bees were fed sucrose solution enriched with ascorbic acid under controlled conditions, and subsequent changes in hemolymph antioxidant levels, antimicrobial activity, and resistance to experimental infection were evaluated.

Our results demonstrate a clear season-dependent effect. In winter, supplementation increased hemolymph ascorbic acid levels and enhanced antimicrobial activity. In contrast, during the active foraging season, when bees naturally obtain sufficient vitamin C from pollen, supplementation led to a decrease in hemolymph levels and did not improve antimicrobial defences. Furthermore, experimental infections with *Serratia marcescens* showed no improvement in the survival of supplemented bees, regardless of season.

These findings highlight that ascorbic acid supplementation represents a double-edged tool in beekeeping. While it may be beneficial under conditions of natural deficiency (e.g., winter or nutritional stress), its application during periods of adequate natural intake may be ineffective or even counterproductive. Targeted and context-dependent use is therefore essential.

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BEES AND MICROBES: AN INTEGRATED STUDY OF MICROBIAL DIVERSITY AND PATHOGENS IN ARGENTINE WILD BEES

P 122

Gregorio Fernandez de Landa[1], Daniele Alberoni[1], Loredana Baffoni[1], Chiara Braglia[1], Leonardo Galetto[2], Carolina Torres[2], Matias D. Maggi[3], Diana Di Gioia[1]

[1]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy
[2]Instituto Multidisciplinario de Biología Vegetal, FCEFyN (CONICET-Universidad Nacional de Córdoba), Córdoba, Argentina
[3]Instituto de Investigaciones en Producción Sanidad y Ambiente (IIPROSAM), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Centro Científico Tecnológico Mar del Plata, CONICET. Centro de Asociación Simple CIC PBA, Mar del Plata, Argentina

Abstract

Wild bees are key pollinators in natural and agricultural ecosystems, yet their microbial ecology and health status remain poorly understood, particularly in the Neotropics. This study investigated gut microbiome diversity and pathogen occurrence in three Argentine solitary wild bees, *Melissodes bruneri* (Eucerini), *Ruizantheda* sp. (Halictidae), and *Diadasia* sp. (*Emphorini*), sampled from three sustainably managed agroecosystems in the Espinal ecoregion (Córdoba Province, Argentina). A total of 94 individuals were analyzed. Gut pathogens (*Nosema ceranae*, *Nosema bombi*, *Crithidia bombi*, and *Apicystis bombi*) were screened by qPCR, whereas bacterial communities were characterized by NGS sequencing of the 16S rRNA gene (V5–V7) on the Illumina MiSeq platform.

All three bee taxa harbored highly diverse and largely undescribed gut bacterial communities dominated by Enterobacteriaceae and Lactobacillaceae families. More than half of the detected bacterial strains showed ≤98.5% identity with known sequences, indicating numerous novel taxa, including putative new genera and families. Alpha diversity metrics (Shannon, richness, evenness, Faith's PD) did not differ significantly among host genera or sampling sites, suggesting a conserved overall diversity structure. In contrast, beta diversity analyses revealed significant compositional differences among host genera and sites, particularly driven by *Ruizantheda* and site-specific patterns in Manantial Norte. Microbiome analysis revealed 205 bacterial strains in *M. bruneri* (46 clusters), 140 in *Ruizantheda* (24 clusters), and 140 in *Emphorini* (28 clusters), with >50% undescribed taxa. Enterobacteriaceae dominated (up to 63%), with key clusters reaching 39.9% relative abundance, highlighting strong host-specific bacterial structuring.

Finally, the tested pathogens, typical of *Apis mellifera* and *Bombus terrestris*, remained undetected, showing that spill-over from managed bees may not occur in this specific context.

Overall, Argentine solitary bees host rich and unique gut microbiomes, with conserved diversity but context-dependent compositional shifts, underscoring their importance as reservoirs of unexplored microbial diversity in South American agroecosystems.

P 123 EFFECTS OF EUROPEAN FOULBROOD ON THE GUT MICROBIOTA OF OVERWINTERED SWEDISH HONEYBEES (*APIS MELLIFERA*)

Erik Gustafsson, Niclas Norrström, Sonja Leidenberger

University of Skövde, School of Bioscience, Skövde, Sweden

Abstract

One of the most critical periods in Swedish beekeeping is overwintering (October to March/April). During this time, the colony keeps the queen warm and is completely dependent on the food it has collected or received from the beekeeper in autumn. Most Swedish beekeepers overwinter their honey bees (*Apis mellifera*) on sugar water (60% solution), since the honey harvest is a significant source of income. High winter mortality is costly for the beekeeping sector. Additionally, poor bee health, for example, in colonies stressed by European foulbrood (*Melissococcus plutonius*), can have long-term ecological consequences, such as reduced pollination in the surroundings. In this study, 12 different honeybee colonies overwintered on three different feeding strategies (1) own honey alone, (2) sugar water alone and (3) a combination of sugar and honey ("mix"). Gut samples from each colony were taken twice, during the autumn (before wintering) and during the spring (after wintering). Using Illumina sequencing (shotgun metagenomics), we investigated the relationship between gut microbiome composition and (a) diet (honey, sugar, mix) and (b) colony health status (affected vs non-affected by European fouldbrood). Additionally, we analyzed if the gut microbiome is affecting spring development (pollen collection and drone production), and the occurrence of bacterial pathogens.

The results indicate that bee health during winter is a variable factor influenced, among other things, by weather conditions, and that it affects not only the mortality but also the strength and colony development in the spring. Therefore, good bee health in autumn and after overwintering is important for the community's ability to start collecting pollen and nectar for the next season. The microbiome seems to be strongly linked to the bees' ability to process nutrition and defend against pathogens, which directly affects their survival and the success of the colony in the following year.

ACCESSIBLE QUEEN REARING UNDER PRACTICAL CONDITIONS: PRELIMINARY FINDINGS FROM A STUDENT PROJECT

P 124

Marc H. M. Hillen

Master Beekeeping Certificate Program, University of Montana, 2025, Missoula, Montana, United States

Abstract

Queen quality strongly influences colony performance, brood production, and practical beekeeping success. This study examined whether high-quality *Apis mellifera* queens could be produced using simple and accessible queen-rearing methods under practical field conditions. The project was carried out during the first three weeks of July in Mandal, Norway, using three *Apis mellifera carnica* cell starter/cell builder colonies kept under comparable conditions. Larvae of approximately 12 hours of age were grafted into wax queen cups primed with diluted royal jelly, and queen quality was assessed by acceptance rate, cell closure, hatching success, queen cell weight, and post-hatching queen weight. Because this work was conducted as a student project associated with the Master Beekeeping Certificate Program at the University of Montana in 2025, each treatment was tested in one colony only and should therefore be regarded as a preliminary pilot study.

Across the project, queens with an average post-hatching weight above 210 mg were successfully produced. Mean post-hatching queen weight was 250 mg, with group means of 221 mg, 247 mg, and 254 mg. No clear differences were observed in queen cell length or queen cell weight, whereas lower queen weight and poorer overall performance were observed in one colony. The control colony performed unexpectedly well, suggesting that under the tested conditions, colony condition may have been more important than supplemental feeding. These findings indicate that high-quality queens can be produced with relatively simple methods, while also highlighting the need for replicated studies before stronger conclusions can be drawn.

P 125 BOTANICAL ORIGIN AND NUTRITIONAL QUALITY SHAPE HONEY BEE PESTICIDE-STRESS RESPONSES

Bhanu Janam[1], Chiara Braglia[2], Daniele Alberoni[2], Soraia I. Falcão[3], Abdulrahim T. Alkassab[4], Diana Di Gioia[2], Sergio Angeli[5,6]

[1]Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Bolzano, Italy
[2]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy
[3]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[4]Institute for Bee Protection, Federal Research Centre for Cultivated Plants, Julius Kühn-Institut, Braunschweig, Germany
[5]Faculty of Agricultural, Environmental, and Food Sciences, Free University of Bozen-Bolzano, Bolzano, Italy
[6]Competence Centre for Plant Health, Free University of Bozen-Bolzano, Bolzano, Italy

Abstract

Chronic dietary exposure to contaminated pollen represents a complex stress pathway for honey bees (*Apis mellifera* L.). While pesticide residues are often considered primary drivers of toxicity, the interplay between pollen origin and nutritional composition remains poorly characterized. In this study, we evaluated the long-term effects of monofloral pollens, from *Malus domestica*, *Phacelia tanacetifolia*, and *Taraxacum* sp. collected from contrasting agroecosystems and alpine habitats, alongside a commercial dietary supplement, on honey bee survival, physiology, and gut microbiota. Chemical analyses among pollen types revealed distinct contamination profiles, including pesticides and trace metals, as well as substantial variation in amino acids and secondary metabolites. Marked differences in biological responses were observed that were not consistently explained by pesticide load alone. For instance, contaminated apple pollen supported high survival and enhanced vitellogenin levels, indicating physiological resilience despite chemical exposure. Similarly, *Taraxacum* pollen from the apple orchard supported high survival, regardless of moderate contamination. In contrast, *Phacelia* pollen with low pesticide residues but elevated copper levels resulted in increased mortality, highlighting the role of non-pesticide contaminants. Notably, alpine *Taraxacum* pollen, despite negligible pesticide contamination, caused the highest mortality and was associated with marked microbial disruption. An overall increase in gut microbiota abundance was observed in pollen-fed bees compared to control, whereas elevated pathogen levels, including *Nosema ceranae* and *Serratia marcescens,* were associated specifically with *Taraxacum*-based diets. Overall, our findings demonstrate that pollen-mediated effects on honey bee health are governed by multiple interacting factors, including contamination, botanical origin, and nutritional composition. Overall, these findings underscore the need for an integrative approach that addresses various environmental factors and their interactions, thereby strengthening risk assessment.

TAILOCINS IN THE HONEY BEE GUT: DIVERSITY, DISTRIBUTION, AND ROLES IN BACTERIAL INTERACTIONS

P 126

Elisa Kathe, Sascha Patz, Yvonne Becker, Matthias Becker, Silvio Erler

Julius Kühn-Institut, Braunschweig, Germany

Abstract

Flowers are hotspots for diverse bacterial communities and key interfaces between plants, insects, and microorganisms. During foraging, honey bees ingest a wide range of bacteria, including plant-associated bacteria and pathogens. Maintaining a stable and functional gut microbiome therefore requires effective antagonistic mechanisms that protect both the microbial community and its host. One well-characterized mechanism mediating interspecific bacterial interactions in the honey bee gut is the type VI secretion system (T6SS), which enables bacteria to eliminate competitors by injecting toxic effectors into neighbouring cells.

Another antagonistic strategy involves tailocins, a subtype of contractile phage tail-like particles (CPTPs) produced by certain bacteria. In contrast to T6SS, tailocins are released in large quantities upon cell lysis and kill closely related bacteria by puncturing their cell wall, and disrupting the vital proton motive force. Despite their potent antibacterial activity, the role of tailocins in interspecific bacterial interactions among honey bee gut symbionts, ingested plant-associated bacteria, and pathogens remains poorly understood.

Here, we investigate the diversity and distribution of tailocin-producing bacteria associated with honey bees and their environment. Notably, comprehensive analyses of our PLaBAse database provide, for the first time, an integrated overview of the abundance and distribution of CPTPs across the honey bee gut microbiome, as well as in bee-associated pathogens. Our results reveal a highly diverse molecular repertoire of antagonistic systems among bee-associated bacteria, with strong differences in gene abundance between bee symbionts and pathogens, highlighting tailocins as an important yet underexplored factor in shaping microbiome dynamics and host-associated microbial interactions.

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NUTRITIONAL CONSTRAINTS IN QUEEN TRANSPORT: IMPLICATIONS OF PROTEIN SUPPLEMENTATION FOR ATTENDANT WORKER PHYSIOLOGY AND SUPPORT CAPACITY

Kathryn A. Knowles, Shelley Hoover

University of Lethbridge, Lethbridge, Canada

Abstract

Highly fertile queens (*Apis mellifera*) are critical to Canadian beekeeping operations, particularly for minimizing overwintering losses and maximizing pollination services. To achieve full colony productivity, many beekeepers rely on purchasing imported mated queens for splits and requeening, especially in spring. This practice represents a substantial financial investment and involves transporting large numbers of queens and attendant workers over long distances. During transport, bees are typically provided with a sugar-based diet which sustains survival but may limit the ability of attendant workers to physiologically support queens, potentially affecting queen condition and reproductive readiness upon introduction.

In this study, four protein-enriched diets (locally sourced pollen, royal jelly, a combination of pollen and royal jelly, and a yeast-based pollen substitute) were compared to a conventional sugar-based control. Worker mortality and diet consumption were recorded for each treatment. In addition, worker heads were collected on days 5 and 10, preserved in 2% paraformaldehyde PBS, and dissected to expose hypopharyngeal glands. Images were captured and the width of 20 acini per bee was measured (n = 300). Worker survival differed significantly among diets, with several protein-containing treatments improving survival relative to both the control and the pollen substitute. Hypopharyngeal gland size was also significantly affected by diet, particularly after 10 days of treatment exposure. These results indicate that protein-enriched diets can enhance the physiological capacity of attendant workers, with potential benefits for queen support during transport. This work provides new insight into nutritional constraints during queen shipment and supports further investigation into protein supplementation strategies.

DOMINANCE OF FRUCTOSE-ASSOCIATED FRUCTOBACILLUS IN THE GUT MICROBIOME OF BUMBLEBEES (*BOMBUS TERRESTRIS*) INHABITING NATURAL FOREST MEADOWS

P 128

Indriķis Krams, Ronalds Krams, Sergejs Popovs, Tatjana Krama

Daugavpils University, Daugavpils, Latvia

Abstract

Bumblebees are key pollinators in agricultural landscapes. However, little is known about how gut microbial communities respond to anthropogenic changes. We used commercially produced colonies of buff-tailed bumblebees (*Bombus terrestris*) placed in three habitats. Whole guts (midgut, hindgut, and rectum) of *B. terrestris* specimens were dissected from the body and analyzed using 16S phylogenetic community analysis. We observed significantly different bacterial community composition between the agricultural landscapes (apple orchards and oilseed rape (*Brassica napus*) fields) and forest meadows, whereas differences in gut communities between the orchards and oilseed rape fields were nonsignificant. Bee-specific bacterial genera, such as *Lactobacillus*, *Snodgrassella*, and *Gilliamella*, dominated the gut communities of *B. terrestris* specimens. In contrast, the guts of *B. terrestris* from forest meadows were dominated by fructose-associated *Fructobacillus* spp. Bacterial communities of workers were the most diverse. At the same time, those of males and young queens were less diverse, possibly reflecting greater exposure to the colony's inner environment compared to the environment outside the colony, as well as bumblebee age. Our results suggest that habitat quality, exposure to environmental microbes, nectar quality and accessibility, and land use significantly affect gut bacterial composition in *B. terrestris*.

P 129 EFFECTS OF PRIMER CHOICE ON POLLEN METABARCODING PERFORMANCE

Jorge Langa[1], Aitor Albaina Vivanco[1], Marin Kovačić[2], Annely Brandt[3], Gennaro Di Prisco[4], Cecilia Costa[5], Melanie Parejo[1], Iratxe Zarraonaindia[1,6]

[1]University of the Basque Country UPV/EHU, Leioa, Spain
[2]University of Osijek, Faculty of Agrobiotechnical Sciences, Osijek, Croatia
[3]LLH-Bee Institute Kirchhain, Kirchain, Germany
[4]IPSP-CNR Institute for Sustainable Plant Protection, Naples, Italy
[5]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[6]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Abstract

The assessment of pollen and bee bread composition is the way to identify and quantify what honeybees are actually foraging and feeding. The most straightforward method is the grain-by-grain examination under a microscope, carried out by a highly skilled and experienced palynologist.
However, advances in genetic techniques and the plummeting costs of DNA sequencing have made possible to analyze this material through metabarcoding: the sequencing of DNA markers across broad portions of the Tree of Life.
Multiple DNA fragments are proposed in the literature to study plant metabarcoding. In particular, the literature favors two of them and causes a dilemma: ITS2 because of its high taxonomic resolution but poor correlation to pollen abundance, and rbcL because of its high correlation to pollen abundance but low taxonomic resolution. Moreover, several alternative primers are available for both markers.
To identify the optimal primer combinations for assessing European honeybee foraging behaviour within the Bee-Guards EU project, we conducted a digital PCR screen across the entire NCBI Non-Redundant DNA database, encompassing both plant and non-plant taxa, to determine which sequences could or could not be amplified in the laboratory using the selected ITS2 and rbcL PCR primers. Once the most suitable primers were identified *in silico*, pollen and bee-bread samples provided by BEE-GUARDS project partners were sequenced. Subsequently, we employed the standardized QIIME2 metabarcoding workflow to quantify and taxonomically classify the recovered DNA.
We found that the most commonly used ITS2 primers amplify a high percentage of non-target sequences (fungi), and that standard metabarcoding pipelines perform poorly when assigning taxonomies based on the rbcL marker. Based on our results, we propose the combination of ITS2F-UniPlantR and rbcL2-rbcLaR. This study aims to clarify these issues, provide benchmarking evidence, and offer guidance for future research on pollinator nutritional landscapes.

CULTIVABLE YEASTS ASSOCIATED WITH *APIS MELLIFERA* MALES AND THEIR FUNCTIONAL TRAITS

P 130

Vilija Lapinskaitė, Živilė Strazdaitė-Žielienė, Elena Servienė

State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania

Abstract

The honeybee (*Apis mellifera* L.) is a eusocial insect well known for its essential role in pollination and maintaining plant biodiversity. Colonies are highly organized social units comprising thousands of individuals, including a few hundred males (drones), thousands of female workers, and a single queen. The honeybee life cycle includes four stages: egg, larva, pupa, and adult. Bees are colonized by a diverse range of microorganisms, both beneficial and pathogenic, which significantly contribute to colony health. Microorganisms with biocontrol potential act as natural regulators of the bee microbiota. While most research has focused on the microbial communities of worker bees, relatively little is known about the microorganisms associated with drones.

In this study, we aimed to perform a screening of yeasts associated with different developmental stages of honeybee drones collected in Lithuania and explore their biocidal and probiotic properties. Cultivable yeasts were isolated based on morphological characteristics and identified using molecular methods. Their functionality was assessed through antimicrobial activity, autoaggregation, cell surface hydrophobicity, and biofilm formation assays.

More than 86 yeast isolates were distinguished from different developmental stages of honeybee drones. It was determined that sealed larvae harbored the greatest diversity of yeasts, with prevailing *Starmerella* and *Metschnikowia* species. *Metschnikowia* spp. were present at all developmental stages of drones. Functional assessment showed that *M. pulcherrima* and *M. fructicola* displayed the strongest biocontrol potential, along with high autoaggregation and cell surface hydrophobicity. *Starmerella apis* and *M. reukaufii* exhibited the highest autoaggregation and strong hydrocarbon adherence. Yeasts of the *Starmerella* genus also demonstrated a pronounced ability to form strong biofilms.

A balanced microbial community is crucial for honeybee colony health, and the isolated yeasts with beneficial traits could serve as potential candidates for enhancing hive wellbeing.

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THE HONEY BEE DRONES - MICROBIAL AND ANTIMICROBIAL RESISTANCE GENES RESERVOIRS

Vilija Lapinskaitė[1], Juliana Lukša-Žebelovič[1], Edita Misevičiūtė[1,2], Elena Servienė[1]

[1]State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania
[2]Vilnius Gediminas Technical University, Vilnius, Lithuania

Abstract

The microbiota of a honey bee colony (*Apis mellifera* L.) forms a complex community of microorganisms inhabiting both the bees and various components of the hive environment. These microbes contribute to colony health by supporting immune function, participating in metabolic processes, and aiding in the maturation of bee products. The major components of the honey bee microbiota are transmitted through direct contact with nestmates, interactions with the hive environment, and while collecting food. Honey bees' microbiota can accumulate antimicrobial resistance genes (ARGs) in their microbiome and may act as reservoirs and disseminators of antimicrobial resistance.

This study aimed to evaluate bacterial communities distributed throughout the entire body of drones and assess the occurrence of ARGs. The metagenomic DNA was extracted from different stages of honey bee drones, and bacterial communities were revealed by Next Generation Sequencing of the PCR-amplified V3-V4 region of the 16S rRNA gene. The members of the *Enterobacteriaceae* family, represented by bacteria of the *Frischella* and *Gilliamella* genera, prevailed in unsealed larvae, pupae, and adult samples. Lactobacillaceae was the most dominant taxon in sealed larvae samples, represented by *Lactobacillus*, *Bombilactobacillus*, and *Apilactobacillus*. High frequencies of ARGs conferring resistance to tetracycline and sulfonamide were detected by PCR, with the highest diversity and detection rate in larvae metagenomic samples.

The microbiota of honey bee drones may affect the host and the entire honey bee community's health and performance, as well as play an important role in maintaining biodiversity and supporting the stability of the ecosystem. By harboring diverse microorganisms, honey bees can transport various ARGs and thus impact the increasing prevalence of antimicrobial resistance, possess a significant global burden.

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UNBALANCED DIETARY OMEGA-6:3 RATIO INCREASES THE DURATION OF ORIENTATION FLIGHTS BUT NOT FORAGING FLIGHTS IN HONEY BEES

P 132

Danny Minahan, Sharoni Shafir

The Hebrew University of Jerusalem, Rehovot, Israel

Abstract

Unbalanced consumption of essential fatty acids affects the ability of animals to perform tasks necessary for survival and reproduction, with excess consumption of omega-6 relative to omega-3 fatty acids negatively impacting learning and memory in mammals and invertebrates. Honey bee adults that consume an unbalanced omega-6 to omega-3 diet (ratio=5) show impaired associative learning ability and nursing behavior relative to bees that consume a balanced omega-6:3 diet (ratio=1) when tested within 1-week following diet consumption. In this experiment we tested whether an unbalanced omega-6:3 diet consumed during the first week after emergence has a negative impact on ontogeny and learning that persists into later life stages. Using a common garden design, we placed barcodes on 1-day old bees and fed them either a balanced or unbalanced diet in separate cages for 7-days, after which bees were released into a shared hive. Transits in and out of the hive were monitored to determine the age at first orientation and first foraging flights, and lifespan, along with the duration and number of flights performed. Bees which consumed an unbalanced diet had longer orientation flights relative to bees fed a balanced diet, but with no difference in the duration of foraging flights, nor in the number of orientation or foraging flights, or ages at task transition. These results suggest that an unbalanced omega-6:3 diet impairs the rate of information acquisition and processing in honey bees, and that this impairment will persist for the lifetime of the bee.

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DOSE-DEPENDENT EFFECTS OF CHITOSAN AND BEE VENOM NANOFORMULATIONS ON HONEY BEE LARVAL SURVIVAL, DEVELOPMENT, AND MICROBIOTA DYNAMICS *IN VITRO*

Aslı Özkırım[1], Dilek Kabakcı[2]

[1]Hacettepe University, ÇANKAYA, Turkey
[2]Muş Alparslan University, MUŞ, Turkey

Abstract

In vitro larval rearing of honey bees provides a controlled platform to investigate nutritional interventions and their impacts on development, survival, and microbiota. Chitosan-based compounds, widely used in veterinary applications, have gained attention in apiculture due to their antimicrobial and immunomodulatory properties. This study evaluated the dose-dependent effects of chitosan and bee venom, alone and in nanoformulation, on larval performance and microbial dynamics under in vitro conditions. Larvae were reared in triplicate groups (n = 45 per group) and subjected to different dietary treatments. Survival rates, development to adulthood, and mortality were recorded. Larval feces were collected and cultured on selective and differential media for microbial identification. Statistical analyses were performed using Tukey's HSD post hoc test. High-dose continuous chitosan exposure (0.1 g/10 g diet) resulted in complete mortality within six days, accompanied by minimal microbial detection. Short-term exposure (first 3 days) followed by a normal diet reduced mortality, supporting the hypothesis that early microbiota disruption can be partially reversible. Delayed chitosan administration also caused progressive mortality, indicating cumulative toxicity. These findings suggest that excessive chitosan induces dysbiosis, likely through combined antimicrobial and prebiotic effects leading to excessive defecation and microbiota collapse. In contrast, reduced chitosan doses (0.02 g/10 g diet) supported normal larval development with lower mortality, indicating a shift from dysbiotic to beneficial microbiota modulation. Bee venom alone showed normal development but delayed mortality, likely due to its dual proliferative (melittin-mediated) and toxic effects. The nanochitosan–bee venom formulation demonstrated the most favorable outcomes, with significantly higher survival and adult emergence rates compared to the control. This effect is attributed to controlled venom release via chitosan nanoparticle encapsulation, reducing toxicity while enhancing microbiota diversity through prebiotic activity. These results highlight that low-dose chitosan supports larval immunity and microbiota, while nanoformulations offer a promising strategy for optimizing honey bee health and development.

THE EFFECT OF EXTRACTS FROM AGRO-INDUSTRIAL WASTE ON MODULATION OF THE HONEY BEE TRANSCRIPTOMIC RESPONSE TO *NOSEMA* INFECTION

P 134

Marko Ristanić[1], Uroš Glavinić[1], Branka Zukić[2], Biljana Stanković[2], Bojan Ristivojević[2], Marina Jelovac[2], Zoran Stanimirović[1]

[1]University of Belgrade, Faculty of Veterinary Medicine, Department of Biology, Belgrade, Serbia
[2]University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia

Abstract

Nosema spp. are important microsporidian parasites of the honey bee (*Apis mellifera*), associated with impaired bee health, reduced colony performance, and overwintering losses. Since current control options are limited, sustainable approaches aimed at improving host resilience are of growing interest. Here, we examined transcriptomic changes associated with *Nosema* infection and evaluated the effects of supplementation with natural extracts derived from grape pomace and mushroom production by-products. A total of 36 samples were collected from four experimental groups (n=9 per group): uninfected controls, *Nosema*-infected bees, uninfected supplemented bees, and infected supplemented bees. Libraries were prepared using Illumina Stranded mRNA Prep and sequenced on the NextSeq 550Dx platform, targeting approximately 10 million paired-end reads per sample. Reads were quality filtered, aligned to the *A. mellifera* reference genome (Amel_HAv3.1) with *STAR* aligner, quantified at the gene level with *featureCounts* tool, and analyzed for differential expression with R packages *DESeq2* using a model including infection, treatment, and their interaction. For function enrichment analysis, R package *clusterProfiler* was used. Preliminary analysis of the first 12 samples revealed a pronounced effect of supplementation on global gene expression. Differential expression analysis identified 2514 genes associated with treatment and 1323 genes associated with the infection–treatment interaction, whereas the effect of infection alone was limited in this subset. Functional and enrichment analyses of differentially expressed genes in the supplementation group, as well as in the interaction group of supplementation and infection, indicated involvement of membrane and transporter functions, RNA processing, transcriptional regulation, ribosome-associated functions, and mitochondrial biology, together with broader themes related to stress adaptation, detoxification, energy metabolism, and cellular protection. These preliminary findings indicate that natural extracts from agro-industrial by-products may affect key molecular pathways in honey bees and could represent a sustainable, supportive approach under *Nosema* challenges.

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IMPROVING HONEY BEE COLONY STRENGTH WITH MOLYBDENUM SUPPLEMENTATION: FIELD EVIDENCE IN EUROPE

Clémence Riva[1], Ilia Belotserkovsky[1], Mathieu Holmiere[1], María Benito-Murcia[2], Eduardo Garcia-Vicente[3], Ana Pérez[3], María Martín Domínguez[4], Précillia Cochard[5], Leonidas Charistos[6], Fani Hatjina[6], Salomé Martínez-Morcillo[7], Benjamin Poirot[5], David Risco[2,3], Sébastien Floquet[8,1]

[1]Oligofeed SAS, Gif-sur-Yvette, France
[2]Department of Animal Medicine, Faculty of Veterinary, Universidad de Extremadura, Caceres, Spain
[3]Neobeitar S.L., Caceres, Spain
[4]Department of Animal Health, Infectious Diseases Unit, Veterinary Faculty, University of Extremadura, Caceres, Spain
[5]Apinov, Scientific Beekeeping & Training Center, Lagord, France
[6]Division of Apiculture Hellenic Agricultural Organization DEMETER, Nea Moudania, Greece
[7]Unit of Toxicology, Veterinary Faculty, University of Extremadura, Caceres, Spain
[8]Institut Lavoisier de Versailles, CNRS UMR 8180, Univ. Versailles Saint Quentin en Yvelines, Univ. Paris-Saclay, Versailles, France

Abstract

Adequate nutrition supports honey bee health, reduces colony mortality and mitigates the effects of other stressors, while nutritional limitation can lead to increased disease susceptibility. Micronutrients, including trace elements (TE), are essential for biochemical and physiological processes. Studies on the importance of TE for honey bees are scarce. Essential TE cannot be produced by bees and the needs are mostly met by pollen. However, the availability of micronutrients varies in space and time due to differences in pollen composition. Among TE, molybdenum (Mo) plays an essential role in insects as it is a cofactor of a wide variety of enzymes. We investigated how supplementation with a bioassimilable Mo-based complex commercialized under the brand Pep'up can improve colony performance. First, we assessed the effect of supplementation on winter mortality, within six apiaries in France and Spain, using in total 247 hives. Supplementation with Pep'up resulted in a significant reduction in winter colony losses by 44% on average. Next, we evaluated the impact on bee populations and honey production in spring through field trials in Spain and Greece. A significant increase in honey reserves of 107% was observed in the Greek apiary, whereas no comparable effect was detected in Spain. We found no effect on the bee population in both studies. These results suggest that local environmental conditions or management practices may influence the effect of Pep'up on honey production. Based on these promising results, further experiments are necessary to clarify the mode of action of the Mo-complex in the honey bee.

DIFFERENTIAL SENSITIVITY AND BREAKDOWN OF SUBSPECIES-SPECIFIC GUT MICROBIOTA SIGNATURES IN *APIS MELLIFERA* EXPOSED TO FLUPYRADIFURONE

P 136

Annelise Rosa-Fontana[1,2], Claudio Alba[3], Juan Miguel Rodríguez Gómez[3], Simone Tosi[4], Dora Henriques[1], Maria Alice Pinto[1], Mariano Higes[2], Raquel Martín-Hernández[2]

[1]Instituto Politécnico de Bragança, Bragança, Portugal
[2]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha, Marchamalo, Spain
[3]Complutense University of Madrid, Madrid, Spain
[4]University of Turin, Turin, Italy

Abstract

The honey bee gut microbiota is a key indicator of host health, yet its role in mediating subspecies-specific responses to pesticides remains poorly understood. This study investigated the differential sensitivity and gut microbiota responses of newly emerged *Apis mellifera iberiensis* (AMI), *A. m. ligustica* (AML), and *A. m. carnica* (AMC) workers to the insecticide flupyradifurone (FPF, 36 ppm). Following OECD 245 guidelines (10-day chronic oral exposure), bees were exposed to FPF alongside negative (NC) and acetone (AC, 1%) controls. Microbiota was characterized using full-length 16S rRNA PacBio Sequel II sequencing. Results revealed marked differential sensitivity in adult bee survival: AML exhibited 100% mortality following exposure, while AMI and AMC showed different resilience levels, with survival rates of 81% and 15%, respectively ($p < 0.0001$). In surviving subspecies, taxonomic profiling of the 8 core gut genera demonstrated divergent dysbiosis shifts. In AMI, FPF caused a total depletion of *Snodgrassella* (13.1% to 0%) and *Bartonella* (28.1% to 0.2%), with a compensatory expansion of *Lactobacillus* (23.8% to 48.6%). Conversely, in AMC, the dominant *Lactobacillus* (55%) and *Bifidobacterium* (7%) remained stable, while *Snodgrassella* increased from 0.3% to 17.5%. Despite these rearrangements, alpha diversity (Shannon index) showed no significant shifts ($p > 0.05$), indicating community turnover rather than a loss of overall diversity. However, beta diversity analysis (PCoA) confirmed a breakdown of subspecies-specific gut microbiota signatures. While subspecies harbored distinct profiles under control conditions (NC: $p=0.001$; AC: $p=0.001$), FPF acted as a potent leveling factor, rendering the surviving communities statistically indistinguishable (FPF: $p=0.08$). These findings highlight that pesticide impact on gut homeostasis is profoundly influenced by host genetic background. Our results underscore the critical importance of intraspecific diversity for bee health and resilience, suggesting that subspecies-specific responses should be considered in pollinator risk assessments.

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MIDGUT HISTOMORPHOLOGY OF HONEY BEES FED WITH FUNGAL EXTRACTS

Leticia Salvioni Ansaloni[1], Caio E. da Costa Domingues[1], Marija Gregori[2], Andrej Gregori[2,3]

[1]University of Maribor - FKBV, Maribor, Slovenia
[2]MycoMedica LTD, Kranjska Gora, Slovenia
[3]University of Ljubljana - Biotechnical Faculty, Ljubljana, Slovenia

Abstract

The agricultural system has been exposing bees to different stressors that vary with crop type and region. These stressors include habitat loss, limited floral resources, parasites and pathogens, climate change, and pesticides, which pose a significant risk to bees. In this context, there is a growing search for natural alternatives that help maintain the honey bees health, with fungal extracts emerging as one of the approaches under investigation due to their potential benefits. Our studies assessing effects of *Ganoderma lucidum* (GL), *Hericium erinaceus* (HE), *Inonotus obliquus* (IO), and *Trametes versicolor* (TV) extracts on honey bees midgut-histomorphology. Newly emerged Carniolan honey bees (*Apis mellifera carnica*) were fed 4% of each fungal extract for 7, 14, and 21 days. The midguts were sampled and histologically processed, and qualitative and semi-quantitative analyses were performed. Fungal extracts in bee diet induced no changes at tissue and cellular level; and indicative the histoarchitecture of the intestine did not differ from that of untreated bees. The midgut lesion index also showed no variation among fungi extracts treatment. The results demonstrate that the use of these extracts in honey bee diet does not cause damage to the midgut of bees over the analyzed period, suggesting their potential as a complementary tool for promoting colony health.

Keywords: bee health, dietary supplementation, fungi, histoarchitecture, midgut morphology.

EFFECTS OF NUTRITIONAL SUPPLEMENTATION ON HONEY BEES: FROM INDIVIDUALS TO THE COLONY SCALE

P 138

Carolina Sosa Labadie[1], Karina Antúnez[2], Guillermo Bragunde[1], Ciro Invernizzi[3], Carmen Rossini[1], Mariana Seveso[1], Belén Branchiccela[4]

[1]Laboratorio de Ecología Química, Departamento de Química Orgánica, Facultad de Química, Universidad de la República, Montevideo, Uruguay
[2]Laboratorio de Microbiología y Salud de las Abejas, Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
[3]Sección Etología, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay
[4]Sección Apicultura, Instituto Nacional de Investigación Agropecuaria, Montevideo, Uruguay

Abstract

Pollen is the main source of proteins, amino acids and lipids for honey bees (*Apis mellifera*). However, in the current scenario of agricultural production, it does not necessarily provide bees with their nutritional requirements. Thus, beekeepers supplement their colonies to mitigate this nutritional stress. Here, we evaluated a nutritional supplement (NS) under development at both the individual and colony levels.

Newly emerged bees were reared under controlled conditions and assigned to three groups: NS, a polyfloral pollen patty (PP, positive control), or no protein (C–, negative control). All groups were provided with sugar syrup. Bees fed with NS and PP showed higher survival than C– bees, with no differences between the two treatments. These treatments significantly increased *vitellogenin* expression and marginally increased *glucose oxidase* expression at both 7 and 14 days. In 14-day-old bees, NS and PP significantly decreased the expression of *hymenoptaecin* and *prophenoloxidase*, and increased glutathione S-transferase activity compared to C–. Additionally, hypopharyngeal glands were significantly larger in protein-fed bees.

Under field conditions, honey bee colonies were established in a *Eucalyptus grandis* plantation, which provides monofloral and low-quality pollen. All colonies were allowed to forage freely but were assigned to three treatments: NS supplementation, PP (positive control) supplementation and no supplementation (C-). Colonies provided with protein treatments tended to be more populated and to produce more honey than C- colonies, nevertheless these differences were not statistically supported.

Considering the promising results observed under controlled conditions and the atypical seasonal conditions experienced during the field study, future assays should be performed to analyze the effects of NS and to optimize its dose and application form if necessary.

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IMPACT OF CHRONIC RADIOACTIVE EXPOSURE ON THE GUT MICROBIOTA OF TWO POLLINATOR SPECIES IN FUKUSHIMA

Laurie Thouvenel, Coline Monchanin, Marlène Botella, Mathilde Lacombrade, Matthias Desmolles, Loic Goulefert, Blandine Mahot-Castaing, Kristine Abenis, Mathieu Lihoreau, Amélie Cabirol

Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI), CNRS, Toulouse University, Toulouse, France

Abstract

The nuclear accident at Fukushima Daiichi power plant that occurred in March 2011 triggered a massive release of radioactive cesium, durably affecting terrestrial and marine ecosystems. Abnormal morphology and behavior have been reported in insects, including the domestic honey bee (*Apis mellifera*) and the wild giant hornet (*Vespa mandarinia*), which are directly exposed through their lifestyles and diet. Beyond direct effects on host organisms, chronic cesium exposure might have durably altered their gut microbiota, with possible consequences on host physiology. Indeed, microorganisms are also affected by ionizing radiation, as exemplified by the reduction in biomass and taxonomic diversity within the soil microbiota inside the Fukushima exclusion zone.

Here we investigated how radioactivity shapes the insect gut microbiota by analyzing the bacterial communities of honey bees and hornets foraging along a cesium contamination gradient. Using 16S rRNA gene amplicon sequencing, we characterize shifts in microbial composition across exposure level. Preliminary results reveal marked changes in the dominant bacterial taxa in hornets populating highly contaminated zones, likely reflecting a switch in dietary behavior. By linking environmental contamination to host-associated microbiomes, our study aims to reveal how changes in the microbiome can alter the stability of entire ecosystems exposed to lasting perturbations.

BEEWELL AMINOPLUS ENHANCES HEMOLYMPH BIOCHEMICAL AND IMMUNE MARKERS IN WORKER HONEY BEES (*APIS MELLIFERA* L.)

P 140

Ivana Tlak Gajger[1], Josipa Vlainić[2], Srebrenka Nejedli[1], Maja I. Smodiš Škerl[3]

[1]Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia
[2]Rudjer Boskovic Institute, Zagreb, Croatia
[3]Agricultural Institute of Slovenia, Ljubljana, Slovenia

Abstract

Insufficient diet diversity and quality compromise honey bee immunity and increase susceptibility to pathogens, which is why dietary supplements enriched with amino acids and B-complex vitamins are increasingly used in practice. Previous studies demonstrated that plant-based and amino acid-rich vitamin supplements can enhance honey bee colony strength, reduce *Nosema* spp. spore loads, and enhance antiviral defenses. However, there is still limited data on their direct effects on biochemical and immune parameters in hemolymph under controlled laboratory conditions. We conducted a laboratory cage experiment with newly emerged worker *Apis mellifera*, randomly assigning them to the BeeWell AminoPlus or sugar syrup control groups and maintaining them under standardized microclimatic and feeding conditions. Hemolymph was sampled on 13th and 22nd day post-feeding, and glucose, trehalose, total proteins and vitellogenin were quantified spectrophotometrically. Data were analysed using appropriate parametric tests to compare treatment and control within and between sampling days. Additional feeding significantly reduced glucose oxidase activity compared to controls at both time points, indicating lower induction of this component of social immunity under supplement feeding. In contrast, supplemented bees showed higher or better-maintained total protein and vitellogenin levels, particularly on 22nd day, suggesting improved nutritional status and immune competence during prolonged cage confinement. Trehalose concentrations in the BeeWell group were more stable between 30 and 60 minutes of measuring on 22nd day post-feeding, indicating a more balanced energy metabolism with supplement administration. Our findings demonstrate that BeeWell AminoPlus modulates hemolymph biochemical and immune-related markers in worker honey bees, supporting protein reserves, vitellogenin level and energetic homeostasis under laboratory stress conditions. Such effects are consistent with field reports on amino acid–vitamin supplements improving honey bee colony performance and resilience and indicate that targeted dietary interventions can be a useful tool to mitigate nutritional and pathogen-related stress in contemporary apiculture.

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HOW DOES EARLY EXPOSURE TO PESTICIDE MIXTURES AFFECT THE PRE-IMAGINAL DEVELOPMENT OF HONEY BEES AND THEIR LARVAL GUT MICROBIOTA?

Diego E. Vázquez, Eugenia Vaz, Karina Antúnez

Laboratorio de Microbiología y Salud de las Abejas. Departamento de Microbiología & Centro de Investigación en Ciencias Ambientales (CICA), Instituto de Investigaciones Biológicas Clemente Estable (IIBCE), Montevideo, Uruguay

Abstract

Honey bee colonies have been increasingly exposed to pesticides due to agricultural intensification over the past few decades. Thus, mixtures of pesticide residues were detected in honey and beebread. Therefore, bees of different ages and developmental stages are exposed to contaminated food. Particularly, larval development is a susceptible period to pollutants because this is the only time when bees grow. Hence, the first line of defence in larvae is the gut and its microbiota. In recent years, the pesticides most frequently detected at the highest maximum concentrations in hives are glyphosate, chlorpyrifos, and various acaricides. Currently, the bioacaricide oxalic acid is one of the most sustainable options, with widespread implementation in Latin America. Therefore, our study aimed to assess how early exposure to these pesticides and their mixtures can alter the pre-imaginal development of honey bees and their larval gut microbiota. For this, we grafted 54 fourth stadium larvae from 3 colonies per treatment and reared them under lab conditions (partial in vitro rearing). This modified protocol allows larvae to be inoculated with native microbiota from their colonies. After 1 day of acclimation without pesticides, we carried out 8 treatments with different combinations of exposure to glyphosate (1.6 mg/L), chlorpyrifos (950 µg/L), and oxalic acid (280 mg/L) for 24 h. Then, we measured larval growth, dissected guts for bacterial diversity analysis and monitored survival during pre-imaginal development. Our preliminary results showed sub-lethal effects on survival and microbiota during exposure, but no synergistic interactions. However, all combinations showed late lethal effects during pupal development. Moreover, chlorpyrifos and its mixtures showed a high teratogenicity during pupation. Therefore, future studies need to assess a greater number of mixtures and integrate multiple endpoints and exposure conditions to understand the anthropogenic impact on bees.

Plasticity and genomic variation to overcome threats

P 142 RELATIONSHIP BETWEEN HYGIENIC AND THE DEFENSIVE BEHAVIORS AND DETERMINING THEIR LINEAGE IN LOCAL HONEY BEE COLONIES OF LATAKIA, SYRIA

Menos M. Assaad

Former Researcher at General Commission for Scientific Agricultural Research, Syria, Latakia, Syrian Arab Republic

Abstract

The study investigated the relationship between hygienic and defensive behaviors in local honey bee colonies from the Syrian costal region, in addition to determining their lineage assignment using a set of discriminative morphometric traits. Twelve standardized colonies were evaluated at the Agricultural Research Apiary in Latakia, Syria, during 2022. Hygienic behavior was measured using the pin-Killed brood assay, while defensive behavior was assessed using a standardized behavioral rating test that included frame- abandoning tendency, agitation level, attack tendency, and stinging propensity. Each colony was tested three times at one- day intervals under blind condition. Lineage assignment was determined using five morphometric traits: cubital index, tarsal index, wax mirror posterior margin shape, discoidal shift direction and abdominal tergite coloration pattern.

The results showed that colonies were classified into four aggressiveness categories: very aggressive, aggressive, moderately aggressive, and gentle, representing 41.66%, 25%, 16.66%, and 16.66% of sample, respectively. The Kruskal-Wallis test revealed significant differences among these categories (H=10.07,P= 0.018). Regarding hygienic behavior, 50% of colonies removed more than 95% of Pin-Killed brood within 48 hours and were classified as highly hygienic, while 16.6% showed moderate hygienic behavior and 33.33% exhibited low hygienic performance. A strong positive correlation was detected between hygienic and defensive behaviors (Spearman's coefficient= 0.99)

Lineage determination revealed genetic admixture with *Apis mellifera carnica* and *Apis mellifera ligustica*. Colonies genetically closer to *Apis mellifera syriaca* (41.66%) exhibited higher levels of both hygienic and defensive behavior, consistent with local adaptation. In contrast, colonies showing genetic admixture with the Carniolan and Italian subspecies (58.33%) displayed more variable defensive and hygienic patterns, suggesting that such admixture may reduce defensiveness while maintaining moderate to high hygienic performance. These findings highlight the potential of targeted selection within *A. m. syriaca* to maintain high hygienic behavior and ensure colonies that are easy to handle and inspect.

GENETIC DIVERSITY OF THE IVORY COAST HONEYBEE, *APIS MELLIFERA* ADANSONII, AND IMPLICATIONS FOR SUSTAINABLE MARKET FARMING

P 143

Kamila Canale Tabet[1], Ivalda R. Nzala Koyo[1,2], Pierre Faux[1], Emilie Deletre[2], Rahim Ouedraogo[2], Thibaud Martin[2], Alain Vignal[1]

[1]GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet Tolosan France
[2]Cirad UPR HortSys, Université de Montpellier, Montpellier, France

Abstract

In Côte d'Ivoire, beekeeping follows practices that differ from those commonly observed in Western countries, with honey production relying largely on wild colonies, often attracted into empty hives placed to encourage the natural settlement of swarms. In this context, integrating beehives into market gardening systems appears to offer a multifaceted solution by helping to address declining yields and deteriorating product quality, while also raising market gardeners' awareness of the risks associated with excessive pesticide use. As a first step towards understanding Ivorian honey bees within this context, we aim at characterising their genetic diversity. We therefore collected honey bees from five Ivorian agroecological zones, completed with field surveys of beekeepers. Despite a few publications in the literature suggesting higher resistance or tolerance to Varroa in African populations, we found a substantial prevalence of the parasite. The samples were sequenced with Illumina short reads. Diversity and population structure analyses were done using autosomal SNP and analyses of complete de novo mitochondria assemblies. This approach for the mitogenome was followed rather than the conventional alignment of reads to a reference, as the latter causes high error rates due to the presence of large nuclear mitochondrial DNA insertions (NUMTs). Initial results confirm that the Ivorian bee population belongs to the mtDNA African lineage A, forming a distinct group from other global lineages, and highlight sporadic introgression events from Northern bees. This highlights the existence of an intact local genetic heritage, which may to contribute to natural resistance to parasites and environmental variations. Our results provide the first large-scale genomic data on an unmanaged West African honey bee population and underscore the importance of conserving local genetic resources. Combined with field survey data, these findings will provide actionable levers for improving beekeeping practices, strengthening agroecosystem resilience, and supporting sustainable development in Côte d'Ivoire.

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CUBITAL INDEX VARIABILITY IN *APIS MELLIFERA*: EFFECTS OF SEASON AND QUEEN MATING STRUCTURE

Taja Falnoga, Ajda Moškrič, Katarina Mole, Jernej Bubnič, Andraž Marinč, Klavdija Ceglar, Janez Prešern

Agricultural Institute of Slovenia, Ljubljana, Slovenia

Abstract

The cubital index is an established morphometric parameter of honey bee wing venation, defined as the ratio of the lengths of two cubital veins on the forewing. Due to its stability and diagnostic value, it is widely used in taxonomic studies to distinguish between species and subspecies within the genus *Apis*. Numerous studies confirm its usefulness in analyzing population differences and geographic variability.

In this study, nine *A. mellifera carnica* colonies were monitored: two headed by artificially inseminated queens mated with multiple drones and seven headed by queens mated with a single drone. Colonies were sampled repeatedly throughout the season, and the cubital index was determined for each worker on both the left and right forewing.

We examined seasonal variability of the cubital index, differences between left and right wings, and variability among colonies. In particular, we investigated whether differences exist between colonies headed by single-drone–mated queens and those headed by multi-drone–mated queens, and whether the number of fathers influences the variability of the index. In this paper, we discuss the observed patterns and present new insights into the use of the cubital index as a morphometric tool in the honey bee *Apis mellifera*.

POPULATION GENOMICS OF ASCOSPHAERA APIS REVEALS GENETIC DIVERSITY AND RECOMBINATION ACROSS HONEY BEE COLONIES WITHIN APIARIES

P 145

Barbara Hočevar[1], Darja Kušar[2], Metka Pislak Ocepek[1], Irena Zdovc[2], Bojan Papić[2], Rene Rus[1], Monika Kozar[1], Lucija Žvokelj[1], Cene Gostinčar[3]

[1]Institute of Pathology, Forensic Veterinary Medicine, Wildlife, Bees and Aquaculture, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia
[2]Institute of Microbiology and Parasitology, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia
[3]Chair of Molecular Genetics and Biology of Microorganisms, Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Abstract

Chalkbrood is a fungal disease of honey bee (*Apis mellifera*) larvae caused by *Ascosphaera apis*. Despite its global distribution and impact on colony health, the population genetic structure and evolutionary dynamics of this pathogen remain poorly understood. In particular, little is known about genome-wide genetic variation and the role of recombination in natural populations of *A. apis*. This study aimed to investigate genetic diversity, population structure, and signatures of recombination in isolates of *A. apis* collected from honey bee colonies.

Chalkbrood mummies were collected from two apiaries: one hive from apiary A and two hives from apiary B, with three mummified larvae sampled per hive. From each larva, fungal colonies were cultured and three hyphal isolates were obtained, yielding a total of 27 isolates. DNA was extracted from single mating type *A. apis* mycelia, followed by paired-end Illumina whole-genome sequencing and identification of single nucleotide polymorphisms (SNPs). Population genomic analyses included estimates of nucleotide diversity, multivariate analyses of population structure (PCA and DAPC), haplotype network reconstruction, linkage disequilibrium (LD) decay analysis, recombination detection tests and assessment of selection pressure using dN/dS ratios.

Genome-wide SNP analysis revealed genetic variation among isolates. Multivariate analyses showed genetic clustering consistent with population structure among sampling units. Haplotype network analyses identified multiple haplotypes and their hierarchical relationships. Some larvae were represented by a single dominant haplotype, while others showed evidence of multiple haplotypes. Linkage disequilibrium decreased with increasing genomic distance, and recombination tests rejected a strictly clonal model of evolution. Estimates of dN/dS indicated that purifying selection predominates across coding regions.

These results demonstrate that *A. apis* populations in honey bee colonies are genetically diverse and exhibit signatures of recombination. Genome-wide analyses provide new insights into the evolutionary processes shaping *A. apis* populations and contribute to a better understanding of chalkbrood disease.

P 146 THE ROLE OF THE ANG FOUNDATION IN CONSERVING *APIS MELLIFERA CAUCASICA* AND *APIS MELLIFERA ANATOLIACA* IN TÜRKIYE

İrfan Kandemir

Ankara University, Ankara, Turkey. ANG Foundation, Istanbul, Turkey

Abstract

Türkiye being a pristine area attracts all attention to its very rich geographic characteristics, rich flora and fauna and also being a bridge connecting three continents. This virgin geography on the spice and silk routes is also the intersection of the honey bee subspecies and is a beekeeping paradise due to harboring many honey bee subspecies and ecotypes. The conservation of native honey bee subspecies is of critical importance for maintaining biodiversity, ecological resilience, and sustainable apiculture. In Türkiye, the ANG Foundation has undertaken significant initiatives to protect and preserve the genetic integrity of two important honey bee subspecies, *Apis mellifera caucasica* and *A. m. anatoliaca*. These subspecies represent valuable genetic resources adapted to diverse ecological conditions and contribute substantially to pollination services and honey production.

The ANG Foundation's conservation strategy focuses on the protection of native populations through the establishment of isolated breeding areas, implementation of controlled queen breeding programs, and promotion of sustainable beekeeping practices. Particular emphasis has been placed on reducing genetic introgression caused by uncontrolled queen and colony movements. Genetic monitoring, collaboration with research institutions, and awareness-raising activities among beekeepers have further strengthened conservation efforts. For *A. m. caucasica*, conservation activities have concentrated on maintaining genetically pure populations within designated protected regions (Camili-Artvin and Posof-Ardahan), while for *A. m. anatoliaca*, efforts have focused on preserving locally adapted honey bee ecotypes in central Anatolia (Kırkırca-Kızılcahamam-Ankara).

The ANG Foundation's work demonstrates the importance of integrating scientific, regulatory, and community-based approaches in honey bee conservation. These efforts contribute not only to the preservation of Türkiye's apicultural heritage but also to the long-term sustainability of beekeeping and ecosystem services. Continued conservation and monitoring programs are essential to safeguard the genetic diversity and adaptive potential of these native honey bee subspecies in the face of increasing environmental and anthropogenic pressures.

WORKER TASK, NOT AGE, DETERMINES IMMUNE RESPONSE IN THE PAPER WASP *POLYBIA PAULISTA*

P 147

Tatjana Krama[1], Indriķis Krams[1], André Rodrigues de Souza[2]

[1]Daugavpils University, Daugavpils, Latvia
[2]University of São Paulo, São Paulo, Brazil

Abstract

In social insects' immune function is often assumed to decline with age due to immunosenescence. However, behavioural roles may also play a key role in shaping immune investment, particularly when individuals differ in exposure to pathogens. In this study we investigated the relative importance of age and task in determining immune response in workers of the paper wasp *Polybia paulista*.

We measured melanotic encapsulation response, a key component of insect immunity, in workers performing different tasks (nurses, guards and foragers) under natural colony conditions. In addition, we assessed immune response in individuals reared under laboratory conditions, where behavioural specialization was experimentally prevented. Age was estimated using both, relative and absolute measures.

Under field conditions immune response differed among tasks, but not with age. Foragers exhibited significantly higher melanotic encapsulation than guards, regardless of age, while nurses showed similar levels to foragers. In contrast, under laboratory conditions, where behavioural specialization was absent, immune response declined with age, indicating immunosenescence in the absence of task differentiation.

These results demonstrate that immune function in social wasps is primarily shaped by behavioural roles rather than chronological age. Elevated immune investment in foragers likely reflects increased exposure to pathogens outside the nest, supporting the exposure risk hypothesis.

Our findings highlight the importance of behavioural plasticity in modulating physiological traits and suggest that task-dependent immunity may represent an adaptive mechanism to mitigate infection risk in social insects.

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MAPPING MUTATIONAL DENSITY ACROSS HONEY BEE CYTOCHROME P450S

Fernanda Li[1], Daniela Barbosa[1], Sana Bashir[1], Carlos A. Yadró García[1], Anais Rodriguez[2], Annelise Rosa-Fontana[1], José A. Rufino[3], Raquel Martín-Hernández[4], MEDIBEES Consortium[5], Maria Alice Pinto[1], Dora Henriques[1]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[2]Centro de Investigaciones Apícolas, La Habana, Cuba
[3]CeDRI, SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[4]Centro de Investigación Apícola y Agroambiental (CIAPA). Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF), Marchamalo, Spain
[5]Various institutions, Bragança, Portugal

Abstract

Honeybees, which are exposed to a wide range of xenobiotics, rely on detoxification machinery to cope with chemical environmental challenges. Among the key enzymes involved in this process, cytochrome P450 monooxygenases (P450s) play a central role, as they catalyse the first oxidative step in xenobiotic biotransformation and are key determinants of metabolic capacity toward a broad range of exogenous substances. P450 proteins share a conserved catalytic core but display considerable variability in their substrate recognition sites (SRSs), which are directly responsible for substrate binding and specificity. In humans, nonsynonymous variants within these regions are known to be drivers of inter-individual variation in metabolic capacity toward xenobiotics. However, whether analogous functional polymorphisms shape metabolic phenotypes in honeybees remains an open question.

In this study, we investigated honeybee P450s and characterized the distribution of naturally occurring mutations across these proteins. Using the dataset generated by the MEDIBEES project (1467 drones, 25 countries, and 18 subspecies), we focused on nonsynonymous single-nucleotide polymorphisms (SNPs) to construct a residue-level map of mutational density across P450s. Our working hypothesis is that the catalytic core would be highly conserved, whereas most mutations would occur in structurally flexible regions, including SRSs.

Consistent with our predictions, SNPs were concentrated in flexible loop regions (48%) while residues in the catalytic core remained invariant. In addition, we assessed SNP functional impact in the best-characterised pesticide detoxification P450s, as allelic differences here may explain variation in pesticide response. High-impact SNPs were rare (<1%), and, when frequent, never occurred in isolation. This suggests epistatic buffering, where co-occurring SNPs mitigate the effects of more deleterious substitutions. Together, these findings advance our mechanistic understanding of substrate access and catalytic specificity in *A. mellifera* P450s. This knowledge may help predict xenobiotic metabolism in honeybees and guide the development of bee-safe agrochemicals.

MOLECULAR INSIGHTS INTO HONEY BEE ADAPTATION: STRUCTURAL EFFECTS OF MISSENSE SNPS IN RESPONSE TO CLIMATE CHANGE

P 149

Daniela Lima[1,2], Ana R. Lopes[1], Demetris Taliadoros[3], Carlos A. Yadró García[1], Raquel Martín-Hernández[4], José A. Rufino[5], Dirk C. de Graaf[6], Matthew T. Webster[7], Dora Henriques[1], Maria Alice Pinto[1]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[2]Pontificia Universiade De Minas Gerais, Belo Horizonte, Brazil
[3]Department of Medical Biochemistry and Microbiology, Sciencefor Life Laboratory, Uppsala University, Uppsala, Sweden
[4]Centro de Investigación Apícola y Agroambiental (CIAPA). IRIAF. Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Marchamalo, Spain, Marchamalo, Spain
[5]CeDRI, SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[6]Ghent University, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium
[7]Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Uppsala University, Uppsala, Portugal

Abstract

Climate change poses significant challenges to honey bee populations by intensifying thermal and environmental stress, making it crucial to understand how populations respond to these pressures or exhibit local adaptation to contrasting climatic conditions. Populations may adapt through regulatory changes affecting gene expression or through non-synonymous mutations that directly alter protein structure and function. This study investigated missense candidate single-nucleotide polymorphisms (SNPs) in European honey bee genomes associated with climate variables to evaluate their potential contribution to local adaptation under varying climatic conditions. From an initial dataset spanning 115 candidate genes, structural analyses were conducted on 17 high-confidence proteins (AlphaFold quality score >85). Each variant was assessed using complementary computational approaches, including SIFT and PolyPhen-2 (functional prediction), I-Mutant, DynaMut2, and Rosetta (stability estimation), and Missense3D (structural assessment). Variants flagged as potentially disruptive in any of these analyses were subsequently subjected to molecular dynamics simulations. Although most variants appeared tolerated or benign, a small subset showed convergent evidence for structural or functional impact. One representative mutation, Y483C in XP_006570411 (LOC552695), exhibited increased local flexibility, higher solvent-accessible surface area, a slight increase in radius of gyration, and reduced hydrogen bonding in 5×100 ns MD simulations, consistent with local packing disruption. LOC552695, although uncharacterized in *A. mellifera*, contains predicted RCC1 and BTB/POZ domains typically associated with cell-cycle regulation, chromatin interaction, protein–protein binding, and ubiquitin-mediated proteolysis. Variants affecting this gene, particularly those disrupting stability, solvent exposure, or residue mobility, may influence how populations respond to environment-specific pressures. These results highlight a focused set of high-priority candidates for functional validation and provide mechanistic insight into the genomic basis of climate-related adaptation in European honey bees.

P 150 MITOCHONDRIAL DIVERSITY OF HONEY BEES ACROSS EUROPE REVEALS HUMAN-MEDIATED DISRUPTION OF THE NATIVE LINEAGES

Ana R. Lopes[1], Fernanda Li[1], Maíra Costa[2], Carlos A. Yadró García[1], Dora Henriques[1], Laima Blažytė-Čereškienė[3], Robert Brodschneider[4], Valters Brusbardis[5], Norman L. Carreck[6], Róbert Chlebo[7], Mary Coffey[8], Ellen Danneels[9], Constantin Dobrescu[10], Vincent R. Douarre[11], Anna Dupleix-Marchal[12], Mariia M. Fedoriak[13], Janja Filipi[14], Anna M. Gajda[15], Linn F. Groeneveld[16], Fani Hatjina[17], Jes Johannesen[19], Michał Kolasa[19], Marin Kovačić[20], Preben Kristiansen[21], János Körmendy-Rácz[22], Grace P. McCormack[23], Raquel Martín-Hernández[24], Maritta Martikkala[25], Robert J. Paxton[26], Marco Pietropaoli[27], Benjamin Poirot[28], Aivar Raudmets[29], Zheko Radev[30], Ivo Roessink[31], Gabriele Soland[32], Maja I. Smodiš Škerl[33], Dalibor Titera[34], Jacques van Alphen[35], Andri Varvana[36], Flemming Vejsnæs[37], Matthew T. Webster[38], Dirk C. de Graaf[9], Maria Alice Pinto[2]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal. [2]LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal. [3]Nature Research Centre, Vilnius, Lithuania. [4]University of Graz, Graz, Austria. [5]Latvian Beekeepers Association, Jelgava, Latvia. [6]Carreck Consultancy Ltd, West Sussex, United Kingdom. [7]Slovak University of Agriculture in Nitra, Nitra, Slovakia. [8]Department of Agriculture, Food and the Marine, Celbridge, Ireland. [9]Ghent University, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium. [10]ROMAPIS, București, Romania. [11]FEdCAN, European federation for the conservation of dark bee, Pont-de-Montvert, France. [12]Laboratoire CRISES EA 4424, Université Paul Valéry, Montpellier, France. [13]Department of Ecology and Biomonitoring, Chernivtsi, Ukraine. [14]University of Zadar, Zadar, Croatia. [15]Warsaw University of Life Sciences, Warszawa, Poland. [16]Norges Birøkterlag (Norwegian Beekeepers Association), Lillestrøm, Norway. [17]Institute of Animal Science & Department of Apiculture, Chalkidiki, Greece. [18]Fachzentrum Bienen und Imkerei, DLR-Westerwald-Osteifel, Mayen, Germany. [19]Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, Poland. [20]Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia. [21]Apinordica AB, Tjällmo, Sweden. [22]Hungarian Apitherapy Society, Nagykovácsi, Hungary. [23]University of Galway, Galway, Ireland. [24]Centro de Investigación Apícola y Agroambiental (CIAPA). IRIAF. Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Marchamalo, Spain. [25]Finnish Beekeepers' Association, Helsinki, Finland. [26]Martin Luther University Halle-Wittenberg, Halle, Germany. [27]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Viterbo, Italy. [28]APINOV, Lagord, France. [29]Estonian Beekeepers Association, Rapla, Estonia. [30]Tobacco and tobacco products institute, Agricultural Academy, Markovo, Bulgaria. [31]Wageningen Environmental Research, Wageningen, Netherlands. [32]Apigenix, Institute for beegenetics, Twann, Switzerland. [33]Agricultural Institute of Slovenia, Ljubljana, Slovenia. [34]Bee Research Institute, Libčice nad VItavou, Czechia. [35]Naturalis Biodiversity Centre, Leiden, Netherlands. [36]Cyprus University of Technology, Limassol, Cyprus. [37]Danish Beekeepers Association, Fulbyvej, Denmark. [38]Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden.

Abstract

The European free market offers significant advantages across many commercial sectors. However, in beekeeping, it poses a potential threat to the genetic integrity of native subspecies. Due to their behavioural traits, being docile and high honey producers, subspecies from the maternal lineage C, *A. m. carnica* and *A. m. ligustica*, have been widely introduced into the native range of lineage M, *A. m. mellifera*. Here, we present a large-scale survey using mitochondrial DNA to assess the current status of genetic introgression across Europe. A total of 1,305 colonies from 35 countries were analysed. DNA was extracted from the thorax of one worker per colony, and subjected PCR amplified at the mitochondrial tRNAleu-COX2 intergenic region, followed by sequencing of the amplicon. Sequences were aligned to A-, M-, and C-lineage reference databases to assign haplotypes and variants using MEGA12. Our dataset revealed a high dominance of C-lineage colonies (65.4%), followed by M-lineage (25.4%) and A-lineage (9.2%). High levels (>80% of colonies) of C-lineage introgression were observed in Finland, the Netherlands, Belgium, Sweden, Germany, and France. M-lineage colonies were mainly confined to conservation apiaries (93.5% of the 139 colonies sampled across France, Sweden, Norway, Switzerland, the UK, and the Netherlands) and to the Iberian Peninsula, where A-lineage colonies were also prevalent. We identified 41 haplotypes (22 M, 13 A, 6 C) and 190 variants, of which 115 are reported here for the first time. Additionally, eight novel haplotypes were detected (1 A, 4 M, 3 C). Unbiased haplotype diversity was highest in the Netherlands (0.872) and lowest in Ukraine (0.08), while unbiased variant diversity peaked in French conservation apiaries (0.941) and was lowest in North Macedonia (0.389). These findings highlight complex patterns of maternal diversity and the widespread genetic replacement of native honey bee populations across much of Europe.

EVALUATING HONEYBEE MATING CONTROL SUCCESS USING *CSD* ALLELIC VARIATION AND GENOTYPE-BY-SEQUENCING

P 151

Ajda Moškrič[1], Katarina Mole[1], Jana Obšteter[1], Jernej Bubnič[1], Andraž Marinč[1], Janez Prešern[1], Maja I. Smodiš Škerl[1], Anja Pavlin[1], Lenka Jerele[1], Taja Falnoga[1], Klavdija Ceglar[1], Črt Zega[1], Agnieszka Laszkiewicz[2], Joanna Niedbalska-Tarnowska[2], Kinga Adamczyk-Węglarzy[2], Natalia Romek[2], Kristina Jancelowska[2], Patryk Skinderowicz[2], Malgorzata Cebrat[2]

[1]Kmetijski inštitut Slovenije, Ljubljana, Slovenia
[2]Laboratory of Molecular and Cellular Immunology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wroclaw, Poland

Abstract

In honeybee breeding, effective mating control is essential for genetic improvement, yet its evaluation remains slow and labor-intensive. Queens mate with multiple drones and store sperm for lifelong use, so mating success is typically assessed indirectly by genotyping worker brood. This approach delays evaluation and requires large sample sizes to accurately resolve patriline composition. The GenoDrone project addresses this limitation by developing a faster method based on genotyping sperm from the queen's spermatheca. We propose using allelic diversity at the complementary sex determiner (*csd*) locus as a proxy for patriline composition and identification of paternal genotypes.

This study evaluated the utility of *csd* variation for resolving patriline structure and assessing mating control success. We analyzed colonies from two controlled contexts: geographically isolated mating experiments and instrumentally inseminated colonies with known drone origin. Samples included queens, worker brood, drones, and drone semen. Methods comprised DNA extraction, *csd* fragment amplification, T-RFLP analysis, Sanger sequencing, cloning of diploid *csd* alleles, and genome-wide SNP genotyping (GBS).

Results revealed high allelic diversity at the *csd* locus and demonstrated its strong potential as a single-marker tool for patriline identification. In controlled mating colonies, *csd*-based analyses showed good concordance with genomic data in identifying fathers and estimating patriline structure. In instrumentally inseminated colonies, all *csd* variants present in the insemination set were recovered in worker offspring, confirming high discriminatory power. Although genome-wide SNP data provided higher resolution, particularly among related drones, *csd* analysis proved to be a rapid, informative, and cost-effective alternative.

These findings support *csd* genotyping as a promising tool for early evaluation of mating control success and a basis for spermatheca-based diagnostics in honeybee breeding and conservation.

P 152 GAME OF DRONES: ASSESSING FINE-SCALE POPULATION STRUCTURE OF *APIS MELLIFERA MELLIFERA* USING MEDIUM-DENSITY SNP DATA

Markus Neuditschko[1], Dylan Elen[2]

[1]Agroscope, Posieux, Switzerland
[2]ZwarteBij.org, Gavere, Belgium

Abstract

The assessment of fine-scale population structure is critical for the conservation and management of the European dark honey bee (*Apis mellifera mellifera*). Wing geometric morphometrics and molecular marker panels remain the principal tools for identifying honeybee origins and delineating subspecies. However, previous studies have primarily focused on quantifying admixture levels in Western honeybees (*Apis mellifera*), while assessments of inbreeding and genetic contribution and its potential consequences for population integrity have received far less attention. To address this gap, we complemented admixture analyses with estimates of individual genetic contributions and inbreeding levels of *Apis mellifera* and integrated these metrics into a high-resolution network visualization. We analyzed 380 honey bees (283 workers and 97 drone-pools) from 14 European breeding populations, including recently established Bosland population of *Apis mellifera mellifera*, genotyped at 70,814 genome-wide SNPs. Runs of homozygosity analysis revealed considerable variation within the Bosland population, where a subset of drone-pools and workers exhibited low genomic inbreeding levels, reflecting high admixture, whereas C-lineage (*Apis mellifera* carnica and *Apis mellifera ligustica*) workers also exhibited low inbreeding. In contrast, *Apis mellifera mellifera* from three French islands and *Apis mellifera mellifera* drone-pools showed on average the highest inbreeding values. High-resolution network visualization effectively resolved population structure, clearly distinguishing M- from C-lineage, and captured fine-scale genetic relatedness, enabling the identification of relationships among sister workers and sister queens, as well as between queens and their offspring. Overall, our results provide a robust framework for genomics-informed monitoring and conservation of *A. m. mellifera* and demonstrate the utility of medium-density SNP data for resolving both fine-scale population structure and genomic inbreeding.

EXPRESSION AND ISOFORM DIVERSITY OF *DSCAM* TRANSCRIPTSIN THE HONEY BEE

P 153

Joanna Niedbalska-Tarnowska, Patryk Skinderowicz, Natalia Romek, Agnieszka Laszkiewicz, Kristina Jancelowska, Weronika Laszkiewicz, Malgorzata Cebrat

Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Abstract

Dscam (Down syndrome cell adhesion molecule) is a protein that plays an important, yet not fully understood, role in insects and crustaceans. It is crucial for nervous system development as well as for immune processes. *Dscam* transcript undergoes extensive alternative splicing, which may generate a very large number of isoforms, potentially exceeding 18,000 in the honey bee. This remarkable diversity results from the presence of three clusters of alternatively spliced exons within the *dscam* gene. The variability between isoforms is primarily located in three immunoglobulin-like domains, while alternative splicing of the transmembrane region further contributes to functional diversity.

The aim of this study is to investigate differences in the expression levels and diversity of *Dscam* isoforms among honey bee individuals, families, developmental stages, and organs. To assess *Dscam* isoform diversity, transcripts were sequenced using Oxford Nanopore long-read sequencing technology, enabling the identification of complex splicing patterns and characterization of isoform variability. Differences in *dscam* expression levels between honey bee organs and individuals at different developmental stages were quantified using real-time RT-PCR, allowing for precise comparison of overall transcript abundance across samples.

To our knowledge, such a comprehensive analysis of *dscam* expression and isoform diversity in the honey bee has not been performed before. This study may provide new insights into the role of extensive isoform diversity in developmental and immune processes.

The results will help address the question of whether *dscam* splicing is largely stochastic or regulated by specific factors that contribute to organ-specific expression patterns. Understanding the mechanisms governing *Dscam* isoform diversity may provide new insights into honey bee immune competence and its potential role in colony health and resilience to pathogens.

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A QUANTITATIVE PERSPECTIVE ON SEX-SPECIFIC FEMINIZER TRANSCRIPTS EXPRESSION IN HONEY BEE DEVELOPMENT

Joanna Niedbalska-Tarnowska[1], Agnieszka Laszkiewicz[1], Ajda Moskric[2], Janez Prešern[2], Kinga Adamczyk-Węglarzy[1], Natalia Romek[1], Malgorzata Cebrat[1]

[1]Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
[2]Agricultural Institute of Slovenia, Ljubljana, Slovenia

Abstract

Sex determination in the honey bee (*Apis mellifera*) is a key process shaping colony structure and function, yet the molecular dynamics underlying this pathway remain incompletely understood. The complementary sex determiner (*csd*) gene initiates sex-specific splicing of the downstream *feminizer* (*fem*) transcript, but previous studies have reported inconsistent patterns of *fem* expression between sexes.

In this study, we developed and optimized a robust RT-qPCR approach to accurately quantify female-specific (fem^F) and male-specific (fem^M) transcripts across developmental stages. Particular attention was given to technical challenges associated with AT-rich sequences, repeated regions, and cDNA instability, which can compromise reliable transcript detection and quantification. Using this optimized approach, we analyzed transcript levels in eggs, larvae, and pupae of both sexes.

We show that fem^F expression is strongly female-biased, with approximately 100-fold higher levels in females compared to males. In contrast, fem^M exhibits weaker sex specificity, with only ~10-fold higher expression in males. Notably, fem^F transcripts are detectable in males, while fem^M expression increases in females during late pupal stages. Quantitative comparisons further reveal that fem^M levels in males are comparable to fem^F levels in females, suggesting that the male transcript - despite containing a premature stop codon - is not efficiently degraded by mRNA surveillance mechanisms.

These findings demonstrate that fem transcript dynamics are more complex and less strictly sex-limited than previously assumed. Our results provide a validated framework for studying sex determination at the molecular level and have important implications for interpreting gene expression data in honey bee development, reproduction, and colony fitness.

CHARACTERIZATION OF IONOTROPIC RECEPTORS INVOLVED IN HOST DETECTION IN *VARROA DESTRUCTOR*

P 155

Sapir Nutkewitz, Eitan Reuveny

Weizmann Institute of Science, Rehovot, Israel

Abstract

Varroa destructor is the most destructive ectoparasite of honeybees and a major contributor to colony losses worldwide. Current control methods rely largely on acaricides, but the emergence of resistant mite populations highlights the urgent need for selective, sustainable alternatives. Because *Varroa* relies on chemical cues to locate hosts, reproduce, and survive within the hive, its chemosensory system represents an attractive yet largely unexplored target for intervention. Here, we present a systematic molecular characterization of the ionotropic receptor (IR) repertoire of *V. destructor*. Using comparative genomic analyses, complemented by a published transcriptomic dataset, protein domain annotation, and phylogenetic reconstruction, we identified fifteen candidate IR-family proteins, including conserved homologs of the co-receptors IR25a and IR93a. To investigate receptor organization, all candidates were cloned and screened for pairwise interactions using Förster Resonance Energy Transfer (FRET) in heterologous *Drosophila* S2 cells. This analysis generated the first interaction map of putative *Varroa* IR complexes, revealing several candidate receptor assemblies. To validate these findings *in vivo*, custom antibodies against Vd_IR25a and Vd_IR93a were generated and used to localize both proteins to sensory structures within the mite forelegs, the principal chemosensory organs of *Varroa*.

Together, these findings present the first attempt to characterize the molecular framework of ionotropic receptor organization in *V. destructor*. The receptor complexes identified here provide the foundation for a functional ligand-screening platform to identify compounds that activate or inhibit *Varroa* chemosensory receptors. By targeting the sensory mechanisms that enable *Varroa* to locate and exploit honeybee hosts, this work lays the foundation for future sensory-based strategies for sustainable *Varroa* management.

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STUDY OF THE F290L MUTATION IN *VARROA DESTRUCTOR*: EIGHT-MONTH PREVALENCE AFTER AMITRAZ APPLICATION AND ITS RELATIONSHIP WITH ACARICIDE RESISTANCE

Antonio Pérez-Pérez[1], Carolina Bartolomé[2], Soledad Sagastume[1], Julio M. Maside[3], Raquel Martín-Hernández[1], Mariano Higes[1]

[1]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Centro de Investigación Apícola y Agroambiental de Marchamalo, (CIAPA), Laboratorio de Patología Apícola, Marchamalo, Spain
[2]Fundación Pública Galega de Medicina Xenómica (FPGMX), Hospital Clínico Universitario de Santiago, Edificio de Consultas, Galicia, Santiago de Compostela, Spain
[3]Departamento de Zoologia, Genética e Antropologia Física, Faculdad de Biología Universidad de Santiago de Compostela, Galicia, Santiago de Compostela, Spain

Abstract

Varroa destructor causes significant mortality in honeybee colonies worldwide. Currently, chemical control remains the predominant strategy, with amitraz being one of the most widely used acaricides. Prolonged use of amitraz may promote the emergence of resistant mites through mutations in the octopamine receptor (Octβ2R), although the evidence supporting this relationship is inconclusive.

To investigate the association between amitraz application and the frequency of the F290L mutation, 40 colonies were selected from a professional beekeeper in Marina Alta (Alicante, Spain), treated with amitraz between October and November 2023. The colonies were then moved to the experimental apiaries of IRIAF-CIAPA (Marchamalo, Guadalajara, Spain), to prevent external reinfestation and ensure controlled conditions. After adapting to these conditions without further acaricide treatments, mites were collected using trays placed on the hive floors, which were inspected weekly throughout August 2024. Genetic sequences were obtained from 28 randomly selected mites from four colonies.

Molecular analysis revealed the presence of the F290L mutation in 53.6% of the mites, with significant variability between colonies (0–100%). These results do not show a direct and uniform relationship between amitraz application and the frequency of the mutated allele, suggesting that acaricide resistance mechanisms under field conditions are complex and multifactorial in cases of therapeutic failures.

These findings highlight the need for further research to understand the molecular processes that may contribute to potential amitraz resistance and emphasize the importance of integrated pest management strategies that reduce selective pressure on *V. destructor*, promoting the sustainability of beekeeping in Spain and internationally.

MAINTENANCE OF HETEROZYGOSITY IN THE PARASITIC CAPE HONEYBEE *APIS MELLIFERA CAPENSIS*

P 157

Johanna T. Pieplow[1], Michael H. Allsopp[2], Christian W. W. Pirk[3], Abdullahi A. Yusuf[3], Ezette E. du Rand[3], Eckart Stolle[1]

[1]Leibniz Institute for the Analysis of Biodiversity Change (LIB), Bonn, Germany
[2]Agricultural Research Council (ARC), Stellenbosch, South Africa
[3]University of Pretoria, Pretoria, South Africa

Abstract

The Cape Honeybee *Apis mellifera capensis* is a subspecies of the Western Honeybee, native to the fynbos biome in South Africa. Some Cape Honeybee workers are able to produce female offspring from unfertilized eggs via thelytoky with central fusion, a form of parthenogenesis where diploidy is restored by the fusion of meiotic products. While this mode of reproduction may be adaptive, e.g. to re-queen the colony from worker-laid eggs, it is predicted to result in a loss of heterozygosity (LOH) and therefore in a reduction of genetic variation and fitness. Despite this detrimental effect, a permanently thelytokous, parasitic lineage of Cape bees is persisting for over 30 years, while large fractions of its genome retained heterozygosity. Yet it remained unclear whether this is a consequence of reduced recombination or strong selection against homozygotes and what long-term effects there are.

Using whole genome sequence data of families as well as developmental and population timelines of South African Honeybees from the native population and the parasitic lineage, we estimate recombination rates in queens and workers, the level of selection against LOH during development and the long-term consequences of thelytokous reproduction in the parasitic lineage. We show that Recombination is largely reduced in thelytokous Cape bees and how selection against homozygotes may play a role over long periods of time.

The Cape Honeybee represents an ideal study system to investigate mechanisms of preserving heterozygosity despite asexual reproduction and serves as a model for other thelytokous organisms.

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SARDINIA AND CORSICA: GENETIC DIFFERENCE, DESPITE GEOGRAPHICAL PROXIMITY

Giovanna Salvatore[1], Pierre Faux[2], Valentino Palombo[1], Mariasilvia D'Andrea[1], Alain Vignal[2]

[1]Department of Agriculture, Environment and Food Sciences, University of Molise, Campobasso, Italy
[2]GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet Tolosan, France

Abstract

A. m. mellifera and *A. m. ligustica* are the historically native honey bee subspecies respectively in France and Italy and their genetic differentiation is generally attributed to postglacial expansion from distinct refugia and long-term geographic isolation across the Alpine barrier. However, morphometric studies suggest that a similar differentiation is found between the two islands of Sardinia and Corsica, two Mediterranean islands distant by only 12 km. A first small-scale structure analysis of Corsican bees showed this population to be admixed between *A. m. mellifera* (~80%) and *A. m. ligustica* bees (~20%). We present here results from an extended sampling of Corsican and Sardinian bees, analysed by whole genome sequencing and compared to reference populations, including from mainland France and Italy. Analyses on the nuclear genome show that the additional samples of Corsican bees are admixed in similar proportions as the previous ones, while Sardinian bees are monomorphically of the Italian *A. m. ligustica* background. However, results on the mitochondrial genome show a contrasting pattern, with all Corsican samples having an *A. m. mellifera* mitogenome and Sardinian ones having both *A. m. mellifera* and *A. m. ligustica* ones, reflecting a pattern also found in mainland Italy.

HOW DO HONEY BEE WORKERS CHOOSE THEIR QUEEN?

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Shimshon T. Sefcik, Sivan Leibovitch, Hagai Y. Shpigler

Volcani Institute of Agriculture, Beit Dagan, Israel

Abstract

The queen bee is the primary reproductive unit of the honey bee colony. Every fertilized egg possesses the genetic potential to develop into a queen, as the developmental trajectory is determined by the nutritional and behavioral care provided by the workers. This study addresses the scientific question of larval selection: how do worker bees determine which larva to raise into a queen when multiple candidates are available?

To study this question, we developed a new controlled laboratory trial using worker cohorts of approximately 230 mixed-aged bees in cages placed in an environmental control room with day-old larvae to track queen-rearing output. In the first experiment, a single larva was introduced per cage to establish a baseline for rearing capacity; the workers successfully raised a queen in 90% of cases. In the second experiment, two larvae were grafted per cage to evaluate if the workers raise two queens or select only one. Our results indicated that workers prioritized the development of only one larva in 92% of cases. The observation that workers prioritize a single larva suggests a specific strategy of resource allocation. By focusing on a single individual, the group likely maximizes the probability of successful queen development.

We used these findings to investigate whether workers distinguish between larvae based on genetic kinship. We presented groups of bees from a single colony with a choice between nestmate larvae and larvae of foreign origin. Our results across multiple trials showed that workers selected the larva from their own hive in 38% of the cases, suggesting that the workers do not prioritize larvae based on their genetic origin but by other parameters. In conclusion, these laboratory experiments provide a new framework to study honey bee behavior and the specific mechanisms workers use to choose a larva for queen-rearing.

P 160 ACCELERATED BREEDING IN HONEY BEES: AN INNOVATIVE APPROACH INTEGRATING NATURAL SELECTION FOR VARROA RESISTANCE

Aleksandar Uzunov[1,2,3], Raffaele Dall'Olio[4], Martin S. Gabel[5], Małgorzata Bieńkowska[6], Andreas Hoppe[7], Vittorio Capano[8], Richard Bernstein[7], Marina D. Meixner[5], Jesús Yániz[9], Goran Aleksovski[1], Ralph Büchler[10], Cecilia Costa[8]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Skopje, North Macedonia
[2]Macedonian Academy of Sciences and Arts, Research Center for Environment and Materials, Skopje, North Macedonia
[3]State Key Laboratory of Resource Insects, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, China
[4]BeeSources, Bologna, Italy
[5]LLH Bee Institute Kirchhain, Kirchhain, Germany
[6]National Research Institute of Horticulture, Puławy, Poland
[7]Länderinstitut für Bienenkunde Hohen Neuendorf, Hohen Neuendorf, Germany
[8]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[9]Universidad de Zaragoza, Huesca, Spain
[10]ApiPhil, Kirchhain, Germany

Abstract

In the BEE-GUARDS project (WP2 - Innovative breeding), we are testing a fast-track honey bee breeding concept that compresses the traditional 24-month cycle into just 12 months. The approach also combines performance testing with controlled exposure to *Varroa*, introducing a natural selection component to reveal resilient genotypes more quickly. Since 2023, four countries: (MK, IT, PL, and DE) each run two parallel apiaries: a conventional "business as usual" colony management with regular frequent *Varroa* control, and an innovative *Varroa*-challenged concept with limited interventions to specifically increase selective pressure. Multiple traits are measured in performance testing apiaries, which also include drone- producing colonies. Generations are already advancing: F2 in MK and DE, F3 in IT, using isolated mating stations, and thanks to exclusive instrumental insemination, F4 in PL. Further analyses and molecular sampling are ongoing, and the next phase will involve beekeepers who will test the selected genotypes under a production-oriented management through a full-season Citizen Science Investigation. In parallel, WP2 is developing innovative reproductive technologies, including sperm and embryo cryopreservation, to support long-term genetic conservation and accelerated breeding strategies.

CONSERVATION STATUS OF THE HONEY BEE SUBSPECIES NATIVE TO THE MEDITERRANEAN ISLANDS

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Carlos A. Yadró García[1,2,3], Dora Henriques[1], Marion Zammit Mangion[4], Thomas Galea[5], Giovanni Cilia[6], Antonio Nanetti[6], Mustafa Necati Muz[7], Androulla Varnava[8], Fani Hatjina[9], Leonidas Charistos[9], José A. Rufino[10], Raquel Martín-Hernández[11], Maria Alice Pinto[12]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal. [2]Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Porto, Portugal. [3]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Porto, Portugal. [4]Department of Physiology and Biochemistry, University of Malta, Msida, Malta. [5]Research and Advisory Services Directorate Rural Affairs Department, Ministry for Agriculture, Fisheries and Animal Rights, Qormi, Malta. [6]CREA Research Centre for Agriculture and Environment, Bologna, Italy. [7]Department of Parasitology, Faculty of Veterinary Medicine, University of Namik Kemal, Tekirdag, Turkey. [8]Department of Agricultural Sciences, Biotechnology and Food Science, Faculty of Geotechnical Sciences and Environmental Management, Cyprus University of Technology, Limassol, Cyprus. [9]Department of Apiculture, Institute of Animal Science, Hellenic Agricultural Organization 'DEMETER', Nea Moudania, Greece. [10]CeDRI, SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal. [11]Centro de Investigación Apícola y Agroambiental (CIAPA). IRIAF. Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Marchamalo, Spain. [12]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal.

Abstract

The Mediterranean islands have been a stage for honey bee diversification, hosting four of the 31 recognised subspecies: *Apis mellifera siciliana* (Sicily), *A. m. ruttneri* (Malta), *A. m. cypria* (Cyprus), and *A. m. adami* (Crete). In a previous study based on whole-genome data, we identified varying degrees of introgression in the four subspecies. Here, we followed up with analyses of their evolutionary history and genomic diversity. Using whole-genome data from 252 samples collected across the four islands, along with reference populations, we investigated gene flow dynamics and introgression variation across the genome to identify regions resistant or susceptible to introgression. We reconstructed phylogenetic relationships based on mitochondrial and nuclear SNPs and characterised patterns of genomic diversity through nucleotide diversity (π), Watterson's theta (Θ_w), Tajima's D, and IBD-based kinship estimates. Gene flow analysis confirmed the previous introgression patterns and additionally identified gene flow between Sicily and Malta, and between Crete and Cyprus. In Maltese and Sicilian samples, a highly permeable region to introgression was identified on chromosome 5, and regions with highly conserved native ancestry were found on chromosomes 4, 6, and 9. Phylogenetic reconstructions based on nuclear SNPs from pure samples clustered the samples in the four lineages previously described by Ruttner (1988). *A. m. cypria* was clustered in lineage O, contrary to previous studies that assigned it within the C lineage. Mitochondrial-based phylogenies recovered only two main groups corresponding to the lineages A and C+O. Samples from Sicily and Malta showed unexpectedly high diversity and kinship, likely due to the elevated genetic diversity common in the A lineage, combined with introgression from the highly divergent C lineage, and possibly the inbreeding effect of conservation strategies. Overall, Mediterranean island honey bees exhibit complex evolutionary trajectories, shaped by introgression, lineage diversity, and conservation practices, resulting in distinct genomic landscapes.

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GENOMIC INSIGHTS INTO THE HYBRID ORIGIN AND DIVERSITY OF CUBAN HONEY BEES

Carlos A. Yadró García[1,2], Dora Henriques[1], Anais Rodriguez[2], Telma Gonçalves[1], Alejandro Pérez-Morfi[2,3], Adolfo Pérez-Piñeiro[2], Maria Alice Pinto[1]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal
[2]Centro de Investigaciones Apícolas, La Habana, Cuba
[3]Centro de Investigaciones en Abejas, Universidad de Guadalajara, Guadalajara, Mexico

Abstract

Cuban honey bees represent a hybrid of the different subspecies introduced into the country over the past 250 years. Previous microsatellite- and mtDNA-based studies have suggested an admixed origin involving A, M, and C lineage subspecies. Due to its insular location, introductions have always been limited compared to the mainland, a situation further reinforced by the 1960 ban on honey bee imports. Here, we analysed the population structure, genetic diversity, and gene flow on 33 Cuban honey bees using whole-genome data. ADMIXTURE at K2 revealed a clear hybrid origin from M and C lineages. However, at K3, Cuban honey bees exhibited a stable and exclusive genetic background, with minimal hybridisation from *A. m. ligustica* and *A. m. caucasia*. PCA further confirmed this pattern, placing Cuban samples between the M and C clusters, slightly shifted toward M. Gene flow analysis revealed the complex genetic origins of the Cuban population, with considerable contributions from all reference populations.

Compared to reference samples of *A. m. ligustica*, *A. m. carnica*, *A. m. mellifera* and *A. m. iberiensis,* Cuban samples also presented the highest median values of nucleotide diversity (π) and Watterson's (Θ_w), reflecting their genetic background originating from several diverging subspecies and lineages. Simultaneously, Cuban bees showed the highest median values of kinship and Tajima's D. These patterns likely result from several factors, including bottlenecks associated with the founding events and colony losses recorded during the early years of *Varroa* invasion. Additionally, the population's isolated nature, combined with various selection and breeding programs, has contributed to increased inbreeding and left a signal of selection. Overall, the Cuban honey bee population exhibits a hybrid origin shaped by multiple lineages, with insular isolation, genetic drift, bottlenecks, and selection collectively shaping its unique genetic diversity.

INVESTIGATION OF PROBOSCIS LENGTH OF THE PANNONIAN ECOTYPE OF *APIS MELLIFERA CARNICA* IN HUNGARY

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Edit Zajácz, Orsolya Bellus, Szilvia Varga, Tibor Cserháti, Réka Balázs, Nóra Pálinkás-Bodzsár

Institute for Farm Animal Gene Conservation, National Centre for Biodiversity and Gene Conservation, Gödöllő, Hungary

Abstract

The Pannonian ecotype of the Carniolan honey bee (*Apis mellifera carnica*) is native to Hungary and in terms of its productive and behavioural characteristics, is well adapted to the local climate and environmental conditions. Due to the high honey bee density in the country, different subspecies often interbreed, leading to hybridization. This complicates the maintenance of the Pannonian bee as a pure population, thus posing challenges for both gene conservation and breeding. Proboscis length is an important morphological trait that varies among bee subspecies and plays a key role in interactions between bees and flowering plants, as it determines the range of accessible food sources. The aim of this study was to investigate the variability of proboscis length and its influencing factors in the Hungarian Pannonian bee. Subspecies identification was based on morphological characteristics (cubital index, abdomen coloration, proboscis length) which were compared with reference values established in Hungary. The combined use of morphological and molecular genetic analyses may provide a more reliable assessment of bee subspecies and ecotype identification. The data indicate that proboscis length exhibits considerable variability even within populations. Individuals from the same colony preparing for winter tended to have shorter proboscis lengths, whereas spring-emerged individuals showed higher values. The observed differences are likely related to seasonal environmental effects and variations in developmental conditions, which we plan to investigate through further morphological and genetic analyses. These findings are consistent with international studies, indicating that morphological traits show substantial phenotypic variability and are not determined solely by genetic factors. Variation in proboscis length alone is not always a reliable criterion for selection, therefore, a complex morphological and genetic approach is necessary.

Relationship between managed and wild bees

DIVERSITY AND PHYLOGENY OF MICROSPORIDIAN AND TRYPANOSOMATID PARASITES IN BEES REVEALED BY METABARCODING APPROACH

P 164

Pavla Bartošová-Sojková[1], Martina Lisnerová[1], Michal Perlík[1,2], Karolína Svobodová[4], Sagar Nayak[1,2], Dragomir Damnjanović[1,2], Alena Lövy[1], Monika Burgerová[1,2], Hana Pecková[1,2], Šimon Zeman[3], Jan Votýpka[1,3], Ivan Fiala[1], Alena Krejčí[2,4]

[1]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Ceske Budejovice, Czechia
[2]Faculty of Science, University of South Bohemia, České Budějovice, Czechia
[3]Faculty of Science, Charles University, Prague, Czechia
[4]Institute of Entomology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czechia

Abstract

Bees are essential pollinators that support both natural ecosystems and agricultural production, yet they host a wide range of parasites that can influence their health and population dynamics. While parasites of honey bees and bumble bees have been relatively well studied, those infecting wild bees remain poorly understood. This project examines the diversity, prevalence and evolutionary relationships of microsporidian and trypanosomatid parasites across honey bees, bumble bees and wild bees using a metabarcoding approach.

A total of 1577 bee tissue samples from 90 species (including 944 samples of *Apis mellifera*), 155 honeybee product samples and 41 *Varroa* mite samples were analyzed. DNA was extracted, followed by parasite-group-specific PCR amplification and high throughput sequencing using the Oxford Nanopore MinION platform. The metabarcoded data were used to assess parasite diversity across hosts and environmental sources.

Microsporidian parasites showed high diversity across bee hosts. In *Apis mellifera*, the most prevalent species was *Nosema ceranae*, detected in approximately 40% of samples. Other taxa, including *Nosema bombi* and *Nosema invadens*, were rare and *Nosema apis* was not detected. *N. ceranae* DNA was also found in *Varroa* mites and bee products. Across all bees, 47 microsporidian species or lineages were identified, greatly expanding known diversity of this parasite group. Wild bees represent a major reservoir, with many lineages found exclusively in solitary species.

Trypanosomatid parasites were also common, with ten species detected, including *Lotmaria passim*, *Crithidia mellificae* and *Crithidia bombi*. Overall prevalence in bee tissues was about 26%, with *L. passim* reaching 67% prevalence in honey bees. These parasites were not detected in bee products or *Varroa* mites.

Overall, our results reveal a much broader diversity of bee associated microsporidian parasites than previously recognized and highlight wild bees as important reservoirs of parasite diversity. Future research will clarify host-parasite evolution and impacts of emerging parasites on pollinator health.

P 165 STATEMENT FOR THE CONSERVATION AND RESTORATION OF *APIS MELLIFERA MELLIFERA*

Laima Blažytė-Čereškienė[1,2], Jacques van Alphen[2,3], Frankie de Dobbelaere[2,4], Vincent R. Douarre[2,5], Natuschka M. Lee[2,6], Markku Pöyhönen[2,7], Jo Widdicombe[2,8], John Greenaway[2,4]

[1]State Scientific Research Institute, Nature Research Centre, Vilnius, Lithuania
[2]Conservation team, SICAMM, Switzerland
[3]Naturalis Biodiversity Center, Leiden, Netherlands
[4]Native Irish Honey Bee Society, (NIHBS), Ireland
[5]Fédération européenne pour la Conservation de l'abeille noire, (FedCAN), France
[6]Swedish University of Agricultural Sciences, Uppsala, Sweden
[7]Suomen Tumman Mehiläisen Hoitajat, Helsinki, Finland
[8]Bee Improvement & Bee Breeders Association, (BIBBA), United Kingdom

Abstract

The dark European honey bee (*Apis mellifera mellifera*) is a native subspecies that has evolved over a million years to adapt to the specific climatic conditions and vegetation of Northern, Western, and Central Europe. Despite its essential role in maintaining biodiversity and a healthy ecosystem, it is currently threatened throughout its natural range due to the widespread importation of non-native subspecies and the resulting hybridization.

The Statement by the SICAMM Conservation team outlines a comprehensive strategy to protect and restore this unique subspecies. The strategy encompasses four distinct scenarios (ranging from largely undisturbed populations to those that are heavily hybridized or living in wild forests) to ensure a targeted approach across different European countries.

Key conservation tools include:

1. Strategic bans on the import of non-native honey bees to ensure biosecurity and promote sustainable beekeeping.
2. The establishment of reserves and protected areas to preserve genetic diversity under natural selection conditions.
3. Restoration programs aimed at increasing the proportion of native honey bee alleles in hybridized populations through distribution of verified native bee resources and education of beekeepers.
4. Protection of free-living populations in forests so that natural selection can operate freely in the face of new diseases, parasites and other environmental issues.
5. The free distribution of genetically verified colonies in and around buffer zones to support the expansion of native alleles, encourage beekeeper engagement, and create a living protective ring around core areas.

By integrating scientific data with beekeeper collaboration and policy advocacy, SICAMM aims to ensure the long-term survival and resilience of the dark European honey bee. The model provided by the Statement is flexible and can be applied to other honey bee subspecies within their native ranges.

ASSESSING COMPETITION AMONG POLLINATORS: A SYSTEMATIC REVIEW OF METHODOLOGICAL APPROACHES

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Gherardo Bogo[1], Danilo Bevk[2], Annely Brandt[3], Robert Brodschneider[4], Giles E. Budge[5], Simone Cutajar[6,7], Grecia S. de Groot[8], Fani Hatjina[9], Jacek Jachuła[10], Lena Ladewig[3], Ioannis Manthos[11], David Mifsud[6], Carolina L. Morales[8], Aleksandra Splitt[10], Tadej Verbič[2], Cecilia Costa[1]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]National Institute of Biology, Ljubljana, Slovenia
[3]LLH-Bee Institute Kirchhain, Kirchhain, Germany
[4]University of Graz, Department of Biology, Graz, Austria
[5]School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom
[6]Institute of Earth Systems, University of Malta, Msida, Malta
[7]Department of Agricultural and Food Science, University of Bologna, Bologna, Italy
[8]INIBIOMA (Universidad Nacional del Comahue-CONICET), Bariloche, Argentina
[9]ELGO DIMITRA, Inst. of Animal Science, Department of Apiculture, Nea Moudania, Greece
[10]The National Institute of Horticultural Research, Skierniewice, Poland
[11]ELGO DIMITRA, Inst. of Plant Breeding & Genetic Resources, Department of Nut Trees, Lamia, Greece

Abstract

Inter-specific competition and resource overlap among pollinators have attracted increasing scientific attention, although consensus on the nature and magnitude of these interactions remains elusive. Yet the diversity of methodological approaches used to assess such interactions makes it difficult to compare findings across studies and draw general conclusions. Here, we present a systematic review of the methodologies employed to evaluate inter-specific interactions among pollinators, encompassing both resource and niche overlaps and competitive dynamics across managed and wild species. Following the PRISMA guidelines, a literature search retrieved 3,335 records in total, reduced to 1,051 after removal of duplicates, and further screened to a final set of 183 peer-reviewed studies presenting original data on inter-specific pollinator interactions. For each study, we extracted bibliographical features, different pollinator descriptors, ecosystem type, methodological approach and design, endpoints assessed, and additional parameters considered. Our review reveals that a wide range of methodological approaches has been used to assess pollinator interactions, each capturing different facets of competition and resource overlap. While these approaches are complementary rather than mutually exclusive, they are seldom used in combination, resulting in fragmented assessments that fall short of a comprehensive understanding of competitive dynamics. Furthermore, studies applying similar methodological frameworks show strong internal heterogeneity, with key methodological details frequently insufficiently reported. Importantly, parameters related to the environment and resource availability, such as land use, landscape carrying capacity, and floral resource availability, are often not considered, despite their central relevance to the interpretation of competitive interactions. Together, these shortcomings severely constrain comparability across studies and our ability to synthesise evidence at broader scales. We conclude that the field would greatly benefit from the development of standardised, comprehensive methodological guidelines, which would improve the rigour, transparency, and comparability of future research on pollinator interactions.

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A HIDDEN THREAT? ASSESSING THE POTENTIAL OF WILD INSECTS AS RESERVOIRS FOR *MELISSOCOCCUS PLUTONIUS*, THE CAUSATIVE AGENT OF EUROPEAN FOULDBROOD

Manon Bovier[1], Alexis L. Beaurepaire[2], Vincent Dietemann[2,3], Lars Straub[1], Benjamin Dainat[2]

[1]Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[2]Swiss Bee Research Centre, Agroscope, Bern, Switzerland
[3]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

Abstract

The impact of pathogens from Western honey bees (*Apis mellifera*) in the ongoing decline of wild pollinators is increasingly scrutinized. By contrast, the extent to which wild insects may act as reservoirs or vectors of honeybee pathogens remains poorly investigated. *Melissococcus plutonius*, the causative agent of European foulbrood (EFB), stands among the most impactful bacterial diseases affecting honeybees worldwide. Despite strict and resource-intensive control measures - such as the destruction of colonies showing clinical signs and the disinfection of affected and neighbouring colonies - EFB is frequently re-occurring at the same locations or spreading to nearby apiaries. One possible explanation for these phenomena is the presence of environmental reservoirs of the pathogen in the vicinity of apiaries. Potential reservoirs include wild insects and the flowers visited both by managed and wild pollinators. Supporting this hypothesis, a recent study in the USA detected *M. plutonius* in 25% of the screened wild bee and wasp species, suggesting a wider prevalence of the pathogens among wild insects than previously assumed. The aim of the current study was to screen for the presence of *M. plutonius* over time and at varying distances of EFB-affected apiaries in Switzerland, a country that recently experienced an increased frequency of outbreaks of the disease. The results allow us to evaluate whether current control measures - colony destruction, interdiction zones around affected apiaries and repeated colony health inspections within a 1 km radius - are sufficient to prevent disease recurrences and spread, and to better assess the potential role of wild bees and flowers in pathogen transmission.

EXPLORING MICROBIAL COMMUNITIES IN NATIVE BEES ACROSS LATIN AMERICA

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Loreley Castelli[1,2], Ingrid Aguilar[3], Leopoldo Alvarez[4,5], Marina P. Arbetman[6], Daniela Arredondo[1,2,7], Nolberto Arismendi[8], Marina Basualdo[9], Pedro Vale de Azevedo Brito[10], Adriana Correa Benítez[11], Andres Delgado-Canedo[12], Juan P. Herrera Cerquera[13], Melisa P. Garrido[5,14], Fernanda Gonzalez[15,16], Josefina Lohrmann[6], Fernando Muller[17,18], Eleazar Pérez-Castro[19], Martin P. Porrini[5,14], Francisco J. Reynaldi[5,20], Estela Santos[21], Mateo Senseber[21], Carlos M. Silva-Neto[22], Sheena Salvarrey[23], Cecilia Smith[24], Jose Carlos Tapia-Rivera[25], Arsened Vargas Guarín[13], Rodrigo Velarde[26], Aurora Xolalpa[27], Filipa Godoy-Vitorino[28], Karina Antúnez[1,2]

[1]Laboratorio de Microbiología y Salud de las Abejas, Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay. [2]Centro de investigaciones en Ciencias Ambientales (CICA), Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay. [3]Centro de Investigaciones Apícolas Tropicales, CINAT. Universidad Nacional, Heredia, Costa Rica. [4]División Entomología, Museo de La Plata, Universidad Nacional de La Plata, Edificio Anexo Museo, Unidades de Investigación FCNyM, La Plata, Argentina. [5]Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina. [6]Grupo de Ecología de la Polinización, EcoPol INIBIOMA (CONICET-Universidad Nacional del Comahue) Bariloche, Argentina. [7]Instituto de Investigación Una Salud, Prorectorado de la Universidad de la República, Montevideo, Uruguay. [8]Centro de Investigación Austral Biotech, Facultad de Ciencias, Universidad Santo Tomás, Valdivia, Chile. [9]PROANVET-Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Argentina. [10]Universidade Federal de Goiás, Goiânia, Brazil. [11]Departamento de Medicina y Zootecnia de Abejas, Conejos y Organismos Acuáticos, Facultad de Medicina Veterinaria y Zootecnia de la Universidad Nacional Autónoma de México, Ciudad Universitaria, Delegación Coyoacán, Ciudad de Mexico, Mexico. [12]Centro Integrado de Pesquisas Biotecnológicas (CIPBiotec), Universidade Federal do Pampa, Campus São Gabriel, San Gabriel, Brazil. [13]Escuela de Ciencias Agrícolas, Pecuarias y del Medio Ambiente (ECAPMA), Grupo INYUMACIZO, Universidad Nacional, Abierta y a Distancia (UNAD), Neiva-Huila, Colombia. [14]Centro de Investigación en Abejas Sociales (CIAS)-Instituto de Investigaciones en Producción Sanidad y Ambiente (IIPROSAM CONICET-UNMdP), Facultad de Ciencias Exactas y Naturales, Centro Científico Tecnológico Mar del Plata-CONICET, Centro de Asociación Simple CIC PBA, Estación Costera J.J. Nágera, Mar del Plata, Argentina. [15]School of Biological, Environmental and Earth Sciences, University of Southern Mississippi, Hattiesburg, United States. [16]Instituto de Virología e innovaciones tecnológicas (IVIT) INTA-CONICET., Castelar, Argentina. [17]Centro de Investigación y Desarrollo Apícola y Maliponicola del Instituto Superior del Profesorado en Ciencias Agrarias y Protección Ambiental (PROCAyPA), Capiatá, Paraguay. [18]Ministerio del Agro y la Producción de la Provincia de Misiones (MAyP), Misiones, Argentina. [19]Facultad de Zootecnia, Universidad Nacional del Centro del Perú, Cusco, Peru. [20]Centro de Microbiología Básica y Aplicada (CEMIBA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (UNLP), La Plata, Argentina. [21]Sección Etología, Facultad de Ciencias. Universidad de la República, Montevideo, Uruguay. [22]Instituto Federal de Goiás, Polo de Inovação, Goiás, Brasil. Universidade Estadual de Goiás, Ipameri, Goiás, Brazil. [23]Sección Etología, Facultad de Ciencias y Departamento de Sistemas Ambientales, Facultad de Agronomía, Salto, Uruguay. [24]Universidad de Lagos, Osorno, Chile. [25]Centro de Investigaciones en Abejas-Centro Universitario del Sur-UDG, Jalisco, Mexico. [26]Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), La Paz, Bolivia. [27]Centro de Innovación para el Desarrollo Apícola Sustentable en Quintana Roo (CIDASQROO), Universidad Intercultural Maya de Quintana Roo, La Presumida José María Morelos, 77870, Quintana Roo, Mexico. [28]Departamento de Microbiología e Inmunologia, Escuela de Medicina, Universidad de Puerto Rico, Recinto de Ciencias Médicas, San Juan, United States.

Abstract

Latin America hosts a great diversity of wild bees, among which stingless bees and bumblebees stand out due to their cultural importance and wide distribution across the American continent. The loss of honey bee colonies and the decline of wild bee populations are a global concern, associated with factors such as agricultural intensification (monocultures and pesticide use), the introduction of exotic species, and habitat destruction.

The gut microbiota plays a key role in bee health and has been recognized as a sensitive indicator of physiological status. In this context, species from the genera *Bombus*, *Melipona*, *Scaptotrigona*, and *Tetragonisca* were studied to characterize their gut microbiota and evaluate the effects of management practices, environmental conditions, and geographic region. A total of 206 worker bee samples were collected from wild nests and managed colonies in low and high impact areas across Argentina, Bolivia, Brazil, Costa Rica, Chile, Mexico, and Uruguay, of which 185 were analyzed. Total DNA was extracted, and the V4 region of the 16S rRNA gene was sequenced using a high-throughput approach.

The results revealed clear differences in gut microbiota composition among the analyzed bee genera. A shared core microbiota was identified across genera, mainly composed of *Snodgrassella* spp., *Gilliamella* spp., *Lactobacillus* spp. and *Bifidobacterium* spp. However, *Melipona* showed an absence or very low abundance of *Gilliamella* spp., in contrast to other genera where this taxon was dominant, highlighting a strong host-associated structuring of the microbiota. Additionally, environmental and management conditions modulated microbial community structure, producing significant shifts in composition without affecting overall diversity, with more pronounced effects in managed colonies than in wild nests.

These findings provide insights into gut microbial assembly of native bees and highlight its potential as an indicator of health and sustainability. Microbiota characterization may contribute to sustainable management strategies and conservation of native pollinators.

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MANAGED AND WILD BEES: ENEMIES OR ALLIES?

Vincent Dietemann[1,2], Jean-Daniel Charrière[3], Alexis L. Beaurepaire[1]

[1]Swiss Bee Research Center, Agroscope, Bern, Switzerland
[2]Department Ecology and evolution, University of Lausanne, Lausanne, Switzerland
[3]Swiss Bee Research Center, Bern, Switzerland

Abstract

Many pollinators are currently declining, calling for appropriate mitigation policies to ensure ecosystem functioning, food security and human well-being. While there is consensus that some factors, such as land management and pesticides, play an important role in these declines, other potential stressors remain more controversial. In recent years, competition for resources with and pathogen transmission from managed bees have been increasingly considered as contributing to wild bee declines, generating conflicts among beekeepers, farmers, conservationists and scientists. We argue that scientific knowledge of the interactions between managed and wild bees is insufficient to resolve the conflicts through contextualized, evidence-based policies. Existing studies are correlational and limited in space and time. This prevents a high level of confidence in the potential effects of managed bees on wild bees and hinders the creation of effective mitigation measures. In addition, effects on wild bee population sizes and genetic diversity, which are key to assessing long-term impacts, were never estimated. Potential negative interactions between managed and wild bees also need to be weighed against other stressors known to affect bees, such as land management, pesticide use, invasive species and climate change. To prioritize mitigation efforts, we advocate increased communication and collaboration between stakeholders to co-create policies integrating ecological and socioeconomic dimensions. To work towards this aim, we present our project BEENERGIA, which combines modern molecular and modelization tools to better understand the interactions among bees in different contexts with stakeholder engagement to reconcile managed and wild bee proponents for the benefit of all bees. The availability of more contextualized and consensual bee conservation measures will allow a productive and sustainable beekeeping activity aligned with pollination requirements and the conservation of biodiversity.

REDEFINING THE HALICTID BEE, SUBGENUS *ACANTHALICTUS* COCKERELL (HYMENOPTERA: HALICTIDAE, LASIOGLOSSUM) BY PHYLOGENETIC AND MORPHOLOGY

P 170

Seunghun Jung, Seunghwan Lee

Seoul National University, Seoul, Korea, Republic of Korea

Abstract

The subgenus *Acanthalictus* Cockerell of the genus *Lasioglossum* Curtis has traditionally been diagnosed by a set of sexual dimorphic characters, including two subapical teeth on the female mandible, a strongly swollen basal elevation with a longitudinal furrow on the male labrum, and distinctive modifications of male metasomal sterna S2 and S6. However, our molecular phylogenetic analysis recovers a well-supported monophyletic *Acanthalictus* clade comprising three species including the type species *L. dybowskii*, which do not uniformly exhibit the previously proposed synapomorphies. Our molecular phylogeny based on multi gene region revealed that certain historically used diagnostic traits lack consistency across the clade and therefore do not serve as reliable synapomorphies. We propose a revised diagnosis for *Acanthalictus* based on more consistent character combination: females with an enlarged preapical mandibular tooth, head wider than long, genal area broader than the compound eye in lateral view, and a serrate inner metatibial spur; males with a strongly swollen labrum bearing a longitudinal furrow and a gently swollen apical portion of S2. This revised character set better reflects the true morphological synaphomorpies based on monophyly of *Acanthalictus*.

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USING BEE-LINING TO FIND WILD-LIVING HONEY BEE COLONIES: NON-LINEAR FLIGHT DURATION FUNCTIONS IMPROVE THE ACCURACY OF NEST SITE PREDICTIONS

Léon Koenig[1], François Rebaudo[1], Patrick L. Kohl[2], Benjamin Rutschmann[3], Fabrice Requier[1]

[1]Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, Gif-sur-Yvette, France
[2]Department of Livestock Population Genomics, University of Hohenheim, Stuttgart, Germany
[3]Agroecology and Environment, Agroscope, Zurich, Switzerland

Abstract

In Europe, the Western honey bee (*Apis mellifera*) has a double nature, as a managed and a wild-living species. Despite substantial decline over the last decades, recent studies have shown that wild-living populations are still present in European forests. The new IUCN Red List assessment has thus classified wild-living populations as endangered in Europe. In this context, locating, estimating the density of wild-living colonies and monitoring their demography are urgently needed to understand the causes of their decline and support conservation efforts.

Bee-lining enables the location of wild-living colonies by observing the flight direction of bees foraging at an artificial field feeder and using their round-trip duration to linearly predict the distance to the colony. We explored the hypothesis of a non-linear relationship between foraging distance and round-trip duration, and tested the inclusion of abiotic variables (e.g. temperature and landscape), to improve the estimation of nest distances of wild honey bee colonies in the field. We gathered bee-lining data from France and Germany, selecting flight durations for forager bees for which we successfully located the colony (n = 592 round trips). We ran the linear models described by Wenner (1992) and Kohl and Rutschmann (2018), comparing them with the non-linear models commonly tested in ecology, and the inclusion of abiotic variables. We compared the models using R^2 and AIC performance criteria.

We found that considering non-linear flight duration functions substantially improved the accuracy of distance prediction models, whereas including abiotic variables did not significantly improve model performance. Using non-linear flight duration functions could improve the efficiency of bee-lining, thereby increasing the likelihood of locating wild-living honey bee colonies in the field. This, in turn, would facilitate the study of these colonies and help to collect the ecological and demographic data needed to assess their conservation status beyond Europe.

THE DEAD CAN TALK: INSIGHTS FROM A PERFECTLY PRESERVED 7TH-CENTURY HONEY BEE TREE-HIVE COLONY

P 172

Michał Kolasa[1], Bartłomiej Molasy[2], Adam Tofilski[3], Marek Krąpiec[4], Artur Górecki[2], Andrzej Oleksa[5], Michał Filipiak[2], Sylwia Jędrzejewska[6], Marcin Wołoszyn[6], Michael Hofreiter[7]

[1]National Research Institute of Animal Production, Kraków, Poland
[2]Jagiellonian University, Kraków, Poland
[3]University of Agriculture, Kraków, Poland
[4]AGH University of Science and Technology, Kraków, Poland
[5]Kazimierz Wielki University, Bydgoszcz, Poland
[6]Rzeszów University, Rzeszów, Poland
[7]University of Potsdam, Potsdam, Germany

Abstract

Interactions between humans and the western honey bee (*Apis mellifera*) have deep historical roots. Yet, while the development of beekeeping practices is well documented, the bees that accompanied past human societies remain largely unknown. In forested regions of Central and Eastern Europe, honey bees were traditionally managed in man-made tree cavities, a form of forest beekeeping known as tree beekeeping. Here, we report the discovery of an exceptionally well-preserved honey bee colony recovered from a tree hive, dated to approximately 1,500 years before present.

The find represents both a cultural artefact and a biological archive. Using an interdisciplinary approach, we reconstructed the history of the tree hive from a living tree growing in a primaeval forest of present-day South-Eastern Poland, through its intentional modification for forest beekeeping, to its preservation in waterlogged riverine deposits. Radiocarbon dating of the wood and bees, combined with palynological analysis of pollen preserved in the combs, independently confirms the age of both the tree hive and the colony.

Analysis of beebread from the colony enabled reconstruction of the bees' diet and allowed us to estimate the season in which the colony collapsed. Moreover, geometric morphometric analysis of wing venation was used to assess the subspecies affiliation of the recovered individuals, providing rare insight into the identity of honey bees managed by the local tribes. In parallel, efforts are currently underway to retrieve genetic information from the preserved specimens.

By directly linking archaeological evidence, ecological reconstruction, and bee morphology, this study demonstrates how insights from the past can help safeguard the future. The potential genomic and phenotypic footprint of historical, locally adapted honey bee populations offer valuable guidance for contemporary conservation efforts, supporting the protection of native honey bee biodiversity in an era of rapid environmental change and increasing genetic homogenisation.

P 173 EFFECTS OF HIGH HONEY BEE DENSITY ON BUMBLEBEE COLONY DEVELOPMENT: A CASE STUDY

Lena Ladewig[1], Hollie Pufal[2], Giles E. Budge[2], Cecilia Costa[3], Gherardo Bogo[3], Annely Brandt[1]

[1]LLH-Bee Institute Kirchhain, Kirchhain, Germany
[2]School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom
[3]CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Honey bee queen breeding mating stations are often located in remote and ecologically sensitive areas and can temporarily host known high numbers of honey bee colonies, potentially increasing pressure on locally available floral resources. This case study investigates, as part of the BEE-GUARDS projekt, how high densities of managed honey bee colonies at a mating station influence the development and fitness of the buff-tailed bumblebee (*Bombus terrestris*).

Thirty standardised *B. terrestris* colonies were distributed along a distance gradient of 100 m, 500 m, and 1500 m from the centre of a honey bee mating station characterised by a high density of *A. mellifera* colonies (1,200 mating nucs and approx. 75 full sized drone colonies). The mating station was located within a protected area with a radius of 7 km, with the mating station at its center, from which all other honey bee colonies were excluded. The honey bee density and flower abundance were observed directly on transects on a weekly basis. Bumblebee colony performance was monitored weekly by recording flight activity at nest entrances and measuring colony weight. At the end of the experiment, bumblebee colony fitness was evaluated using several key indicators, including reproductive output (production of gynes), total worker number, and overall nest development. These metrics were compared across the distance gradient to determine whether proximity to high densities of honey bee colonies influenced bumblebee colony vitality. The results contribute to a growing body of research addressing interactions between managed and wild pollinators and may inform pollinator conservation strategies and beekeepers on the best-practice of the spatial management of honey bee colonies in sensitive landscapes.

BALANCING MANAGED AND WILD BEES IN URBAN BIODIVERSITY HOTSPOTS: DATA AND PLANNING TOOLS FROM VIENNA

P 174

Julia Lanner, Sophie Kratschmer

BOKU University, Department of Integrative Biology and Biodiversity Research, Institute of Zoology, Vienna, Austria

Abstract

Vienna hosts approximately 470 wild bee species and is considered a hotspot of bee diversity. At the same time, urban beekeeping is growing in popularity. This raises concerns about competition for floral resources, as high densities of managed honey bees are thought to deplete nectar and pollen available to other pollinators, especially where resources are spatially or seasonally limited. In a first project (BeEcoVie I, October 2023–April 2024), we compiled over 12,000 wild bee records from 1990–2022 for Vienna from scientific literature, citizen-science platforms, and databases. Through collaboration with the local beekeeping community, we also obtained the first citywide estimates of apiary locations and honey bee colony numbers.

Based on flower-visiting traits, our analyses identified areas where high wild bee diversity coincides with high ecological similarity between wild and managed bees. These conditions, when coupled with dense apiary concentrations, are expected to increase the risk of competition for floral resources. Notably, we found such overlaps predominantly in protected areas and the city center.

To address the issues identified in BeEcoVie I, we designed a follow-up project (BeEcoVie II). We aim to (i) resolve fine-scale, site-specific ecological conditions for bees in under-studied areas and (ii) assess the city's ecological carrying capacity for bees, defined as the levels of wild bee assemblages and honey bee colonies that different landscapes can sustain without inducing foraging competition. Results will be translated into decision support for beekeepers, urban planners, authorities, and other stakeholders via a co-created, open-access platform (hive.city), designed to guide apiary placement that minimizes risks to local wild bee communities and interspecific competition.

BeEcoVie II will provide an evidence base for urban apiculture and wild bee conservation, advancing practical solutions to align beekeeping with long-term habitat quality for all bees.

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WHERE SWARMS SETTLE AND PERSIST: ENVIRONMENTAL DETERMINANTS OF HONEY BEE NESTING IN MOUNTAIN FORESTS

Andrzej Oleksa[1], Bartłomiej Molasy[2], Stanisław Handl[3], Pola Demczak[2], Igor Boruta[2], Wojciech Lipa[1], Michał Kolasa[4], Konrad Zaremba[3,5]

[1]Kazimierz Wielki University, Bydgoszcz, Poland
[2]Jagiellonian University, Kraków, Poland
[3]The State Forests, Warsaw, Poland
[4]National Research Institute of Animal Production, Balice, Poland
[5]Fundacja Ochrony Przyrody i Rozwoju Turystyki ProNaturae, Gniewoszów, Poland

Abstract

Log hives (artificial cavities in tree trunks designed to provide suitable nesting conditions for honey bees) are increasingly used to support unmanaged populations in natural and semi-natural landscapes. Yet, little is known about which environmental factors influence whether these nesting sites are occupied.

We studied log hive occupancy in the Sudeten Mountains (Central Europe) across 254 km², with elevations from 390 to 1420 m a.s.l. A total of 997 log hives have been systematically monitored from 2019 until 2024 (≈ 3.9 hives km⁻²). During the study, 81 log hives were occupied for at least one season, with seasonal occupancy ranging from 12 to 51 log hives (≈ 0.05–0.20 occupied hives km⁻²).

Using high-resolution spatial data, we assessed how topography (elevation, slope, aspect), landscape features (forest cover, distance to water and forest edges), and proximity to human settlements influenced the probability of occupancy. We analyzed the effect of these abiotic variables on the occurrence of occupied log hives using generalized linear models.

Log hive occupancy declined above 650 m a.s.l. and increased near water sources. This elevational pattern likely reflects climatic constraints, including cooler temperatures, shorter vegetation and foraging seasons, and increasing distance to swarm sources. Consecutive-year occupancy exceeded random expectations, suggesting that some hives are consistently preferred across multiple seasons and may be repeatedly colonized, indicating a tendency of swarms to occupy previously used nesting sites.

These findings demonstrate that landscape and fine-scale topography determine where unmanaged honey bees establish nests. They provide practical guidance on the strategic placement of log hives and help improve the availability of nesting sites for wild honeybee populations in mountain forests.

Funding note: This study was conducted within the 'Bartnicy Sudetów' project (funded by WFOŚiGW Wrocław), with support from State Forests and the Biodiversa+ FREE-B project (Polish component funded by NCN grant 2024/06/Y/NZ9/00137).

A MORPHOMETRIC ANALYSIS OF *VARROA* AND *TROPILAELAPS* MITES ON ISOLATED ISLANDS IN THAILAND

P 176

Preeyaporn Phonguttha[1], Rujira Ponkit[1], Sanchai Naree[2], Alexis L. Beaurepaire[3,4], James D. Ellis[5], Guntima Suwannapong[1]

[1]Department of Biology, Faculty of Science, Burapha University, Chon Buri, Thailand
[2]Faculty of Pharmaceutical Sciences, Burapha University, Chon Buri, Thailand
[3]Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[4]Bee Research Centre, Agroscope, Bern, Switzerland
[5]Entomology and Nematology Department, University of Florida, Gainesville, United States

Abstract

Varroa and *Tropilaelaps* parasitism are the most serious threats to honey bee health and are ubiquitous across Thailand, including the mainland and the island areas. Whether the geographic barrier, isolating the islands from the mainland by the Gulf of Thailand, affects the morphological variation of these mites is unknown. The morphometric analyses of *Varroa* and *Tropilaelaps* mites were performed to study the morphological variation across isolated mite populations in Thailand. *Varroa* specimens were sampled on *Apis mellifera* and *A. cerana* in the eastern and southern regions of the mainland and islands. *Tropilaelaps* specimens were sampled on *A. mellifera* and *A. dorsata* in the eastern and southern regions of the mainland, including Samui Island. Morphometric measurements of *Varroa* and *Tropilaelaps* were made on 19 and 17 characteristics, respectively. Despite geographic isolation by the ocean, *Varroa* mites from *A. cerana* on the Chang, Samui, and Pha-Ngan islands did not show separation from *A. cerana* mites, but *A. cerana* mites on both the mainland and islands were separated from *A. mellifera* mites on the mainland. *Tropilaelaps* mites from Samui Island exhibited some morphometric differences from mainland populations; however, they showed partial overlap with the mainland. These findings support the idea that the potential influence of host species is a dominant factor in *Varroa* morphology, whereas *Tropilaelaps* morphology appears to respond to geographic factors rather than to hosts.

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RISK OF VIRUS TRANSMISSION TO WILD BEES DUE TO POOR *VARROA DESTRUCTOR* CONTROL

Anja Probst[1], Orlando Yañez[1], Rebecca Denes[1], Vincent Dietemann[2,3], Peter Neumann[1], Alexis L. Beaurepaire[1,2]

[1]Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland.
[2]Swiss Bee Research Centre, Agroscope, Bern, Switzerland
[3]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

Abstract

Wild bee populations are declining globally, and virus host shifts from managed pollinators may be involved. In particular, inadequate management of ectoparasitic mites *Varroa destructor*, being potent honey bee virus vectors, may foster virus transmission from managed honey bees to other hosts. However, the actual role of *V. destructor* infestation levels in *Apis mellifera* colonies for virus transmission to local wild bees remains unclear. Here, we quantified infection rates of managed and wild bees with two viruses associated with *V. destructor* – Deformed wing virus (DWV) and Acute bee paralysis virus (ABPV) – and one virus which is not thought to be transmitted via *V. destructor*, Black queen cell virus (BQCV). By comparing infection rates, virus prevalence, and viral loads in managed and wild bees collected near highly (above 50 mites per day on the bottom board) and low (control; below 5 mites per day on the bottom board) infested apiaries, we assessed whether *V. destructor* infestations influence virus spillover to sympatric wild bees. We hypothesise that wild bees close to apiaries with high mite infestation levels have higher loads of DWV and ABPV than wild bees close to apiaries with low infestation levels. Further, we hypothesise that there is no difference in the load of BQCV when comparing wild bees collected close to highly infested apiaries to wild bees collected close to low infested apiaries. Altogether, this study will enable us to evaluate the risks associated with beekeeping practices while highlighting the benefits of effective mite control for both managed and wild bee health. The results will be presented and discussed.

SWITCHED TO ORAL: OC 114

RESOURCE OVERLAP: REVEALING POSSIBLE COMPETITION BETWEEN HONEYBEES AND BUMBLEBEES VIA POLLEN ANALYSIS

P 178

Tadej Verbič[1,2], Danilo Bevk[1,2], Ladeja Fajfar[1], Blaž Koderman[1], Danijel Kablar[1]

[1]National Institute of Biology, Ljubljana, Slovenia
[2]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Abstract

Wild bees play a crucial role in pollination and contribute substantially to crop yield and quality. However, pollinator communities are increasingly threatened by habitat loss, limited floral resources, pesticide use, climate change, diseases, and invasive species. Recent studies suggest an additional potential driver of native pollinator decline: high densities of managed honeybees. Pollinator competition is primarily driven by shared floral resources, as honeybees can deplete nectar- and pollen-rich plants. Such resource overlap may alter the foraging networks of wild bees, forcing them to exploit alternative floral resources.

Our preliminary study aims to assess resource overlap between bumblebees and managed honeybees in a resource-limited environment. As both pollinators are generalist foragers, they are expected to exploit a wide range of floral resources. The study was conducted in Slovenia in July 2025. Eight bumblebee colonies were introduced into the study area for a period of 16 days. After the fifth day, 50 honeybee colonies were added, thereby increasing honeybee density, and removed again five days later. Throughout the experiment, pollen samples were collected from both bumblebees and honeybees at the entrances of their colonies.

Palynological analysis of the pollen samples provided insight into the foraging resources of both pollinator groups. Honeybees are likely to focus on the most abundant floral sources, potentially leading to shifts in bumblebee pollen use toward alternative floral resources. Our results aim to provide insight into pollinator competition and may help inform sustainable beekeeping and biodiversity conservation.

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EUROPEAN FREE-LIVING HONEY BEE COLONIES: EARLY INSIGHTS

Mick Verspuij[1], Aicha Meliani-Rodríguez[2], Maria Alice Pinto[2], Joachim R. de Miranda[3], Andrzej Oleksa[4], Fabrice Requier[5], Steve Rogenstein[6], Léon Koenig[5], Grace P. McCormack[1]

[1]University of Galway, Galway, Ireland
[2]Instituto Politécnico de Bragança, Bragança, Portugal
[3]Swedish University of Agricultural Sciences, Uppsala, Sweden
[4]Kazimierz Wielki University, Bydgoszcz, Poland
[5]Université Paris-Saclay, Gif-sur-Yvette, France
[6]Honey Bee Watch, Galway, Ireland

Abstract

Although beekeeping practices are often associated with the maintenance of Western honey bee (*Apis mellifera*) populations, wild populations, also known as free-living colonies (FLCs), have demonstrated the capacity to persist and thrive without human intervention. Despite this resilience, Europe's native subspecies are facing an unprecedented conservation crisis. Consequently, the IUCN Red List now classifies wild honey bee populations as Endangered in the European Union. FLCs occur at exceptionally low densities and have declined by a median of 56% per decade across seven European countries.

The FREE-B project aims to locate and monitor FLCs along a latitudinal gradient from Portugal to Sweden (including France, Ireland, Poland), to establish a comprehensive framework for understanding their distribution, nesting ecology, and long-term survival in response to diverse environmental and climatic pressures. To achieve broad spatial and ecological coverage, the project combines a citizen-science framework with standardized field protocols across all partner countries. Preliminary findings from reported observations reveal that FLCs occupy a wide variety of nesting substrates, with tree cavities standing out as the most characteristic and frequent nesting site, followed by buildings, wall cavities, loghives (artificial tree cavities), and other human structures. These colonies occur across a broad range of landscapes but are predominantly found in agricultural matrices, with additional presence in urban areas and several forest types. Most recorded nests are found at low- to mid-level heights, with only a minority situated high above the ground. These preliminary results suggest that FLCs remain present across diverse European landscapes despite regional and environmental differences. Moreover, the integration of citizen science with partner-led monitoring significantly broadens both geographical and ecological coverage, facilitating the detection of colonies in previously under-sampled regions. Sustaining this temporal data collection will be essential for clarifying how changing landscape configurations and environmental stressors shape colony–survival dynamics over time.

Technological applications for the bee science & Bee-based monitoring networks

P 180 HYDRODYNAMIC ANALYSIS AND OPTIMIZATION OF THE CLEANWAX BEESWAX PURIFICATION EQUIPMENT USING CFD

Ramón Arias, Daniel A. Ramírez, Nicolas Madariaga, Karen Del Pilar Yañez

Universidad Tecnica Federico Santa Maria, Valparaiso, Chile

Abstract

The efficiency of beeswax decontamination processes depends largely on hydrodynamic conditions, particularly phase dispersion and the interfacial area between the molten wax and the solvent. In this study, a computational fluid dynamics (CFD) approach was used to analyse and optimize the mixing performance of a pilot beeswax purification system (CLEANWAX equipment) developed by researchers at the Federico Santa María Technical University. The system consists of a jacketed stirred tank operating with a two-phase mixture of molten beeswax and water at a volumetric ratio of 1:4. Simulations were performed under transient conditions using a multiphase Volume of Fluid model coupled with a sliding mesh approach to resolve impeller rotation. Turbulence was modelled using the standard k–βmodel. Both phases were initially stratified at 80 °C, assuming Newtonian behaviour for the molten beeswax. Model validation was conducted by calculating the power number from torque values obtained from computational fluid dynamics simulations and comparing them with theoretical correlations and experimental data reported in the literature, showing good agreement within an acceptable deviation range of 10%. Several rotational speeds and impeller designs were evaluated to determine their impact on flow patterns, velocity fields, vortex formation, and macroemulsion development. The results indicate that hydrodynamic performance is highly dependent on impeller design, with certain configurations promoting improved axial flow and faster dispersion of wax droplets in the aqueous phase. The optimal configuration for efficient dispersion of molten beeswax in water consists of two hydrofoil impellers mounted on an inclined shaft, operating with downward axial flow at a rotational speed of 300 rpm.

BIOAGRIAPIS: SEVEN YEARS OF BIOMONITORING USING BEES TO ASSESS CONTAMINATION IN AGROECOSYSTEMS

P 181

Marco Bergero[1], Amanda Dettori[2], Barbara Guarino[3], Elena Anselmetti[3], Matteo Bonta[3], Ariele Muzzarelli[4], Paolo Colombatto[5], Simone Tosi[6]

[1]Aspromiele, Turin, Italy
[2]BeeLab - DISAFA, Università degli Studi di Torino, Grugliasco, Turin, Italy
[3]Settore Fitosanitario Regione Piemonte, Turin, Italy
[4]Fondazione Agrion, Manta, Cuneo, Italy
[5]Fondazione Podere Pignatelli, Villafranca Piemonte, Turin, Italy
[6]Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università degli Studi di Torino, Grugliasco, Torino, Italy

Abstract

Biomonitoring using bees is crucial to characterize environmental contamination. Here, we present a 7-year long biomonitoring project (BioAgriApis) based in Piedmont, started in 2018 it is still ongoing. The survey collects multiple bee-related matrices to assess bee exposure to pesticides across multiple agricultural contexts. Monthly (March-September) multiresidue analyses of bee bread revealed a widespread presence of pesticide cocktails, with pronounced variability across season and land use. We used the Risk Quotient (RQ) to assess the cumulative risk for bees. This analysis showed higher values during months of intense agricultural activity, particularly in the fruit-growing area, then in the grape and hazelnut growing areas, and lastly in the areas characterised by horticultural and cereal crops. Additional agronomic activities planned in this project included the use of honey seed mixtures, which helped evaluate the quality of the forage and identify solutions to support biodiversity. The findings confirm the need for a multidisciplinary approach that accounts for the complexity of residual chemical mixtures and their effects on bees. Future developments of the project include expanding the analysed matrices, refining risk assessment methodologies, and integrating the findings into sustainable agroecosystem management programs.

P 182 MONITORING OF BUMBLEBEE QUEENS IN SLOVENIA USING CITIZEN SCIENCE

Danilo Bevk[1,2], Blaž Koderman[1], Ladeja Fajfar[1], Danijel Kablar[1], Mojca Pibernik[2], Tadej Verbič[1], Johanna Robinson[3]

[1]National Institute of Biology, Ljubljana, Slovenia
[2]SloBees – Pollinator Conservation Society of Slovenia, Škofja Loka, Slovenia
[3]Slovenian Institute for Adult Education, Ljubljana, Slovenia

Abstract

Bumblebees are important pollinators. Although their populations are known to be declining, their status and trends are poorly documented. The influence of climate change on activity of queens in spring is also insufficiently understood. To address this, we launched an annual citizen science initiative in Slovenia in 2024, focused on recording bumblebee queens every year from January to April. Participants photograph a queen and upload the image together with information on its activity and location directly in a mobile app. Participants select one of ten species representing the main colour patterns found in Slovenia or indicate that the species is other or unknown. After the campaign, all submissions are reviewed by experts. Species are corrected or added when necessary. We included in the final analysis only entries with an adequate photograph for identification.

The first year primarily served to test the method and instructions, which were then refined in the second year. In 2025, at least 109 people participated, contributing 1,534 records. Data quality improved substantially: while 20% of entries lacked a photograph in the first year, this decreased to only 6% in the second. After the review, 1,330 records were considered suitable for analysis.

In 2025, nearly two-thirds of observations belonged to two groups: *Bombus lucorum/terrestris* and *B. pascuorum*, followed by *B. hortorum* and *B. pratorum*. Relative species frequencies matched expectations and field experience. The lowest misidentification rates were recorded for *B. argillaceus* (95% correct), *B. hypnorum* (94%), and *B. lucorum/terrestris* (92%). The highest error rates occurred for *B. hortorum* (77% correct) and *B. pratorum* (78%).

Based on two years of experience, this citizen science approach proves suitable for monitoring bumblebee queens. It also plays an important role in raising awareness about bumblebees, their foraging needs, and the effects of climate change on their seasonal activity.

A NEW PROPOLIS MOLECULAR COMPLEX TARGETING HUMAN HAIR FOLLICLE DERMAL PAPILLA CELLS FOR ANTI-HAIR LOSS APPLICATIONS

P 183

Athina Boulaka[1], Eleni Panagiotidou[1], Sophia Athanasopoulou[1], Danai Georgiou[1], Marianna Kapetanou[2], Konstantinos Gardikis[1,3]

[1]Research and Development Department, APIVITA S.A., Industrial Park Markopoulo Mesogaias, Athens, Greece
[2]Hellenic Pasteur Institute, Athens, Greece
[3]Department of Pharmacy, Frederick University, Nicosia, Cyprus

Abstract

Hair loss is a multifactorial condition influenced by androgen signaling, oxidative stress, and impaired hair follicle regeneration. Propolis, rich in bioactive polyphenols, exhibits antimicrobial and antioxidant properties relevant to scalp and follicle health. In this study, we evaluated Propolis HG Complex, a novel blend combining: (1) Propolis AmC, an extract titrated in specific molecular fractions of propolis, previously shown to target pathogenic strains of *Cutibacterium acnes* while maintaining scalp microbiome balance and regulating sebum levels; and (2) a liposomal–cyclodextrin delivery system designed to enhance the penetration and bioavailability of propolis polyphenols.

The biological activity of Propolis HG Complex was assessed in human hair follicle dermal papilla cells (HFDPCs) and in a stress-induced premature senescence (SIPS) model using normal human dermal fibroblasts (NHDFs). HFDPCs were treated with the HG complex, followed by real-time PCR evaluation of SRD5A1 gene expression and Western blot analysis of β-catenin protein levels. In NHDFs exposed to H_2O_2, cellular senescence was evaluated via X-gal staining.

Propolis HG Complex significantly upregulated SRD5A1 mRNA in HFDPCs and increased β-catenin protein levels, indicating activation of pathways associated with hair follicle proliferation and anagen phase maintenance. In the SIPS model, treatment reduced the proportion of X-gal-positive senescent cells, demonstrating protection against oxidative stress–mediated cellular aging.

These results highlight the multi-target potential of Propolis HG Complex in supporting scalp homeostasis, enhancing dermal papilla cell activity, and mitigating senescence-related mechanisms involved in hair loss. Further studies are warranted to validate its performance in clinical settings related to hair loss and hair growth.

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FROM LANDSCAPE TO HIVE: A MULTILEVEL APPROACH TO PREDICT DAILY FORAGING ENVIRONMENTAL SUITABILITY IN APIS MELLIFERA

Flavia V. Chianese[1], Roberta Santopietro[1], Viviana Valenzano[1], Lena Ladewig[2], Josipa Štavalj[3], Annely Brandt[2], Marin Kovačić[3], Cecilia Costa[4], Gennaro Di Prisco[1]

[1]CNR-IPSP, The Italian National Research Council Institute for Sustainable Plant Protection, Portici, Napoli, Italy
[2]LLH-Bee Institute Kirchhain, Erlenstr, Kirchhain, Germany
[3]University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia
[4]CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Understanding how environmental conditions shape honeybee foraging activity is critical for sustainable apiculture, yet predictive frameworks that simultaneously integrate meteorological, phenological, and landscape-level drivers remain lacking. This study presents a multilevel approach to characterize and predict daily foraging environmental suitability for *Apis mellifera*.

Two sites in Southern Italy are selected to represent opposite land use contexts: one dominated by intensive agriculture, the other by semi-natural vegetation.

Daily hive weight dynamics, continuously recorded via IoT sensors, are combined with weather data and vegetation indices derived from Sentinel-2 satellite imagery. A key original contribution is the quantification of floral resource availability through a distance-weighted potential honey yield methodology, informed by a purpose-built multi-level land use database linking 1,445 habitat classes to species-specific monthly nectar and pollen production values.

These heterogeneous data streams are first synthesized through an environmental suitability index - grounded in peer-reviewed thresholds for *Apis mellifera* foraging activity - which serves as both an interpretable baseline and a featureengineering layer to train a machine learning classifier. The resulting framework constitutes a reproducible methodology for quantifying the environmental determinants of foraging dynamics, with potential for application across diverse landscape contexts.

EXPLORING THE ROLE OF *APIS MELLIFERA* AS A MONITORING TOOL FOR ANTIMICROBIAL RESISTANCE: A CASE STUDY IN SOUTH TYROL, ITALY

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Giovanni Cilia[1], Silvia Rosa[2], Federico Martignago[3], Franco Mutinelli[3], Michela Bertola[3], Salvatore Barone[4], Lorenzo Brusetti[2], Maria Ludovica Saccà[1]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]University of Bolzano-Bozen, Bolzen, Italy
[3]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy
[4]Azienda sanitaria di Bolzano, Bolzano, Italy

Abstract

Antimicrobial resistance (AMR) is a growing global concern affecting human, animal, and environmental health. Increasing attention is being paid to environmental reservoirs of antimicrobial resistance genes (ARGs) within the One Health framework. Honey bees (*Apis mellifera*), due to their wide foraging range and continuous interaction with air, water, plants, and soil, can act as effective bioindicators of environmental microbial contamination and resistome diversity.

This study investigated the occurrence of antimicrobial-resistant bacteria and ARGs associated with honey bees in South Tyrol (Northern Italy). Honey bee samples were collected from several apiaries located in different environments, including urban, agricultural, natural, and livestock-influenced areas. Sampling was conducted in two seasons during 2024 and 2025 to evaluate possible temporal variations in microbial communities and antimicrobial resistance patterns.

Microorganisms isolated from both the surface and gut of honey bees were identified using MALDI-TOF mass spectrometry. In total, 171 bacterial species were detected (98 in 2024 and 115 in 2025, highlighting a high microbial diversity. Among the isolates were potential plant, animal, and human pathogens, including *Pantoea agglomerans*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. Antimicrobial resistance was assessed phenotypically through minimum inhibitory concentration (MIC) tests and genotypically using PCR assays.

DNA extracted from pooled honey bee samples was screened for 32 ARGs belonging to six major antibiotic classes. Twenty-four ARGs were detected overall in 92.1% of the total investigated samples. The most frequent genes were associated with tetracycline resistance (*tetA, tetM, tetL, tetW*), followed by β-lactam resistance genes (*blaTEM, blaSHV, blaCMY2, blaOXA48*). Genes conferring resistance to fluoroquinolones (*qnrB, qnrS*), aminoglycosides (*strA, aacA1*), sulfonamides (*sul1*), and colistin (*mcr-1*) were also identified.

The frequent co-occurrence of multiple ARGs suggests that honey bees can effectively reflect the environmental resistome, supporting their potential role as bioindicators for environmental AMR surveillance.

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A PROGRAMMABLE, AUTOMATED IN-HIVE AEROSOL DELIVERY PLATFORM-FIELD RESULTS USING OXALIC ACID

Ron Corkidi

ToBe, Tel Mond, Israel

Abstract

Background: *Varroa destructor* remains the most significant biotic threat to honey bee (*Apis mellifera*) colonies worldwide. Although several effective compounds are available, most treatments require repeated manual application and provide limited control over treatment timing and dosage. Hive Master is a programmable, automated in-hive delivery platform, designed in the dimensions of a standard brood frame for installation in conventional hives, for the application of organic, water-based formulations. In addition to *Varroa* treatments, the platform may enable the delivery of nutritional supplements, probiotics, microbial products, and other water-based additives.

Methods: Multiple field trials were conducted using brood-right commercial colonies located in different regions and during different seasons of the year. Colonies were treated with oxalic acid delivered by Hive Master over a two-week period, corresponding to one sealed brood cycle. Treatment performance was compared with commercially available Varroxan® and amitraz strips. *Varroa* infestation levels were measured before and after treatment using standardized monitoring methods. Colony health was assessed throughout the trials by periodic brood measurements and photographic documentation.

Results: Across independent field trials, OA delivery using Hive Master V4 achieved *Varroa* reductions ranging from 82% to 95% after a single two-week treatment. Comparable *Varroa* control to Varroxan® was achieved in one-third of the treatment duration while using less than half the total amount of active ingredient. In comparative field trials, Hive Master V4 also achieved higher efficacy than amitraz strips under the tested conditions.

Conclusions: These results demonstrate that automated in-hive delivery of water-based formulations can provide effective *Varroa* control within a single sealed brood cycle in brood-right colonies. The platform also provides a standardized approach for investigating application schedules and evaluating additional water-based compounds and hive additives in future studies.

EXPRESSION OF OXIDATIVE STRESS AND DETOXIFICATION GENES IN HONEY BEES (*APIS MELLIFERA*) ACROSS AN URBANISATION GRADIENT

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Slobodan Davidović, Katarina Erić, Pavle Erić, Irena Hribšek, Marija Tanasković

[1]Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

Abstract

Urban environments present challenging habitats for pollinators due to multiple stressors such as pollution, habitat fragmentation, and pesticide exposure. Nevertheless, cities may also serve as important refugia for pollinators due to the presence of parks, gardens, and other green spaces with diverse flowering plants. In Belgrade, a long-standing tradition of planting melliferous plant species has contributed to high pollinator diversity, with approximately 250 recorded bee species and a well-established population of free-living honey bees (*Apis mellifera*). However, the physiological mechanisms that enable honey bees to persist under urban environmental pressures remain insufficiently understood.

To investigate molecular stress responses, honey bees were sampled from eight sites along an urbanisation gradient in Belgrade, comprising six urban and two semi-urban locations. In addition, one rural site and one site located near mining tailings were included for comparison. The control group consisted of newly emerged worker bees, not yet exposed to external environmental conditions. Expression of three marker genes associated with detoxification and oxidative stress responses was evaluated: metallothionein (MT), superoxide dismutase (SOD1), and cytochrome P450 monooxygenase (CYP9Q2).

Metallothionein expression was elevated in all environmental samples, with the highest levels recorded at both semi-urban sites. Notably, MT expression in urban and semi-urban locations exceeded the levels observed near the mining tailings deposit. In contrast, SOD1 expression showed no consistent trend and remained largely comparable to control levels. The highest CYP9Q2 expression was detected at one urban site, followed by the rural site.

These results indicate that honey bees inhabiting urban and semi-urban environments experience significant exposure to environmental contaminants, particularly metals, and respond by increasing their metallothionein expression. The relatively stable SOD1 levels suggest that detoxification processes may mitigate metal toxicity before substantial oxidative stress occurs. Elevated CYP9Q2 expression likely reflects exposure to pesticides associated with urban mosquito control and agricultural practices.

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DETECTION OF HONEY BEE PATHOGENS AND REPLICATIVE DEFORMED WING VIRUS (DWV) IN *V. ORIENTALIS* FROM NORTHEASTERN ITALY, MALTA AND CYPRUS

Sabrina de Leo[1], Laura Zulian[1], Fulvio Bordin[1], Paolo Zucca[2], Giovanni Cilia[3], Laura Bortolotti[3], Manlio Palei[2], Charles Spiteri[4], Franco Mutinelli[1], Anna Granato[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Laboratory for Honey bee Health, Legnaro, Italy
[2]Central Directorate for Health, Social Policies and Disabilities, Friuli Venezia Giulia Region, Trieste, Italy
[3]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[4]Animal Health and Welfare Department, Ministry for Agriculture, Fisheries and Animal Rights, Marsa, Malta

Abstract

The oriental hornet (*Vespa orientalis*), native to Southwestern Asia, the Middle East, Northeastern Africa and the Southeastern Mediterranean, is expanding its range across Europe due to global trade and climate change. Its negative impact on *Apis mellifera* colonies, via direct predation and potential pathogen transmission, has prompted surveillance plans to monitor its spread across Italy, where it is endemic in the southern and central regions.

Between August and October 2024, *V. orientalis* specimens were collected from two endemic areas (8 from Malta; 8 from Turkish Republic of Northern Cyprus) and one Italian region where it is neo-invasive (35 from Friuli Venezia-Giulia) and analysed to assess the presence of the most common honey bee pathogens. Molecular analyses revealed *Vairimorpha ceranae* only in one Maltese specimen. Virus coinfections were detected; Deformed wing virus (DWV) was most prevalent across sites, followed by Black queen cell virus, Acute paralysis virus, Sacbrood virus and Chronic paralysis virus. *Apis mellifera* filamentous virus occurred in all Northern Cyprus samples (100%) and also in Maltese (87.5%) and Italian ones (28.6%). No other tested pathogens were detected (*Vairimorpha apis*; *Lotmaria passim*; *Crithidia mellificae*; Israeli acute paralysis virus, Kashmir bee virus). A strand-specific RT-PCR was used to evaluate the presence of replicative DWV, the most prevalent virus, although no morphological alterations were observed in any specimens. Detection of the DWV negative strand in samples provided evidence of active viral replication in this species.

These findings suggest that *V. orientalis* may act as potential vector of honey bee pathogens. The detection of replicative DWV indicates a dual role, functioning as both a mechanical and biological vector. This highlights the urgent need for enhanced monitoring, control, and, where feasible, eradication efforts—particularly in recently invaded areas—to safeguard honey bee health, colony stability, and the sustainability of the beekeeping industry.

PCR INHIBITORS IN *APIS MELLIFERA* COMPOUND EYES AND THE EFFECT OF PUPAL PIGMENTATION ON GENOMIC DNA QUALITY AND YIELD

P 189

Valérie Deshaies[1], Ségolène Maucourt[1,2], Laurence Plamondon[1,2], Claude Robert[3], Andrée Rousseau[1,2], Pierre Giovenazzo[1]

[1]Laval University, Department of Biology, Québec, Canada
[2]Centre de Recherche des Sciences Animales de Deschambault (CRSAD), Deschambault, Canada
[3]Laval University, Department of Animal Sciences, Québec, Canada

Abstract

Genomic approaches are increasingly used to study the honeybee, *Apis mellifera*, providing key insights into its genetic diversity, evolution, and adaptation to environmental stressors. However, the reliability of these analyses depends on genomic DNA (gDNA) quality and yield, as well as amplification efficiency. Biological factors such as eye pigmentation and developmental stage may influence these parameters, particularly through PCR inhibitors associated with pigments. This study aimed to evaluate the effect of eye pigmentation on quantitative PCR (qPCR) amplification and the influence of pupal pigmentation on gDNA quality and yield.

For the first objective, qPCR amplification of the β-actin gene (*ACTB*) was performed using gDNA extracted from different tissues: drone pupal thorax (positive control), whole adult worker, worker head, whole drone pupa, and drone pupal head. Pupae exhibited pale pink eyes, whereas adult workers had dark, highly pigmented eyes. Each condition was analyzed in five replicates. For the second objective, pools of 30 drone pupal heads were grouped according to body pigmentation (white, lightly pigmented, and dark). gDNA was extracted using the Quick-DNA Tissue/Insect Miniprep Kit, and its quality and yield were assessed by automated electrophoresis.

Results indicate that eye pigmentation affects qPCR amplification. Adult workers with dark eyes showed higher Cq values (number of cycles required for the fluorescence to exceed the DNA detection threshold) than pupae, suggesting partial PCR inhibition, which was more pronounced in isolated heads. No significant differences were observed between drone pupae and the positive control. Additionally, gDNA quality and yield decreased with increasing pupal pigmentation. White pupae produced more intact DNA (higher DIN values) and higher yields than darker pupae, likely reflecting differences in tissue structure and homogenization efficiency.

Overall, white pupal heads represent an optimal biological material for genomic analyses in *Apis mellifera*, providing high-quality and high-yield gDNA suitable for reliable downstream applications.

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DNA BARCODING AND MORPHOLOGICAL IDENTIFICATION TO INVESTIGATE THE BEE FAUNA OF TWO ITALIAN NATIONAL PARKS

Gaia Di Francescantonio[1], Chiara B. Boni[1], Francesca Coppola[1], Simona Sagona[1], Marco Bonifacino[2], Eckart Stolle[3], Giorgio Davini[4], Luciano Di Martino[5], Antonio Felicioli[1]

[1]University of Pisa, Pisa, Italy
[2]University of Florence, Sesto Fiorentino, Italy
[3]Leibniz Institute for the Analysis of Biodiversity Change, Bonn, Germany
[4]Gran Sasso and Monti della Laga National Park, Assergi, Italy
[5]Majella National Park, Sulmona, Italy

Abstract

Bee population monitoring is crucial for bee conservation and requires the identification of specimens at species level; however, traditional identification based on morphology suffers from lack of experts and specialized keys. DNA barcoding can complement morphological identification, although it deeply depends on the completion of reference sequence libraries and is still unreliable for *taxa* whose genomes have not been thoroughly explored.

Our aims were to compare the outcomes of DNA barcoding and morphological analysis for the determination of 486 bees collected in 2024 across 12 sites of the Majella (MNP) and Gran Sasso (GSNP) National Parks in Italy, and to contribute to the bee species' checklist of the two Parks.

All specimens were identified by an expert taxonomist and barcoded. DNA extraction followed the HotSHOT method, Folmer primers were used to amplify the 658 bp fragment of the *cox1* gene, sequencing was performed on an Oxford Nanopore GridION machine, and data were processed via ONTbarcoder.

Barcoding resulted in 440 identifications at species level, 14 at genus level and 32 failed sequencings. According to the morphological analysis, 78% of those 440 species-level identifications was correct, 7% incorrect, and 15% only correct at genus level. 71% of wrong assignments differed from morphological identifications at family level, which suggests contamination might have occurred. Morphological analysis resulted in 443 specimens identified at species level; the remaining 43 were only identified at genus level because diagnostic body parts were missing or the specimen belonged to a cryptic species. Barcoding results for morphologically cryptic specimens suggested a likely-correct identification in 76% of cases.

Investigated methods complemented each other and allowed us to detect 133 species (23 genera); of those, 29 are newly reported for the MNP and 45 for the GSNP. One species from the GSNP, *Andrena probata*, has only been reported twice in Italy before.

DYNAMICS OF THE CHESTNUT NECTAR FLOW AND ITS IMPACT ON HONEY BEE COLONIES

P 191

Ana Diéguez-Antón[1,2,3], Jonathan S. Castaño-Serna[1,2], María Shantal Rodríguez-Flores[1,2], María Carmen Seijo Coello[1,2]

[1]Department of Plant Biology and Soil Science, Faculty of Science, Universidade de Vigo, Ourense, Spain
[2]Instituto de Agroecoloxía e Alimentación (IAA), Campus Auga, Universidade de Vigo, Ourense, Spain
[3]CIMO, Instituto Politécnico de Bragança, Bragança, Portugal

Abstract

Honey bees rely on efficient foraging to sustain colony growth and survival. Worker bees perform diverse tasks, but foraging is crucial, as it provides nectar, pollen, water, and other resources necessary for colony maintenance. The success of foragers directly affects colony nutrition, honey production, and overall performance. Abundant and diverse floral resources determine the quantity and quality of nectar collected. However, climatic variations can alter flowering timing and nectar availability, influencing foraging efficiency and colony health. Monitoring foraging activity and resource intake is therefore key for sustainable beekeeping and optimizing honey production. In this context, precision beekeeping has emerged as an advanced, data-driven approach that integrates sensors, automation, and real-time monitoring to optimize colony management and enhance productivity. This study assesses the abundance of growing stock and its effects on resource availability and evaluates how colony density influences colony weight gain and nectar foraging. The study was conducted in Galicia (Northwest Spain), where scales were placed under two different hives in three apiaries in which, in previous years, beekeepers' honey contained chestnut pollen and where chestnut trees were present nearby. The data were collected over a two-year period. The results showed that there are differences in the beginning of the chestnut flowering period between years and locations, and that the duration of the nectar flow also varies across years and areas. Additionally, colonies that were stronger before the nectar flow period gained weight more quickly than those with lower strength. Hive scales could help beekeepers track colony performance and adapt to variations in nectar flow due to climatic fluctuations, supporting effective management and the production of high-quality monofloral honeys.

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GENTLE MONITORING FOR SPECIES PROTECTION

Melina Eisenring

UniBe, Bern, Switzerland

Abstract

Insects, especially pollinators, are absolutely crucial to maintaining natural biodiversity and human nutrition. For the first time in the history of the earth, wild insects are in massive decline. Evidence-based protective measures are therefore now appropriate, which require reliable monitoring data. Unfortunately, monitoring of wild insects is currently based on captures, which: 1) kills animals worthy of protection, 2) does not allow reliable statements, especially for social insects, since 10 animals can come from one or 10 colonies. Population genetics is therefore particularly essential for social insects to improve their protection. We are therefore proposing a paradigm shift for insect monitoring: collecting genetic data without killing animals or damaging exhibits, to lay new foundations for the protection of wild insects. The method will be established in the laboratory in Bern using bred bumblebees and exhibits from the museum. The first field applications will then be carried out on endangered bumblebee species in Switzerland. After adaptation, the established method can be applied to other species to support species protection for pollinators worldwide with careful monitoring.

BESAFEBEEHONEY: A EUROPEAN NETWORK FOR THE SAFETY, VALORISATION, AND SUSTAINABILITY OF BEEKEEPING PRODUCTS

P 193

Andreia Freitas[1,2], Marta Leite[1,2], Ivana Varenina[3]

[1]Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Vila do Conde, Portugal.
[2]REQUIMTE/LAQV, Porto, Portugal
[3]Croatian Veterinary Institute, Zagreb, Croatia

Abstract

Honey and other beekeeping products are valued for their nutritional, medicinal, and economic importance. At the same time, honeybees, particularly *Apis mellifera*, play a crucial ecological role as pollinators, supporting biodiversity and agricultural productivity worldwide. However, in recent decades, honeybee populations have faced increasing pressures from biotic and abiotic stressors, including diseases, habitat loss, climate change, and exposure to anthropogenic contaminants. These threats have contributed to colony losses, declining honey production, increased dependence on imports, and concerns about the sustainability and safety of the honey value chain. The BeSafeBeeHoney COST Action (CA22105) aims to address these challenges by establishing an interdisciplinary and transnational network focused on the valorisation of beekeeping products and the use of hive matrices as tools for environmental biomonitoring. The Action brings together researchers, veterinarians, beekeepers, policy experts, and other stakeholders across fields such as chemistry, biology, ecology, agrarian sciences, veterinary sciences, nutrition, economics, and policy. Key research themes include the nutritional and medicinal properties of hive products; monitoring environmental contaminants using hive matrices as bioindicators; identification and control of honeybee diseases; evaluation of the socio-economic and ecological impacts of colony losses; and market analysis and evidence-based policy-making. Through coordinated research, knowledge exchange, and stakeholder engagement, the initiative promotes innovative and sustainable solutions.

This work will also present the main activities developed so far within the Action, including networking events, training schools, workshops, and collaborative initiatives, highlighting their role in capacity building and knowledge transfer.

Ultimately, BeSafeBeeHoney aims to strengthen the resilience and competitiveness of the European apiculture sector while supporting biodiversity, food security, and sustainable agricultural systems.

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METHODOLOGICAL APPROACHES TO ASSESS POSSIBLE EFFECTS OF RADIO-FREQUENCY ELECTROMAGNETIC FIELDS EXPOSURE ON HONEY BEE DEVELOPMENT, WILD BEE DIVERSITY AND REPRODUCTION

Fani Hatjina[1], Anastasia Kalapouti[1], Leonidas Charistos[1], Arno Thielens[2], Maria Bouga[3], Niels Bijl[4], Pieterjan De Boose[5], Giovanni Costa[4], Jürg Fröhlich[6], Menno van de Meerakker[4], Maarten Paulides[4], Felipe Oliveira Ribas[5], Kemal Sumser[4], Fabian Schneider[6], Menelaos Stavrinides[7], Zoi Thanou[8], Antonios Tsagkarakis[8], Androulla Varnava[7], Marco Zahner[6], Anke Huss[9]

[1]Institute of Animal Science & Department of Apiculture, Hellenic Agriculture Org. "DEMETER", Nea Moudania, Greece
[2]Photonics Initiative, Advanced Science and Research Center, the Graduate Center of the City University of New York, New York, United States
[3]Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, Athens, Greece
[4]Electromagnetics for Care + Cure Research Lab, Eindhoven University of Technology, Eindhoven, Netherlands
[5]Department of Information Technology, Ghent University, Ghent, Belgium
[6]Fields at Work GmbH, Zurich, Switzerland
[7]Department of Agricultural Sciences, Cyprus University of Technology, Limassol, Cyprus
[8]Department of Crop Science, Agricultural University of Athens, Athens, Greece
[9]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands

Abstract

The current study aims at investigating the effects of RF-EMFs at 3.6 GHz and 24-26 GHz on honey bee development/ fitness and wild bee biodiversity and reproduction under controlled and field conditions (Horizon Europe project 101057216, Exposure To electromAgnetic fIelds and plaNetary health - ETAIN).

An indoors experiment was designed where three honey bee colonies were exposed to a 5G-like modulated RF-EMF signal at 3.6 GHz with an incident rms-power density of 1W/m_2, while three parallel control colonies were not. The two groups of colonies were kept in separate rooms electromagnetically shielded from each other. The colonies were placed on digital scales and equipped with sensors for temperature, humidity, and CO_2. Brood development, colony strength and foraging activity, as well as sensor's data, have been collected.

In parallel, an installation of custom 5G-emulating exposure setup with RF-EMF emissions in two 90° sectors and control in two other 90° sectors was established, as an outdoor experimental station. A total number of 24 honey bee colonies were placed at different distances from the antenna and under different strengths of the RF-EMF signal, at 3.6 GHz and 24-26 GHz. The development of the honey bee colonies, as well as the diversity and abundance of wild bee species collected in pan traps, have been monitored. Additionally, *Osmia bicornis* nests were established at the same locations, and the ratio of males to females *Osmia* bees was also recorded.

The 1st year overall results did not confirm any negative effects on brood development and foraging activity of the honey bees under the indoor conditions at 3.6 GHz. However, a decline of honey bee colony strength was observed under field conditions at 24-26 GHz. Also the males/female ratio of *Osmia bicornis* bees was altered from 0.6 in non-exposed areas to 1.2 in exposed areas.

RELATIVE EFFICACY OF GENETICS, CHEMICAL, AND MECHANICAL CONTROL OF MITES *VARROA DESTRUCTOR*

P 195

Harmen P. Hendriksma[1,2], Dorothee J. Lüken[1], Torsten Ellmann[3]

[1]Julius Kühn Institute, Institute for Bee Protection, Federal Research Centre for Cultivated Plants, Braunschweig, Germany
[2]Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland.
[3]Deutscher Imkerbund e.V., Wachtberg, Germany

Abstract

The ectoparasitic mite, *Varroa destructor,* acting as a potent virus vector is the most important pest of western honey bees, *Apis mellifera*, driving colony losses worldwide. Despite the worldwide use of chemical, mechanical, and genetic control approaches, their relative effectiveness under field conditions remains debated. Here, we evaluated the relative efficacy of these approaches in the field. We conducted a large-scale, experiment using 144 colonies at 6 apiaries established from controlled matings of sister queens representing multiple breeding lines (*A. m. carnica*). Colony genotype affected mite fall, indicating a genetic component shaping colony–mite interactions. In the subsequent season, colonies were subjected to either brood removal as a mechanical control strategy or formic acid treatment as a chemical control. Compared with formic acid treatment, brood removal resulted in a ~40% reduction in adult bee populations, significantly higher mite loads on adult bees, higher DWV levels, and lower colony survival. Virus prevalence CBPV and ABPV did not differ between treatments; however, multivariate analyses revealed a negative association between them, independent of treatment. The DWV infections covaried with mite loads and reduced colony sizes. Overall, these results demonstrate that, under the conditions tested, brood removal did not provide mite control or colony health benefits comparable to formic acid treatment. These findings underscore the limits of mechanical control when applied as a stand-alone strategy and highlight the opportunity to integrate pest management with genetic selection to improve colony health.

P 196 ARE THE FLORAL CHOICES OF WILD BEES EXPLAINED BY POLLEN POLYPEPTIDES, LIPIDS AND AMINO ACIDS CONTENTS?

Anne-Laure Jacquemart, Julien Radoux, Yannick Agnan, Christel Buyens

Université Catholique de Louvain, Louvain-la-Neuve, Belgium

Abstract

Against the backdrop of the widespread decline in pollinators, particularly wild bees, we are studying floral choices in relation to bee health. We are focusing on the availability and nutritional quality of floral pollen resources. Bees depend on these resources throughout their entire life cycle. The chemical compositions of floral pollen are analysed for three types of components: total polypeptides, total lipids, and total and free amino acids.

Since 2021, pollen samples have been collected at several locations (Belgium, France, Sweden, Switzerland) by volunteers. Pollen is collected from fresh flowers to avoid any influence from the bees' saliva when they form pollen loads. Overall, 83 plant species visited by wild bees are currently being analysed on a total of 203 collected plant species.

The main findings point out several key explanatory factors: location (soil type and climate), plant family, species, and the technique used.

When volunteers collect whole flowers, the sieving stages are particularly time-consuming, and this affects both pollen loss and the purity of the samples. The best approach is therefore to dissect flowers with deep corollas or to allow the pollen to fall onto sheets of paper. The IEX chromatography method, which is more efficient than HPLC, was chosen for the amino acid analyses.

Preliminary results showed some trends according to the plant family analysed. Polypeptide content ranged from 11 to 155 µg/mg, while lipid content varied between 9 and 91 µg/mg. Rosaceae and Fabaceae species present pollen with high contents or P/L ratios. Pollen from Boraginaceae species had the highest total amino acid contents, while the lowest were found in Malvaceae species.

STATUS OF THE YELLOW-LEGGED HORNET IN KOREA AND RESEARCH ON NEST DETECTION

P 197

Subae Kim

National Institute of Agricultural Sciences, Rural Development Administration, Wanju-gun, Korea, Republic of Korea

Abstract

The yellow-legged hornet, *Vespa velutina nigrithorax*, was first detected in Busan, Korea, in 2003 and has since spread widely, becoming a serious threat to apiculture and public safety. Trap surveys showed that *V. velutina* is now the dominant hornet species in Korea on an annual basis, although *Vespa mandarinia* is more frequently captured from April to June. Recent monitoring also suggests that its seasonal occurrence pattern is shifting from a gradual increase after July to a bimodal pattern, possibly due to intensified summer heatwaves.

This study summarizes the current status of *V. velutina* in Korea and recent research on nest detection. Meteorological factors, including temperature, precipitation, solar radiation, humidity, and wind speed, were analyzed to develop a damage prediction model for risk-based monitoring and control. In addition, UAV-based nest detection systems were investigated, including observer tracking, rotating antenna systems, small-drone secondary searches, 3D apiary mapping, image recognition, and acoustic analysis of nest-specific sound patterns. These approaches are expected to improve the early detection and precise localization of *V. velutina* nests and support an integrated management strategy for reducing damage to beekeeping and human safety in Korea.

P 198 MODIFIED APPROACH FOR DRY AUTOMATED SAMPLING OF POLLINATORS WITH VANE TRAPS

Kenneth Kuba, Ugo Diniz, Felix Kreuzmann, Sara D. Leonhardt

Plant-Insect Interactions, TUM School of Life Sciences, Technical University Munich, Freising, Germany

Abstract

Automated collection of insect pollinators is a crucial approach in many ecological studies. Not only does it allow sampling over longer time periods and simultaneously across several plots, but it also increases sampling time and thus reduces daytime biases. However, one of the primary drawbacks—besides bycatch and the increased sorting and insect-handling time—is the decreased visibility of identification traits and the loss of ecologically relevant contaminants, such as pollen, chemical residues, and particulate matter.

To address this issue, we designed a new trapping bucket (currently named *KDK Automated Size Stratifier*) for commercially available vane traps, which are widely used in the US and have shown promising collection results. In the design, we integrated an automated stratification approach that separates insects by size into several layers and cells to avoid cross-contamination. Additionally, apart from two commercially available parts, we designed all other elements for 3D printing, enabling easy and nearly identical reconstruction in larger quantities. The traps are currently being implemented at several sites in the Butterfly Horizon project and potentially in the ProPollSoil Horizon project, and the first results will be presented alongside further tests and live demonstrations.

With this trap design, we aim to improve insect pollinator sampling and increase the amount of information that can be obtained from single individuals by collecting them separately and dry rather than collectively in a collection fluid. Furthermore, this method may offer new insights not only into pollinator visitation but also into individual loads of agrochemicals and other contaminants, helping to better understand insect decline and evaluate restoration efforts.

THE SUPERBEELIVE MACROSCOPE, A MONITORING TOOL TO INVESTIGATE THE BEE-ENVIRONMENT RELATIONSHIP

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Maksym Lytvynenko[1], Idriss Jairi[1], Maxime Linard[2], Sandro Andreu[2], Nathalie Chevallier[2], Sébastien Druon[1], Jean-luc Oms[1], Jonathan Bares[3], Jean Triboulet[1], Pascal Poncelet[1], Matthieu Rousset[2]

[1]LIRMM, UMR 5506, University of Montpellier - CNRS, Montpellier, France
[2]IBMM, UMR 5247, University of Montpellier - CNRS, Montpellier, France
[3]LMGC, UMR 5508, University of Montpellier - CNRS, Montpellier, France

Abstract

A combination of environmental stresses including chemical pollution, invasive predatory or parasitic species, or climate change is producing, on a global scale, an undeniable decline in bee colonies. This damage to the main pollinating species is not only a precursor of important losses in agricultural production but also a testimony to the degradation of the Earth's ecosystem. Therefore, the study of bee health must be considered from a holistic perspective and mobilize the concerted efforts of distinct communities of knowledge and expertise.

The "SuperBeeLive" project that we are conducting consists in analyzing in real time the social organization and homeostasis of a bee colony, from birth to death, while monitoring the levels of stress intensity that it undergoes through several biological interactions and exposure to neurotoxic insecticides. The innovation consists in the construction of a macroscope for the observation of bee colonies thanks to the instrumentation of a beehive in order to continuously collect several types of digital and video data streams in parallel, in a systematic, automatic and non-invasive way. This experimental device, which uses IoT, computer vision and neural network technologies, is intended (i) to decipher the etiology of the bee's disorders by correlating observed behavioral colony disturbances with the variations of these stressors (ii) to increase our knowledge of the functioning of the bee superorganism.

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FIRST ASSESSMENT OF FREE-LIVING COLONIES IN PORTUGAL

Aicha Meliani-Rodríguez[1], Grace P. McCormack[2], Joachim R. de Miranda[3], Andrzej Oleksa[4], Fabrice Requier[5], Steve Rogenstein[6], Maria Alice Pinto[1]

[1]CIMO – Centro de Investigação de Montanha, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[2]Zoology, School of Natural Sciences and Ryan Institute, University of Galway, Galway, Ireland.
[3]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[4]Faculty of Biological Sciences, Kazimierz Wielki University, Bydgoszcz, Poland
[5]Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, Gif-sur-Yvette, France
[6]Honey Bee Watch, Galway, Ireland

Abstract

The Western honey bee (*Apis mellifera*) is a key pollinator and plays a fundamental role in ecosystem services and agricultural productivity. However, beyond managed colonies, wild honey bee populations, also known as free-living colonies (FLCs), form an important yet critically understudied component of Europe's native biodiversity. In this context, the multinational FREE-B research consortium, with partners from France, Ireland, Poland, Portugal and Sweden, aims to understand how FLCs survive and persist across Europe.

Here, we provide a first assessment of the occurrence, density and winter survival of FLCs at a study site in Portugal, employing two complementary field methods: systematic transect surveys targeting cavity-bearing trees, and the bee-lining technique, which consists of the flight paths of foraging bees back to their nest. Using these approaches, a total of 13 FLCs were identified. All colonies were found nesting in cavities of old oak trees (*Quercus ilex*). Colony survival was also assessed through repeated field monitoring visits conducted across the season, revealing that 5 out of 13 colonies survived the winter 2025-2026 (38.5%), a winter survival rate that we hypothesized reflects the impact of severe winter conditions. To expand spatial coverage, a citizen-science approach was also implemented, resulting in the identification of 28 additional colonies across Portugal during the first year. Most of these colonies were located in tree cavities, while the remaining were found in buildings. Additional information was collected for each colony, including metadata on cavity characteristics, entrance height and orientation, and other relevant ecological parameters.

These findings provide valuable baseline data on FLCs' distribution, nesting preferences, and early survival dynamics in Portugal, with density estimates potentially exceeding the European average of 0.26/km². Overall, this work marks an initial step toward understanding FLCs in the country and their potential role in biodiversity conservation.

TRACKING PESTICIDE EXPOSURE THROUGH BEE-COLLECTED POLLEN

P 201

Niks Ozols[1], Maksims Filipovičs[1], Betija Rubene[1], Sabīne Močāne[1], Baiba Tikuma[2], Jānis Gailis[1], Viktorija Zagorska[1]

[1]Latvia University of Life Sciences and Technologies, Institute of Plant Protection Research "Agrihorts", Jelgava, Latvia
[2]Latvia University of Life Sciences and Technologies, Institute of Soil and Plant Sciences, Jelgava, Latvia

Abstract

Honey bees (*Apis mellifera*) are widely used as bioindicators of environmental contamination, reflecting pesticide exposure across agricultural landscapes. While pesticide residues are frequently detected in bee pollen, their temporal dynamics and periods of increased risk remain insufficiently understood. This study assessed pesticide residues in pollen collected from six apiaries across Latvia during the 2025 flowering season. A total of 40 composite pollen samples were analysed using LC-MS/MS and GC-MS/MS multi-residue methods. Nineteen active substances were detected. Herbicides were the most frequently detected group, with glyphosate occurring most often. Among fungicides, boscalid, azoxystrobin and tebuconazole were commonly detected. The highest concentrations were recorded for glyphosate (840 µg kg^{-1}) and fluazifop-P-butyl (660 µg kg^{-1}). Insecticides, including acetamiprid, flupyradifurone and cypermethrin, were detected less frequently but had a greater impact on toxicological risk. Pesticide exposure was evaluated using the Pollen Hazard Quotient (PHQ). While median PHQ values remained below the commonly used threshold (PHQ<50), several individual samples exceeded this level, with a maximum PHQ of 1173.9 driven primarily by cypermethrin. Elevated PHQ values were most frequently observed between mid-May and early July, corresponding to peak crop flowering and pesticide application periods. Compared to previous observations, pesticide residues in 2025 were distributed more evenly across the sampling period, indicating extended exposure. Apiaries located in agriculturally dominated landscapes tended to show higher contamination levels; however, no statistically significant relationship between landscape composition and PHQ values was detected. These results demonstrate that pesticide exposure in pollinators is characterized by periods of increased risk rather than consistently low average levels. Such exposure may have greater biological relevance than median values alone. Our findings emphasise the importance of accounting for temporal variability in pesticide risk assessment and support the use of honey bee-collected pollen as an effective tool for large-scale environmental monitoring.

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SURVEILLANCE OF *VESPA VELUTINA* IN PIEDMONT: THE VESP PROJECT

Alberto Pesavento[1], Alberico Franzin[2], Cristina Pogolotti[3], Luca Anselmo[4], Elena Biasibetti[2], Luca Allais[1], Simone Tosi[3], Luca Carisio[2]

[1]Aspromiele – Associazione Produttori Miele Piemonte, Alessandria, Italy
[2]Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Torino, Italy
[3]University of Turin - Department of Agricultural, Forest and Food Sciences, Grugliasco, Italy
[4]University of Turin - Department of Life Sciences and Systems Biology, Torino, Italy

Abstract

Vespa velutina is an invasive alien hornet and a predator of honey bees and other insects. The species is now established across much of Europe. In the Piedmont Region (Northwest Italy), only a limited number of nests and adult individuals have been reported, mainly in areas near the southern border shared with the Liguria Region and France, where the hornet has long been established.

In this context - where *V. velutina* is not yet widely established - early detection of nests through surveillance and their prompt removal are considered the most effective strategies to prevent spread and permanent establishment.

Surveillance of *V. velutina* in Piedmont (VESP) is a project of the "Task Force Velutina Piemonte" aimed at developing a surveillance system capable of: (i) integrating information from different surveillance components (e.g., voluntary monitoring by beekeepers and targeted surveillance campaigns); (ii) estimating surveillance sensitivity; and (iii) informing surveillance decision-making.

We implemented a surveillance system considering beekeeper apiary inspections and hornet trapping as formal tests to validate the absence of *V. velutina*. The system is currently under development, with different parameter scenarios being evaluated for sensitivity calculations. Test sensitivity parameters are based on literature data, the number of tests derived from beekeeper distribution, and behavioural information collected through a questionnaire (more than 500 respondents). Estimates of *V. velutina* prevalence are derived from species distribution modelling.

The surveillance system provides a dynamic, multi-level map defining risk levels and areas across the entire study region. An assessment of the map's effectiveness, using data from intensive trapping surveys, is currently underway.

The VESP project is planned to be ready for field testing in 2026. Subsequently, it could be scaled up and replicated in other regions, and potentially adapted for surveillance plans targeting other invasive species.

AN UPDATED CHECKLIST OF THE WILD BEES OF ITALY: TAXONOMIC REVIEW, DISTRIBUTIONAL ASSESSMENT AND PERSPECTIVES FOR FUTURE RESEARCH

P 203

Marino Quaranta[1], Maurizio Cornalba[2]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]Department of Mathematics, University of Pavia, Pavia, Italy

Abstract

Italy hosts one of the richest wild bee faunas in Europe, yet a comprehensive and critically revised national checklist has been lacking in recent decades. Here we present an updated checklist of the wild bees of Italy, based on an extensive review of both historical and recent literature, online databases, and unpublished data. Notably, the very process of preparing this checklist has already stimulated a significant increase in research activity on the Italian wild bee fauna.

A total of approximately 1,350 nominal species were examined. For each taxon, we assessed two main aspects: (i) taxonomic validity and (ii) confirmed presence in Italy. Species not presenting taxonomic or distributional issues were retained without comment, whereas problematic cases were critically discussed. This process was supported by the examination of museum collections, field experience, and consultation with taxonomic specialists.

To provide a synthetic ecological interpretation, each confirmed species was assigned to one of four abundance categories, integrating information on geographic distribution, frequency of occurrence, and habitat specialization. Given the well-known limitations of available data, these categories are based on expert assessment rather than strictly quantitative measures, and should be interpreted as working hypotheses.

The resulting checklist represents a substantial update over previous national inventories, incorporating numerous taxonomic changes, new records, and revised distributional data. It also highlights persistent knowledge gaps, particularly concerning species complexes in need of revision and poorly documented regions.

This work provides a robust baseline for future taxonomic, biogeographical and conservation studies on Italian wild bees, and contributes to a broader understanding of European bee diversity.

WITHDRAWN

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PERFORMANCES OF SELECTIVE MECHANICAL TRAPS FOR AUTUMN CONTROL OF THE INVASIVE HORNET *VESPA VELUTINA NIGRITHORAX* IN WESTERN AND SOUTHERN EUROPE

Amanda Riveros[1], Marco Pietropaoli[1], Camilla Pedrelli[1], Giovanni Brajon[1], Dirk C. de Graaf[2], Carlos Marín Barcáiztegui[3], María Shantal Rodríguez-Flores[4], Ester Ordóñez[5], Martina Girola[1], Giovanni Formato[1]

[1]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma, Italy
[2]Laboratory of Molecular Entomology and Bee Pathology (L-M EB), Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium
[3]Independent Researcher, Ribera de Arriba, Spain
[4]Agroenvironmental Systems Research Group, Faculty of Sciences, University of Vigo, Ourense, Spain
[5]Group for the Development and Training of the Galician Beekeeping Sector (Agrupación Apícola de Galicia), Ourense, Spain

Abstract

Vespa velutina nigrithorax, commonly known as the yellow-legged hornet, is an invasive species originating from Southeast Asia that has rapidly expanded across Europe since its introduction in 2004. Its predation on the honey bee (*Apis mellifera*) threatens apiculture, biodiversity, and ecosystem stability. Mechanical trapping is widely used as a control strategy; however, many traps lack selectivity and capture large numbers of non-target insects. This study evaluated the effectiveness and selectivity of five trap–bait combinations for improving the capture of *V. velutina* while minimizing impacts on non-target species. Field trials were conducted during autumn 2024 (October–November) in northern Spain (Galicia and Asturias) and Italy (Liguria and Tuscany) using a citizen science approach involving 12 beekeepers. Two bait types were tested: a commercial attractant and a homemade mixture of water, sugar, and yeast. Five trap–bait combinations were assessed: VelutinaTrap with commercial bait (BC), VelutinaTrap with homemade bait (BH), Vespa Catch Selet with commercial bait (VC), Vespa Catch Select with homemade bait (VH), and GardApis Sentinel with homemade bait (GH). Traps were installed near apiaries at heights of 1–1.5 m, spaced approximately 3 m apart, and inspected weekly for four weeks. Capture rate was calculated as the number of *V. velutina* individuals per trap per day, while selectivity was measured as the ratio of *V. velutina* to non-target insects captured. Results showed that the VelutinaTrap with homemade bait achieved the highest capture efficiency. Overall, trap design emerged as the primary factor influencing trapping performance. These findings indicate that mechanical trapping can represent a sustainable management strategy for *V. velutina*, provided that trap selectivity is improved to reduce impacts on native insect fauna.

WINTER BROOD DYNAMICS IN US HONEY BEE COLONIES: A FOUR-SEASON STUDY

P 205

Stephanie R. Rogers[1], Rogan Tokach[2], Ben De Moras[1], Geoffrey R. Williams[1]

[1]Auburn University, Auburn, United States
[2]North Dakota State University, Fargo, United States

Abstract

Understanding the temporal dynamics and patterns of brood production is fundamental to effective honey bee colony management. The presence or absence of capped brood is a critical determinant in optimizing the timing of chemical treatments for *Varroa* and is increasingly relevant for identifying regions at risk of Tropilaelaps infestations. However, spatial and temporal variability in brood breaks, expectedly governed by local weather and climatic conditions, complicates the development of standardized integrated pest management (IPM) and early response strategies for *Varroa* and *Tropilaelaps*, respectively. Although brood monitoring is routinely conducted during warmer periods, winter brood production remains insufficiently characterized due to limited sampling, as inspections are often minimized to reduce colony disturbance. To address this knowledge gap, a US-nationwide collaboration involving 16 land-grant universities or community colleagues, one statewide cooperative extension system, five USDA-ARS laboratories, and six beekeepers, set out to monitor winter brood activity. From November to February over four consecutive winters (2022–2026), bi-weekly estimates of capped brood and adult bees were recorded in sentinel managed colonies. These data were used to produce static and dynamic bi-weekly maps, made publicly available online, to help beekeepers estimate local brood breaks and better time their mite treatments.

Preliminary spatial analysis using the first three years of data reveals consistent winter brood breaks across several regions of the country, though annual weather variability significantly influences brood development. Year four data was used to test the accuracy of predictive models to determine their efficacy in predicting brood patterns across the US, now and into the future. The results of this work will be used to further refine location-specific IPM recommendations and support long-term honey bee health.

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A THREE-YEAR NATIONAL MONITORING NETWORK AND WEB BASED DIAGNOSTIC TOOL FOR *VARROA DESTRUCTOR* MANAGEMENT IN SLOVENIA

Rene Rus[1], Lucija Žvokelj[1], Barbara Hočevar[1], Monika Kozar[1], Mira Jenko Rogelj[2], Ivo Planinc[2], Suzana Skerbiš[2], Alenka Jurić[2], Mateja Ratiznojnik[2], Vida Lešnik[2], Anita Vraničar Novak[2], Metka Pislak Ocepek[1]

[1]Institute of Pathology, Forensic Veterinary Medicine, Wildlife, Bees and Aquaculture, Veterinary Faculty, University of Ljubljana, Slovenia, Ljubljana, Slovenia
[2]National Veterinary Institute, Veterinary Faculty, University of Ljubljana, Slovenia, Ljubljana, Slovenia

Abstract

Varroa destructor remains a leading cause of honeybee colony losses worldwide. Monitoring varroa infestation rates requires considerable effort. Consequently, many beekeepers avoid it, leading to delayed treatments and colony collapses. To encourage beekeepers to adopt proactive varroa control, we established a continuous national monitoring network across Slovenia. Moreover, we developed a web platform to track regional varroa dynamics, along with interactive diagnostic calculators for beekeeper's colony assessment.

From 2023 to 2025, we operated a monitoring network of eight test apiaries (each with five *Apis mellifera carnica* colonies) across Slovenia. Every three weeks during active season, we measured varroa infestation using natural mite drop, brood decapping and sugar roll tests. We also evaluated colony strength, brood extent and food reserves, and treated all colonies according to veterinary protocols. We integrated all diagnostic data into our web platform for real-time tracking. The platform also provides beekeepers with extensively illustrated diagnostic guides and interactive calculator to determine their own colony infestation rates.

Our monitoring revealed high infestation variability, most evident between individual colonies within the same apiary rather than across regions. We observed exponential late summer mite growth and autumn reinvasions, common in Slovenia's high colony density. Additionally, we recorded colony food shortages during the poor 2023 and 2024 seasons.

By publicly sharing regional data and diagnostic calculators, we provide beekeepers with critical reference points for optimising varroa treatments. We demonstrated that tracking colony conditions alongside diagnostic methods is essential. For example, during broodless periods (brood interruption, queen absence) all mites are phoretic, causing temporarily higher counts in sugar roll test that rapidly decrease once new brood is reared. Understanding colony dynamics enables beekeepers to accurately interpret infestation severity. Finally, publishing our field data on an accessible digital platform encourages proactive apitechnical and treatment interventions, ultimately improving managed honeybee survival in Slovenia.

ELEMENTAL COMPOSITION OF THREE HYMENOPTERA SPECIES FROM A SINGLE APIARY

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Ljubiša Stanisavljević[1], Nenad M. Zarić[1,2], Sofia Bouchebti[3], Walter Goessler[4], Ratko Pavlović[3]

[1]University of Belgrade, Faculty of Biology, Belgrade, Serbia
[2]BOKU University, Tulln, Austria
[3]Mitrani Department of Desert Ecology, Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Midreshet Ben Gurion, Israel
[4]University of Graz, Graz, Austria

Abstract

Insects are increasingly used as biomonitors of environmental pollution, yet comparative studies among co-occurring taxa under identical environmental conditions remain limited. This is a case study that investigated interspecific differences in elemental composition in three Hymenoptera species, *Apis mellifera*, *Vespula germanica*, and *Vespa crabro*, collected from a single apiary to evaluate their suitability for biomonitoring of trace elements.

Worker individuals were sampled simultaneously and analyzed for essential and potentially toxic elements using ICP-MS. Significant interspecific differences were observed for most elements. Hornets exhibited the highest concentrations of numerous trace and toxic elements, including Al, V, Mn, Fe, Ni, Cu, As, Cd, Sb, Ba, and Pb, whereas wasps generally showed the lowest concentrations. Honey bees displayed intermediate values but were characterized by significantly higher concentrations of nutritionally important macroelements such as Mg, P, K, Ca, and Zn. Multivariate analysis (PCA) clearly separated species based on their elemental profiles, with trace elements driving the distinction of hornets and macroelements differentiating bees from wasps.

These differences likely reflect species-specific ecology, including trophic position, foraging range, and dietary preferences. Predatory behavior in hornets may contribute to higher bioaccumulation through trophic transfer, while honey bees integrate environmental exposure over larger spatial scales but may also be influenced by hive-related sources.

Our findings demonstrate that even closely related and co-occurring insect species can exhibit different elemental signatures under shared environmental conditions. This highlights the importance of species selection in biomonitoring studies and supports the use of multi-species approaches to improve the accuracy and ecological relevance of environmental assessments. However, the single-apiary design limits generalization. The observed differences should therefore be considered site-specific and preliminary, although shared exposure enabled direct taxon comparison. Different environments may change absolute concentrations and relative enrichment through variation in pollution sources, soil composition, vegetation and prey availability across sampling sites.

P 208 USING POLLINATION NETWORKS TO UNDERSTAND THE HEALTH STATUS OF A PROTECTED HABITAT

Elena Tafi, Daniele Calabrese, Matteo Dal Cin, Gianni Henson, Samuele Pignedoli, Massimo Nepi

Department of Life Sciences, University of Siena, Siena, Italy

Abstract

Pollination networks describe the interactions between pollinators and flowering plants within an ecosystem, providing understanding of its functioning and stability. Wild bees (Hymenoptera: Apoidea: Anthophila) are one of the most important Angiosperm pollinator groups, considered "key-stone species" for the ecosystems. Since networks are sensitive to disturbances in the ecosystem they can be used not only to provide a picture of the current state of the communities in each environment, but also as a tool for monitoring and conserving biodiversity and ecosystem integrity. This study investigated the pollination network of a protected habitat included in the Habitats Directive as 6210* "Semi-natural dry grasslands and scrublands facies on calcareous substrates (Festuco-Brometalia and important Orchid sites)". The study area covered 1 km_2 in the site of Monte Labbro (Grosseto, Italy) which was sampled over two years (2024-2025) during the flowering season. The sampling protocol comprised Focal Floral Observations in ten 1 m_2 plots and three 200 m transects per each sampling date. Specimens of Lepidoptera, Diptera and Coleoptera were also included in the sampling. A vegetation survey was also carried out. The data enabled the characterisation of the Apidofauna of Monte Labbro and the identification of species of conservation interest (e.g., *Melitta tricincta*). In the two years, more than 70 bee morphospecies were identified, interacting with more than 60 plant species, resulting in a total of 3670 observed interactions. Metrics calculated from the pollination network highlighted a moderate generalisation degree within the system and a certain vulnerability to the loss of pollinator species, given the modest average number of links per plant species. This preliminary study provided an initial assessment of the health status of Monte Labbro, but further monitoring over the coming years would be desirable to identify and predict any risk factors for the conservation and preservation of this habitat.

HONEY ENVIRONMENTAL DNA CAN DISCLOSE PLANT-SUCKING INSECT POPULATION GENETIC DIVERSITY IN AGRICULTURAL AND FOREST LANDSCAPES

P 209

Valeria Taurisano[1], Anisa Ribani[1], Samuele Bovo[1], Kate E. Nelson Johnson[1], Ayça Özkan Koca[2], Giuseppina Schiavo[1], Valerio Joe Utzeri[1], Maria Letizia Calabri[1], Francesca Bertolini[1], Luca Fontanesi[1]

[1]Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy
[2]Department of Gastronomy and Culinary Arts, Maltepe University, Istanbul, Turkey

Abstract

Honey is a valuable source of environmental DNA (eDNA), containing biological traces from organisms involved in its production and surrounding ecosystems, including those of honeydew-producing insects. Honeydew is used by honey bees as a sweet feed source, so honey contains traces of plant-sucking insects that could provide insights into the biological and ecological interactions of insect communities. In this study, we compared two metabarcoding assays targeting mitochondrial DNA regions (COI and CYTB) to profile Hemiptera in 80 honey samples from Italy and Turkey, including citrus, polyfloral, and honeydew honey samples. The two markers showed complementary performance. COI effectively detected dominant taxa such as *Metcalfa pruinosa*, particularly in polyfloral honey samples collected in some Italian regions where it often dominated. In contrast, CYTB improved the detection and taxonomic resolution of aphid diversity, achieving a higher proportion of species-level assignments. Additionally, multiple sequenced reads assigned to some hemipters made it possible to explore within-species diversity. We compiled mitochondrial haplotypes from published literature and NCBI databases to build reference datasets for key species. This enabled the assignment of sequencing reads to distinct haplotypes and the estimation of their relative frequencies across samples. We detected increased haplotype diversity in *Metcalfa pruinosa* compared to previous reports, suggesting either multiple introduction events or ongoing population expansion. For *Thelaxes suberi* and *Cinara cedri*, haplotype numbers were consistent with earlier studies, but our data provided additional insights into their relative abundances and potential geographic structuring. *Aphis gossypii* exhibited high haplotype richness and heterogeneous mitotype fingerprints across samples, reflecting its known genetic complexity and broad ecological adaptability. Overall, combining complementary metabarcoding markers enhances the detection of hemipteran diversity while enabling simultaneous insights into their population genetic structures. These findings support the usefulness of honey-derived entomological fingerprints to explore genetic diversity, highlighting the value for ecosystem-scale analyses.

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A NATIONAL *APIS MELLIFERA* L. COLONY NETWORK FOR ENVIRONMENTAL SURVEILLANCE OF ANTIMICROBIAL RESISTANCE GENES (ARGS)

Rossella Tiritelli[1,2], Ilaria Resci[3], Allegra Sartore[2], Laura Zavatta[4], Sergio Albertazzi[1], Vittorio Capano[1], Valeria Caringi[1], Irene Guerra[1], Laura Bortolotti[1], Dolores Catelan[2], Giovanni Cilia[1]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]Unit of Biostatistics, Epidemiology and Public Health, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua, Padua, Italy
[3]Department of Veterinary Sciences, University of Pisa, Pisa, Italy
[4]Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy

Abstract

Antimicrobial resistance (AMR) is a major public health concern, with the environment acting as both a reservoir and transmission pathway for antimicrobial resistance genes (ARGs). Due to their wide foraging range and interaction with environmental matrices, *Apis mellifera* colonies can serve as effective bioindicators of microbiological contamination.

Within the BeeNet project, this study sampled 339 apiaries across Italy between April and June 2021. DNA extracted from pooled bee samples was screened by PCR for 31 non-chromosomal ARGs linked to eight antimicrobial classes. ARG occurrence was analyzed using Bayesian spatial models. At the same time, environmental drivers were selected using Stochastic Search Variable Selection (SSVS) to identify relevant predictors among livestock farm density, livestock animal density, land use, apiary density, and human population density. The latter models were specified only for positivity to the three most common classes of AMRs.

Overall, 96.16% of samples tested positive for at least one ARG. Tetracycline resistance genes showed the highest mean prevalence (30.4%), with *tetM* (61.7%), *tetG* (58.4%), and *tetL* (50.7%) being the most frequently detected, particularly in northern regions. Colistin resistance genes (*mcr1, mcr2, mcr9*) showed prevalences between 10% and 13%, with clusters in central and southern Italy. Among β-lactam resistance genes, bla_{NDM} was the most widespread (15.3%), with peaks in Emilia-Romagna and Lombardy.

Using a 0.8 prior probability of inclusion, some of the livestock farm density and animal density variables showed a posterior probability of inclusion greater than 0.5. Pig-related variables showed contrasting associations across models, while equine and poultry densities were positively associated with β-lactam resistance. Small ruminant density was negatively associated with tetracycline and β-lactam resistance.

These findings demonstrate widespread environmental circulation of ARGs and support the use of *A. mellifera* colonies as bioindicators at a national scale, aiding hotspot detection and AMR mitigation within a One-Health perspective.

HEM NETWORK (HONEYBEE EPIDEMIOLOGICAL MONITORING) IN REUNION ISLAND: A HEALTH SURVEILLANCE SYSTEM TAILORED TO AN INSULAR CONTEXT

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Iason Tsarmpopoulos, Jean-Marie Chatel, Quentin Diziain, Benoît Jobart, Alexandra Saggiorato, Abalhassani Saïd, Margot Camoin

GDS Réunion, La Plaine des Cafres, France

Abstract

As an isolated oceanic island, Reunion Island presents a unique epidemiological profile for beekeeping. Free of *Varroa destructor* and other major pathogens until 2017—and still free of some diseases such as Sacbrood virus—the island provides an exceptional setting to study the introduction and spread of honeybee pathogens and the impact of Varroa introduction on viral dynamics. Since 2016, the Animal Health Protection Group (GDS) of Reunion Island has operated the HEM network, a system of sentinel hives strategically placed near airports, seaports, and areas with high apiary density. The network includes 34 hives grouped into 7 apiaries belonging to public institutions, all monitored monthly by the GDS as part of a long-term honeybee health surveillance framework.

Monitored variables include *Varroa destructor* infestation loads (VP100), trapping of the small hive beetle (*Aethina tumida*) since 2022, detection of American foulbrood (*Paenibacillus larvae*) since 2024, colony development and strength assessment, and PCR-based detection of major viral, bacterial, and fungal diseases since 2022. DWV and CBPV loads are quantified using RT-qPCR protocols. Monitoring is complemented by recording anti-*Varroa* treatments applied at the apiary level.

Results highlight the impact of *Varroa* introduction on the circulation and seasonal dynamics of honeybee viruses, particularly the increase of DWV. This increase has an impact on the population and the dynamics of other viruses like BQCV and CBPV. Seasonal peaks in opportunist pathogens lie the European foulbrood (*Melissococcus plutonius*) detection were observed during periods of pollen resource scarcity and immediately before treatment period. Differences in pathogen and viral load dynamics according to *Varroa* treatment strategies were also observed. Overall, the HEM network demonstrates the seasonal variation of honeybee diseases in relation to climatic conditions, resource availability, and beekeeping practices, generating valuable baseline data for honeybee health surveillance and disease management in tropical island ecosystems.

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RAPID BROOD DECAPPING – A NOVEL METHOD FOR DETECTION OF *TROPILAELAPS MERCEDESAE*

Aleksandar Uzunov[1,2,3], Chao Chen[1,2], Marin Kovačić[4], Cecilia Costa[5], Irakli Janashia[6]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Skopje, North Macedonia
[2]State Key Laboratory of Resource Insects, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, China
[3]Macedonian Academy of Sciences and Arts, Research Center for Environment and Materials, Skopje, North Macedonia
[4]University of J.J. Strossmayer in Osijek, Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia
[5]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[6]Agricultural University of Georgia, Tbilisi, Georgia

Abstract

The honey bee parasitic mite *Tropilaelaps mercedesae* has recently been reported in southwestern Russia and Georgia, highlighting the growing importance of accessible and reliable detection tools for regions at risk of future spread. Rapid Brood Decapping (RBD) method provides a practical, quick, and non-destructive test for assessing brood infestation by mechanically opening sealed worker cells and stimulating the movement of mites present therein, thus allowing them to be visually identified. The method requires depilation wax strips and basic protective equipment, making it suitable for routine use by beekeepers and inspectors. Field evaluations demonstrated over 90% detection efficacy and similarly high survival of the inspected brood, confirming that RBD is sensitive in terms of detection while minimally disruptive to colony development. These characteristics position RBD as a promising diagnostic technique. Building on this established protocol, the present study explores the potential of integrating simple video-based computer-vision analysis as a complementary support tool. Short recordings captured immediately after decapping have been processed to automate the technique by integrating video and image analysis to detect mites. While still at the development stage, these algorithms illustrate how digital tools might, in the future, assist beekeepers by reducing observer bias, accelerating data interpretation, and enabling more standardized and precise assessments in field conditions. Early trials suggest that automated detection can reliably identify mite movement within the recorded frames. The validated RBD protocol provided a practical basis for scalable *Tropilaelaps* surveillance and the method's simplicity, low cost, and high detection efficacy make it suitable not only for field use by beekeepers but also for integration into National Surveillance Programs, as already done by the Italian Ministry of Health.

HONEYBEE-COLLECTED POLLEN AS A BIOINDICATOR OF MICROPLASTIC CONTAMINATION IN CHILEAN ECOSYSTEMS

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Karen Yañez[1], Gastón Carvallo[2,3], Ramón Arias[1], Valentina Tapia[4], María José Leiva[4], Daniel A. Ramírez[4]

[1]Centro de Biotecnología Dr. Daniel Alkalay Lowitt, Universidad Técnica Federico Santa María, Valparaíso, Chile
[2]Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
[3]Instituto de Ecología y Biodiversidad (IEB), Concepción, Chile
[4]Departamento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María, Valparaíso, Chile

Abstract

The honeybee (*Apis mellifera*) plays a key role in pollination and has been widely recognized as an environmental biomonitor through matrices such as pollen, honey, and wax. In this context, the presence of microplastics in bee-collected pollen represents an emerging concern, as this resource is the primary source of nutrients for the colony and may act as a pathway for anthropogenic contaminants into the hive.

This study integrates the results of one investigation conducted in Chile, aiming to assess the presence, abundance, morphological characteristics, and composition of particle - including microplastics - in bee-collected pollen from different regions. A total of 14 samples were analyzed using chemical extraction protocols based on saline washing and n-hexane extraction. Particles were quantified and morphologically characterized using stereomicroscopy, and a subset was further analyzed by micro-FTIR spectroscopy coupled with microscopy to confirm their chemical composition.

Results revealed a high abundance of particles across all samples, reaching up to 219 particles·g^{-1}, with fragments being the dominant morphology, followed by films and spheres. The particle size distribution was dominated by fine fractions (0.0142 - <0.050 mm), and colors such as purple, blue, and pink were predominant. Compositional analysis indicated that most particles were of natural origin; however, microplastics were confirmed in this study, including polymers such as PET and polyester/epoxy resin, albeit at low relative frequencies.

Overall, these findings provide preliminary evidence that bee-collected pollen can act as both a vector for microplastic entry into the hive and a bioindicator of environmental contamination. This study highlights the need for more advanced analytical approaches and further research on the potential impacts of microplastics on bee health and terrestrial ecosystems.

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HYDROALCOHOLIC SOLVENT-BASED PURIFICATION OF BEESWAX: EFFECTS ON RESIDUE REDUCTION AND FUNCTIONAL ACCEPTANCE BY *APIS MELLIFERA* COLONIES

Karen Yañez[1], Catalina Jara[2], Enrique Rojas[1], Ramón Arias[1], Daniel A. Ramírez[2]

[1]Centro de Biotecnología Dr. Daniel Alkalay Lowitt, Universidad Técnica Federico Santa María, Valparaíso, Chile
[2]Departamento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María, Valparaíso, Chile

Abstract

Beekeeping is a key activity for food security and the balance of agroecosystems, with *Apis mellifera* being the most important species in terms of productivity. However, agricultural intensification has increased colonies' exposure to pesticide and veterinary drug residues, which accumulate in the wax due to their lipophilic nature, compromising bee health and the quality of bee products. In this context, the present study evaluated the level of acceptance and use of different types of purified wax by bee colonies, with the aim of providing evidence for the use of safer materials in the hive.

Four purification treatments (ethanol, isopropanol, butanol, and double cleaning with propanol followed by sterilization) were analyzed, along with a commercial wax as a control. The trial was conducted over 45 days in three hives with sister queens, using biweekly evaluations of three behavioral indicators: initial activity, stretching, and final use. The results showed significant differences in the colonies' response depending on the type of wax. Waxes purified with ethanol, propanol, and butanol showed high levels of acceptance, in some cases exceeding the control, with the ethanol treatment standing out in particular. In contrast, the wax that underwent sterilization consistently received the lowest scores, with an average acceptance level.

The integrated analysis of the indicators suggests that purification using hydroalcoholic solvents does not negatively affect the wax's functionality but may actually promote its incorporation into the hive's dynamics. Although variability in development was observed among hives, all waxes were ultimately used over the long term. These results support the use of purified waxes as a viable strategy for improving the sanitary quality of beekeeping materials, contributing to the development of more sustainable and safer production systems.

ASSESSING EXTRACTION LOSSES AND MATRIX EFFECTS FOR NEONICOTINOIDS AND THEIR METABOLITES IN HONEY BEES

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Nenad M. Zarić[1,2], David Ruso[3], Ljubiša Stanisavljević[2], Ratko Pavlović[4], Rainer Schuhmacher[1]

[1]BOKU University, Tulln, Austria.
[2]University of Belgrade, Faculty of Biology, Belgrade, Serbia
[3]Core Facility Bioactive Molecules: Screening and Analysis, BOKU University, Tulln, Austria.
[4]Ben-Gurion University of the Negev, Midreshet Ben Gurion, Israel

Abstract

A stepwise sequential-spiking approach was used to develop and diagnose an extraction procedure for 18 neonicotinoids and metabolites in whole honey bees. The workflow comprised extraction of 2 g homogenized bees with acidified acetonitrile after water addition, salting-out with magnesium sulphate and sodium acetate, magnesium sulphate clean-up, evaporation, and final reconstitution. To localize analyte losses, samples were spiked at four stages: before extraction, before clean-up, before evaporation, and after final clarification prior to injection. Three extraction variants were compared: no hexane, hexane added before salts, and hexane added after extraction and before magnesium sulphate clean-up. Final clarified extracts reconstituted in methanol or methanol:water were also evaluated.

The bee matrix proved to be the dominant source of analyte loss and variability. Major losses were already evident during extraction and salting-out, while additional instability was introduced during reconstitution and final clarification. Across all compounds and spike points, the no-hexane workflow gave the highest overall mean recovery (55.25%), followed closely by hexane before salts (54.47%) and hexane after extraction (53.71%). In contrast, evaluation of the final post-clarification spike samples showed that the cleanest final extract was obtained with hexane before salts combined with methanol:water, which gave the highest matrix factor (65.82%) and the lowest matrix effect (–34.18%). Importantly, the arm with the best recovery and the arm with the lowest matrix effect coincided for only 4 of the 18 analytes, demonstrating that analyte preservation and matrix suppression were not optimized by the same workflow.

These results show that extraction of neonicotinoids from honey bees is strongly matrix-limited and requires balancing total recovery against final extract cleanliness.

Welfare in bees and ONEHEALTH

DEVELOPMENT OF SUSTAINABLE AND RESILIENT BEEKEEPING PRACTICES IN RESPONSE TO ENVIRONMENTAL CONSTRAINTS IN ALGERIA

P 216

Noureddine Adjlane[1], Soumeya Kaouche[1], Reda Smaoui[2]

[1]Department of Agronomy, University of Boumerdes, Boumerdes, Algeria
[2]Centre de formation professionelle à Bouinane, Blida, Algeria

Abstract

Beekeeping in Algeria faces multiple environmental and health challenges, including water stress, extreme temperatures, scarcity of floral resources, and increasing pressures from parasites and pathogens. These factors limit colony productivity and resilience, threatening the long-term sustainability of the apiculture sector.

Between 2023 and 2025, a field-based study was conducted across diverse agro-ecological zones, including coastal, Tellian, and steppe regions, to develop sustainable and resilient beekeeping practices. Structured surveys were carried out among beekeepers to document current management methods, parasite control strategies, and approaches to floral resource management. Direct observations of colonies were performed to evaluate behavioral traits, colony performance, and the effectiveness of applied practices.

Results highlighted the importance of integrated strategies combining several approaches. Effective sanitary management, including regular monitoring of *Varroa destructor* infestations and prevention of brood diseases, proved essential. Optimizing floral resources through the diversification of melliferous crops and the conservation of natural habitats contributed to colony resilience. Adapting beekeeping practices to local climatic conditions, such as strategic transhumance and supplemental feeding during periods of environmental stress, showed positive outcomes. Furthermore, the integration of sustainable techniques, including the use of natural products for parasite control and reinforcement of colony resistance, complemented these strategies.

Overall, these approaches improved colony survival and enhanced resilience under variable environmental conditions. Developing sustainable and locally adapted beekeeping practices represents a key strategy to increase productivity, maintain colony health, and ensure the long-term sustainability of apiculture in Algeria. Such practices are critical not only for the economic viability of beekeepers but also for supporting ecosystem services, including pollination, under increasingly challenging environmental conditions.

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RNA INTERFERENCE: A LONG-LASTING SHIELD FOR HONEYBEES?

Salvatore Arpaia[1], Valeria Bonina[2], Giovanni Federico[2]

[1]ENEA, Rotondella, Italy
[2]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Reggio Calabria, Italy

Abstract

Double-stranded RNA (dsRNA) utilizing RNA interference (RNAi) is a promising biotechnological tool against honeybee pests and pathogens (e.g. *Vairimorpha ceranae*, Deformed Wing Virus – DWV, Israeli Acute Paralysis Virus - IAPV). This approach offers high species-specificity, leaves no residues in hive products, and is safe for bees.

Following the recent US authorization of Norroa™ targeting *Varroa destructor*, interest in RNAi has accelerated, leading to new dsRNA developments against the invasive beetle *Aethina tumida*. In laboratory trials, both treatments induce limited acute mortality but cause severe sublethal effects, including impaired immature development and reduced adult fecundity.

To evaluate whether these individual sublethal effects translate into broader population-level impacts, we developed stage-structured dynamic simulation models for both *V. destructor* and *A. tumida*. The models assessed the multi-generational population dynamics and the efficacy of dsRNA treatments under various scenarios.

Our simulations demonstrate that dsRNA-based interventions significantly delay population growth and substantially reduce overall pest abundance in both species. Crucially, the model highlights that treatment efficacy is maximized when interventions are applied during the early stages of infestation. These findings validate the integration of RNAi-based tools as a highly effective and viable component of sustainable Integrated Pest Management (IPM) strategies in modern apiculture.

EVALUATION OF PROBIOTIC AND POSTBIOTIC CANDIDATES AGAINST *NOSEMA CERANAE* IN HONEY BEES

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Daniela Arredondo[1,2,3,4], Matilde Alarcón[3,4], Loreley Castelli[3,4], Sofía Palacios[3,4], Diego E. Vázquez[1,3,4], Eugenia Vaz[3], Pablo Zunino[1,3,4], Karina Antúnez[1,3,4]

[1]Instituto de Investigación Una Salud, Prorectorado de la Universidad de la República, Montevideo, Uruguay
[2]Programa de Desarrollo de las Ciencias Básicas MEC-UdelaR, Montevideo, Uruguay
[3]Laboratorio de Microbiología y Salud de las Abejas, Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, MEC, Montevideo, Uruguay
[4]Centro de Investigación en Ciencias Ambientales (CICA), Instituto de Investigaciones Biológicas Clemente Estable, MEC, Montevideo, Uruguay

Abstract

Honey bees face multiple global threats, including infections by pathogens such as *Nosema ceranae*. There is no available treatment recommended for *N. ceranae*, as fumagillin may be toxic for humans, highlighting the need for alternative control strategies. Native intestinal microbial community is essential to maintain adequate nutrition, health and immunity against pathogens. The administration of probiotics and postbiotics as feed additives in honey bees has gained increasing attention in the last decade. However, their effectiveness remains debated, and the outcomes are highly strain-dependent. In this study we evaluated the *in vivo* effect of fifteen bacterial isolates obtained from the honey bee gut microbiota to assess their potential as probiotics or postbiotics. The microorganisms collection includes isolates of the genera *Apilactobacillus, Lactobacillus, Bombella, Bifidobacterium, Bombilactobacillus, Bartonella*, and *Gilliamella*, as well as a previously developed probiotic based on a mixture of 4 *Apilactobacillus kunkeei* strains. Fresh broth cultures were administered 24 hours after emergence at 1×109 CFU/mL in sugar syrup as potential probiotics, and heat-inactivated aliquots were administered in sugar syrup as potential postbiotics. *Nosema ceranae* infection was performed after 36 hours with approximately 35,000 spores per bee, and experiments were conducted in triplicate. Most treatments resulted in increased mortality compared to *N. ceranae*-infected controls, suggesting adverse effects of certain bacterial strains. In contrast, the *A. kunkeei* strains mixture did not increase mortality under infection, while its postbiotic counterpart showed limited efficacy against the pathogen. Additionally, some bacterial strains reduced the spore counts without improving survival. Our results are consistent with previous reports showing that probiotic effects in honey bees are strain dependent and may involve trade-offs between host survival and pathogen load. This variability emphasizes the importance of selecting and screening candidate microorganisms before considering their application in honey bee colonies.

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EVIDENCE FOR IMMUNE MEMORY IN THE HONEY BEE (*APIS MELLIFERA*): IMPROVED SURVIVAL AFTER HOMOLOGOUS PATHOGEN CHALLENGE

Valērijs Ašķeļaņecs[1], Gloria Ruiz-Guzmán[2], Indriķis Krams[1], Jorge Contreras-Garduño[2]

[1]Daugavpils University, Daugavpils, Latvia
[2]National Autonomous University of Mexico, Mexico City, Mexico

Abstract

Understanding immune defence mechanisms in honey bees (*Apis mellifera*) is critical for addressing current challenges in pollinator health and colony losses. While invertebrates lack adaptive immunity, they may exhibit immune priming, a form of innate immune memory that enhances protection against subsequent infections. In this study we investigated whether the honey bee exhibits immune memory within a single generation.

We compared survival and immune responses among bees subjected to different immune challenges: naïve individuals, bees exposed twice to the same pathogen (homologous challenge) and bees exposed to different pathogens (heterologous challenge). Bacterial species used included *Escherichia coli*, *Staphylococcus aureus* and *Micrococcus lysodeikticus*. Immune response was assessed through multiple parameters, including lytic activity, phenoloxidase activity, hemocyte count and antimicrobial peptide expression.

Bees exposed to homologous challenges showed significantly higher survival compared to those subjected to heterologous challenges. In addition, immune parameters differed between groups with increased lytic activity and altered immune profiles in the homologous challenge group. No significant differences were observed between homologous and naïve groups in some parameters, suggesting a complex immune modulation rather than a simple enhancement.

These results provide clear evidence of immune memory in honey bees and support the hypothesis that prior exposure to pathogens can enhance resistance to subsequent infections. This phenomenon may have important implications for pollinator health management, including the potential development of immune priming-based strategies to reduce disease impacts in managed bee populations.

COMPARING POPULATION GROWTH OF *TROPILAELAPS MERCEDESAE* AND *VARROA DESTRUCTOR*, TWO IMPORTANT HONEY BEE PARASITIC MITES

P 220

Dan Aurell[1], Rogan Tokach[2], Bajaree Chuttong[3], Pichet Praphawilai[4], Léna Barascou[1], Chuleui Jung[5], Hyunha Oh[5], Geoffrey R. Williams[1]

[1]Auburn University, Auburn, United States
[2]North Dakota State University, Fargo, United States
[3]Chiang Mai University, Chiang Mai, Thailand
[4]Chiang Mai University, Chaing Mai, Thailand
[5]Gyeongkuk National University, Andong, Korea, Republic of Korea

Abstract

Tropilaelaps mercedesae is an emerging honey bee parasite rapidly expanding through western Asia and eastern Europe. Reports consistently indicate that *T. mercedesae* reproduces faster than *Varroa destructor*, yet concrete evidence is lacking. This limits our ability to comparatively assess the threat posed by these two mites, and to estimate frequency and effectiveness of beekeeper interventions needed to appropriately manage them. To address this knowledge gap, we evaluated *T. mercedesae* population growth in three studies in Thailand and South Korea. We did this by estimating colony mite populations in untreated colonies (n = 39) at the start and end of monitoring periods in which mite populations were allowed to grow. We found that *T. mercedesae* had an average daily rate of increase (r) of 0.04 across these studies, corresponding to a 10.8-fold population increase over 2 months. In contrast, a representative V. destructor daily r of 0.021 corresponded to a 3.5-fold population increase over the same period. Compared to *V. destructor*, populations of *T. mercedesae* appear to grow more rapidly. This may promote colony damage and drive *T. mercedesae* dispersal to new colonies, and likely has implications for beekeeper mite control practices.

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USING ENVIRONMENTAL DNA TO DETECT *TROPILAELAPS MERCEDESAE*

Dan Aurell[1], Rogan Tokach[2], Zach Bateson[3], Bajaree Chuttong[4], Chuleui Jung[5], Missy Berry[3], Megan O'Neil[3], Hyunha Oh[5], Frank Rinkevich[6], Pichet Praphawilai[4], Geoffrey R. Williams[1]

[1]Auburn University, Auburn, United States
[2]North Dakota State University, Fargo, United States
[3]National Agricultural Genotyping Center, Fargo, United States
[4]Chiang Mai University, Chiang Mai, Thailand
[5]Gyeongkuk National University, Andong, Republic of Korea
[6]USDA ARS, Baton Rouge, United States

Abstract

Tropilaelaps mercedesae have spread far beyond their native range, and potentially threaten wild and managed *Apis mellifera* populations worldwide. To prevent and delay *T. mercedesae* infestations of new areas, time-efficient and scalable early detection are needed. Current physical monitoring methods have limited scalability as they are time consuming and depend on trained personnel. Here we studied the influence of sample type and colony infestation level on the use of environmental DNA to detect *T. mercedease*. We did this by collecting samples from 106 *A. mellifera* colonies in Thailand and South Korea, where *T. mercedesae* mites are present. Sample types included adult bees, brood comb, bottom board debris, and honey, as well as swabs of bottom boards, individual brood cells, and brood comb surfaces. We also determined *T. mercedesae* infestation rate for each colony by uncapping worker cells. For each sample type, we tested for T. mercedease DNA using a qPCR assay, and then determined a detection threshold – the infestation of T. mercedesae required to provide 50% probability of detection in the qPCR assay. We found that bottom board swabs collected through the entrance performed consistently well in both countries; it detected *T. mercedesae* in all tested hives in Thailand and had a detection threshold of 2.6% brood infestation (infested cells per 100 cells) in Korea. The second-best method was samples of adult bees, with estimated detection thresholds of 0.42% and 3.9% brood infestation for Thailand and Korea, respectively. Based on these results, we believe that eDNA methods are appropriate for *T. mercedesae* detection because they can be sensitive and involve simple and quick sampling.

EFFICACY AND SELECTIVITY OF SPRING TRAPPING FOR *VESPA VELUTINA NIGRITHORAX* IN WALLONIA, BELGIUM

P 222

Florian Bastin, Cyril Voss, Louis Hautier

Plant and Forest Health Unit, Life Sciences Department, Walloon Agricultural Research Center, Gembloux, Belgium

Abstract

To manage the invasion of the Asian hornet and reduce its impacts, spring trapping appears to be a promising strategy. Early in the season, queens are solitary and therefore more vulnerable to control measures such as trapping or nest removal. In the absence of a species-specific attractant for the Asian hornet, spring trapping poses a risk to non-target insects, particularly pollinators such as bumblebee queens, solitary bees, and native vespids.

To mitigate this risk, a non-lethal spring trap was developed by the CRA-W to selectively capture Asian hornets while allowing smaller species to escape and limiting the entry of larger ones. Over 3,000 CRA-W traps were deployed across southern Belgium (Wallonia) for a 12-week period in spring, using a sugar-based bait absorbed in a bio-absorbent.

A total of 12,852 insect visits were recorded, consisting mainly of honeybees (27%) and other insects such as ants and flies (64%), which were able to access the bait as feeding source and exit traps. Of the remaining 9% that were retained, 802 were Asian hornets, 38 were European hornets, 216 were wasps, and 101 were bumblebees. Based on these 1,157 captured individuals, the selectivity of the trap for the Asian hornet was estimated at 69%, exceeding that of conventional drowning traps.

Asian hornet captures were primarily concentrated in urban areas that had been colonized for several years. Capture rates increased notably in early May, coinciding with the period when all foundresses have emerged from hibernation and are active in the environment.

These preliminary results highlight the need for further research to enhance trap selectivity, particularly through the development of more specific baits. In addition, identifying optimal trapping locations and assessing the impact on the nest density will be essential to determine whether spring trapping represents a sustainable strategy for managing the Asian hornet population.

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MICROBIAL INTERACTIONS AND COLONY HEALTH: THE ROLE OF SERRATIA MARCESCENS IN NOSEMA CERANAE-INFECTED HONEYBEES

Chiara Braglia[1], Daniele Alberoni[1], Melisa P. Garrido[2,3], Martin P. Porrini[2,3], Loredana Baffoni[1], David Mifsud[4], Dara Scott[5], Martin J. Eguaras[2,3], Diana Di Gioia[1]

[1]Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy
[2]Centro de Investigación en Abejas Sociales (CIAS), FCEyN, UNMdP, Mar del Plata, Argentina.
[3]Instituto de Investigaciones en Producción Sanidad y Ambiente (IIPROSAM), CONICET, UNMdP, Mar del Plata, Argentina
[4]Institute of Earth Systems, L-Universita ta' Malta, Msida, Malta
[5]ADVANCE SCIENCE Ltd, Galway, Ireland

Abstract

Global honeybee declines exhibit cyclical patterns of varying intensity and are frequently associated with infectious agents, including the gut microsporidian *Nosema ceranae* and numerous viruses. Nevertheless, these pathogens alone are seldom sufficient to cause complete colony collapse. Instead, colony losses are often the result of multifactorial interactions, where synergistic effects between pathogens and environmental or biological stressors exacerbate disease outcomes. While research has extensively investigated interactions between pathogens and pesticides, considerably less attention has been given to the role of opportunistic, non-core gut bacteria such as *Serratia marcescens* in shaping disease dynamics. This study aims to elucidate the interaction between *S. marcescens* and *N. ceranae*, focusing on the proliferation dynamics of *Serratia* during co-infection and its impact on honeybee survival. Furthermore, we evaluated potential mitigation strategies, including supplementation with beneficial bacteria and antimicrobial treatments. Multiple *Serratia* strains were isolated from weakened bees and experimentally administered to newly emerged, healthy individuals in combination with *N. ceranae*. Bees were maintained under controlled laboratory conditions, and survival rates were monitored. Quantitative PCR analyses were conducted to determine pathogen loads in both gut tissues and hemolymph. In addition, dietary supplementation with beneficial lactic acid bacteria (e.g., Lactobacillaceae) or treatment with fumagillin was tested as a means to control pathogen proliferation. Our findings demonstrate that all tested *Serratia* isolates were capable of translocating across the gut epithelium and multiplying within the hemocoel. Co-infection with *N. ceranae* significantly enhanced *Serratia* expansion in the gut, resulting in accelerated mortality. Notably, supplementation with flower-associated beneficial bacteria, particularly *Apilactobacillus kunkeei*, markedly reduced *Serratia* loads, highlighting its potential as a sustainable microbial management tool readily applicable in commercial beekeeping. Overall, these results emphasize the intricate nature of microbial interactions within the honeybee host and reinforce the importance of investigating multifactorial pathogen–microbiota relationships in colony health decline.

MONITORING OF HONEYBEE POISONING INCIDENTS IN POLAND: 2025 CASE STUDIES AND PESTICIDE RESIDUE ANALYSIS

P 224

Olga Burek, Tomasz Kiljanek, Milena Goliszek

Department of Chemical Research of Food and Feed, National Veterinary Research Institute (PIWET), Pulawy, Poland

Abstract

Poland has had an official system for diagnosing bee poisoning cases since 2014. Samples of dead bees came from apiaries where it was suspected that the poisoning may have been caused by improper use of plant protection products (PPPs) in neighbouring crops. The symptom of the poisoning was a sudden and massive number of dead bees in front of the hives.

Samples of dead bees were subjected to toxicological analysis in order to identify the pesticides that may have caused the poisoning. The pesticides whose residues are analyzed in the samples are active substances found in plant protection products, biocidal products or veterinary medicines. The analyses were carried out using a specially developed analytical procedure, validated and accredited in accordance with ISO 17025.

In 2025, a total of 29 samples of dead bees collected from apiaries where poisoning symptoms were analyzed. The studied cases represented almost 1,200 bee colonies kept in the affected apiaries.

Pesticide residues were detected in every sample of dead bees, and in over 90% of the samples, residues of highly toxic substances were found, which may have been the cause of the bees' poisoning. In total, residues of 45 pesticides from different chemical groups were detected above the limit of quantification. The most frequently identified compound was fipronil and its metabolites, detected in 8 out of 29 samples. Other identified substances included insecticides (e.g., chlorpyrifos, clothianidin, dimethoate), fungicides (e.g., azoxystrobin, prothioconazole-destio, boscalid, tebuconazole, difenoconazole) and herbicides (bentazon, pendimethalin). Among veterinary medicinal products, amitraz metabolites (DMF, DMPF) were most frequently detected.

In the vast majority of cases, the results of toxicological analyses make it possible to identify the potential cause of bee poisoning, which requires further investigation based on the findings of an inspection of the suspected plantation and the results of tests on plant material.

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SMALL HIVE BEETLE IN REUNION ISLAND - FROM INITIAL ERADICATION STRATEGY TO CURRENT MANAGEMENT

Margot Camoin[1], Jean-Marie Chatel[1], Gilbert Piednoir[2]

[1]Groupement de Défense Sanitaire de la Réunion, Plaine des Cafres, France
[2]Groupement de Défense Sanitaire de Bretagne, Ploufragan, France

Abstract

Small hive beetle (SHB) was first detected in the south of Réunion island in July 2022. Following French regulations, an eradication strategy was implemented from July 2022 to July 2023. Control measures applied included destruction of 15 infested apiaries, surveillance of apiaries within a 10 km zone, and honeybee, beekeeping equipment and hive product movement restrictions.

Absence of financial compensation for the production losses due to the restriction of movements and the confirmed presence of small hive beetles in feral colonies largely hindered the success of this plan. Consequently, in mid-2023, the strategy changed drastically and the above-mentioned measures were no longer enforced. According to European regulations, suspicions reported by beekeepers continued to be investigated. And despite presumed underreporting, 26 new cases over a 2-year period were confirmed in apiaries from different parts of the island. Levels of infestation per hive remained quite low, but occasionally tens of larvae would be discovered in protein patties or in syrup that would not be consumed quickly by colonies.

Starting from the second half of 2025, new cases with massive larvae infestations were found more regularly in weak colonies... The recent rise in SHB infestations calls for more frequent hive inspections by beekeepers. To better understand the behavior of this pest, a long-term monitoring survey in a highly infested apiary was set up. Results so far show that live SHB are mainly found on the bottom board (53.9%), compared to lids (43.9%) or frames (0.2%). Trials of oil traps conducted in Réunion Island concluded that traps inserted in the bottom board at the entrance of the hive catch more adults (88.7%) than the ones placed between frames (11.3%). Similar trends have been observed in Madagascar, but more studies are needed to help beekeepers better manage SHB in their hives.

ERUCA SATIVA AND *RESEDA LUTEA* FLOWER EXTRACTS INHIBIT *NOSEMA CERANAE* INFECTIONS IN LABORATORY ASSAYS

P 226

Matteo Carloni[1], Eleonora Pagnotta[2], Laura Righetti[2], Lorena Malaguti[2], Vittorio Capano[3], Antonio Nanetti[3], Nicola Pecchioni[1,4], Manuela Bagatta[2], Luisa Ugolini[2]

[1]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[2]CREA Research Centre for Cereal and Industrial Crops, Bologna/Foggia, Italy
[3]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[4]CREA Research Centre for Cereal and Industrial Crops, Bologna/Foggia, Italy

Abstract

Brassicales represent a large and economically important plant order characterized by the presence of the endogenous glucosinolate (GSL)–myrosinase defense system in their organs and tissues. Isothiocyanates (ITCs), the main hydrolysis products of GSLs, are known for their antimicrobial properties and may represent a sustainable control strategy, offering an alternative to antibiotics against bee pathogens such as the parasitic microsporidian *Nosema (= Vairimorpha) ceranae*. The present study evaluated the efficacy of GSL-based formulations derived from the flowers of *Eruca sativa* and *Reseda lutea*, belonging to the Brassicaceae and Resedaceae families, respectively, in controlling *N. ceranae* infection in worker honey bees under laboratory conditions. Freeze-dried flowers were chemically characterized, and water-soluble GSL-enriched extracts were prepared and formulated in sugar syrup at two different concentrations. Extract administration reduced food consumption and bee survival in a concentration-dependent manner compared with the control group fed syrup alone. The lower concentration was subsequently tested on 6-day-old bees artificially infected with *N. ceranae* spores, showing consumption comparable to the control group, although a slight reduction in survival was still observed. At this concentration, both extracts significantly reduced *N. ceranae* abundance, with no significant differences between plant species. Isothiocyanates were detected in the gut, accumulating primarily in the hindgut and at higher levels in bees fed *R. lutea* extracts. These findings highlight the potential of floral GSL-rich formulations as natural and environmentally sustainable tools to support colony health and reduce pathogen load.

P 227 SUMMER TREATMENT FOR *VARROA* MITES DEPENDING ON THE INFESTATION – TREAT IF NECESSARY

Jean-Daniel Charrière, Benoît Droz

Swiss Bee Research Centre, Agroscope, Bern, Switzerland

Abstract

In many countries in Central and Northern Europe, strategies for controlling *Varroa destructor* involve two summer treatments, mainly using formic acid. A key advantage is that the risk of re-infestation or an initial treatment with poor effectiveness is offset by the second treatment.

However, some beekeepers wish to reduce the number of summer treatments. Yet, if these are simply omitted without any other measures, this leads to a high risk of colony losses. Here we propose an alternative for all beekeepers who wish to treat their bees only when necessary. Based on the number of *varroa* mites found on the hive floors, the decision to treat or not is made for each colony.

Field trials conducted over four years with several hundred colonies aimed to develop a method for reducing the number of summer treatments without the risk of increased colony losses. Between July and September, we defined three observation windows and infestation thresholds. If these thresholds were exceeded, formic acid treatments were applied. The thresholds we defined—5 *Varroa*/day in July, 2 *Varroa*/day in August and September-, made it possible to dispense with nearly half of the formic acid treatments. Winter colony losses ranged from 2.7% to 8.9%, which were lower than the losses recorded during the same years in the general survey on losses conducted in Switzerland (14.9% to 18.9%).

Reducing the number of summer treatments is therefore possible, but one must nevertheless be prepared to invest more time in *Varroa* counting and to manage colonies on an individual basis. This is why this control strategy is primarily aimed at beekeepers with a small or medium-sized apiary who are equipped and experienced in measuring the natural mite fall.

INTRODUCED WITH THE HOST: PATHOGEN CO-INTRODUCTION IN THE FIRST EUROPEAN RECORD OF *APIS FLOREA* IN MALTA

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Giovanni Cilia[1], Cecilia Costa[1], Aleksandar Uzunov[2,3], Matthew Vella[4], David Mifsud[4]

[1]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[2]Ss. Cyril and Methodius University in Skopje, Skopje, North Macedonia
[3]Institute of Apicultural Research, Beijing, China
[4]University of Malta, Msida, Malta

Abstract

The recent detection of the red dwarf honey bee *Apis florea* in Malta represents the first documented occurrence of this Asian species in Europe and provides a valuable opportunity to investigate pathogen dynamics associated with the introduction of a non-native pollinator into an insular ecosystem. This study aimed to characterise the pathogen assemblage present in the *A. florea* colony found in Malta in 2024 and to evaluate whether infections could be introduced with the founding population.

Foragers (n = 160) were individually analysed using quantitative real-time PCR targeting a broad panel of bee pathogens. Viral replication was assessed through strand-specific RT-PCR, and representative amplicons were sequenced for phylogenetic comparison with publicly available sequences.

A very high infection frequency was observed, with 158 of 160 individuals testing positive for at least one pathogen. The most prevalent agents were deformed wing virus A (85.6%) and *Nosema ceranae* (76.9%), followed by acute bee paralysis virus (36.9%), chronic bee paralysis virus (25.0%) and the trypanosomatid *Lotmaria passim* (23.8%). Sacbrood virus and black queen cell virus occurred at lower prevalence. Strand-specific RT-PCR confirmed active replication of all detected viruses. Conversely, deformed wing virus B, *Nosema apis*, and several brood-associated pathogens were not detected. Phylogenetic comparisons showed high similarity with strains previously reported from Asian honey bee hosts.

Overall, the pathogen profile observed in the Maltese *A. florea* colony suggests that infections were most likely co-introduced from the species' native range rather than acquired from the local environment. Considering the epidemiological vulnerability of island pollinator communities, and the challenges faced to conserve the endemic *Apis mellifera* subspecies *A. m. ruttneri*, as well as the potential for interspecific pathogen transmission, continued monitoring will be necessary to assess whether *A. florea* may act as a transient carrier or become an epidemiologically relevant reservoir within the local pollinator network.

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DWV INFECTION STATUS IN APIS MELLIFERA IBERIENSIS IN MAINLAND PORTUGAL: PREVALENCE AND VIRAL LOAD INSIGHTS

Maíra Costa[1], Ana R. Lopes[1], Cláudia A. Rodrígues[1], Sarah de Oliveira Rodrigues[1], Aicha Meliani-Rodríguez[1], Tudor N. Ternar[2], Maria Alice Pinto[1], Dora Henriques[1]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur, Faculty of Animal Science and Biotechnology, Department of Apiculture and Sericulture, Cluj-Napoca, Romania

Abstract

Deformed wing virus (DWV; *Iflavirus aladeformis*) is a highly prevalent pathogen with a wide global distribution that affects honey bee health, being mainly transmitted by its vector, the mite *Varroa destructor*. DWV infection can be subclinical, without causing obvious effects on productivity, although under certain conditions, clinical signs associated with the disease may be evident. The prevalence and viral loads of DWV are known in the Azores, but in mainland Portugal, they have not been assessed. To address this gap, a large-scale survey was conducted from north to south during spring, sampling three colonies per apiary (30 worker bees per colony). After RNA extraction and cDNA synthesis, prevalence and viral load were evaluated by qPCR. Viral loads were categorised based on Cq values as follows: low (5–15), medium (15–25), and high (25–35), with lower Cq values indicating higher viral loads. The results showed that ~90% of the samples were positive for DWV, demonstrating that the virus is widely distributed across Portugal, as observed throughout Europe. Regarding viral loads, ~75% of the samples showed medium to high levels, highlighting not only the high prevalence but also that many positive samples exhibited higher viral loads. Variability between apiaries was also observed, with colonies within the same apiary showing distinct viral loads. These findings provide important insights into colony health, indicating that subclinical infections may coexist with apparently healthy colonies and emphasising the virus's high prevalence across the entire territory.

This work was supported by the project "MITE- Varroa e vírus transmitidos: MonItorização de muTações e dEsenvolvimento de ferramentas moleculares inovadoras" funded by National Beekeeping Programme 2023-2027. FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020).

BIOASSAY-GUIDED CHARACTERIZATION OF ANTIMICROBIAL COMPOUNDS FROM HONEY BEE GUT BACTERIA AGAINST *PAENIBACILLUS LARVAE*

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Buse Dengiz[1], Jiri Killer[1], Pavel Dobeš[2], Pavel Hyršl[2], Jaroslav Havlík[1]

[1]Czech University of Life Sciences, Prague, Czechia
[2]Masaryk University, Brno, Czechia

Abstract

American foulbrood (AFB), caused by the spore-forming bacterium *Paenibacillus larvae*, is one of the most destructive diseases affecting honey bee (*Apis mellifera*) colonies. The persistence of spores and limitations of current control strategies necessitate the development of alternative antimicrobial approaches. In this study, antimicrobial metabolites produced by the honey bee gut bacterium *Lactobacillus helsingborgensis* VSM/RO4 were investigated using a bioassay-guided purification strategy.

Co-cultivation of *L. helsingborgensis* with *P. larvae* PL52 was performed in mBHI medium at 37 °C under anaerobic conditions for 48 h. Cell-free supernatants were obtained, freeze-dried, and subjected to reversed-phase HPLC fractionation, yielding 10 pooled fractions (AMC1–AMC10). Antimicrobial activity was evaluated using a 96-well microtiter assay at concentrations ranging from 64 to 1024 µg/mL. Among the fractions, AMC2 exhibited the strongest activity, inhibiting bacterial growth at concentrations ≥256 µg/mL.

Further RP-HPLC sub-fractionation of AMC2 produced five fractions (A–E), which were tested using disk diffusion assays. Aliquots (20 µL; 0.04–0.32 mg/disc) were applied onto 6 mm disks against *P. larvae* strains PL52 and PL41. Fraction C showed the highest antimicrobial activity, producing inhibition zones of 15 mm at 0.32 mg/disc and maintaining activity across all tested concentrations against PL52. Fractions A and B showed moderate activity at higher concentrations, while fractions D and E were inactive against PL52. Moderate inhibition by fractions C–E was observed against PL41.

These findings demonstrate that antimicrobial activity is localized within a specific chromatographic fraction enriched in antimicrobial compounds. The results highlight the honey bee gut microbiota as a promising source of antimicrobial compounds for sustainable AFB control, with ongoing tandem mass spectrometry-based proteomic analysis aimed at identifying proteins associated with the observed antimicrobial activity.

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MODULATION OF HONEY BEES' SENSITIVITY TO AN ALARM PHEROMONE COMPONENT

Alice M. Durand[1], Ariane Marty[1], Jean-Marc Devaud[1,2]

[1]Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI), Toulouse, France
[2]Institut Universitaire de France, Paris, France

Abstract

Honey bees are eusocial insects living in large colonies, where the workers tend to the colony needs. When the colony is under predatory stress, especially from large predators, honey bees release a pheromone signal to recruit nestmates for colony defense. When such a defense response is not adaptive (i.e. when a beekeeper is visiting the hive), the release of this pheromone leads to unnecessary stress upon the colony, which can in turn be detrimental for the colony well-being. Indeed, in laboratory conditions, bees exposed to isopentyl acetate (IPA), the main component of the alarm pheromone, show increased aggressiveness as well as subsequent decreased performances in a learning task necessary for efficient foraging. However, little is known as to which bees can be recruited for colony defense, and therefore susceptible to these effects. Within colonies, task allocation is mainly age-dependent (yet influenced by other factors): during spring and summer, younger bees carry in-hive tasks while older bees are allocated to outside tasks such as foraging. With the aim of identifying the determinants of collective recruitment for colony defense, we investigated the respective roles of age and current activity of bees in their sensitivity to IPA. In addition, we tested whether exposure to specific floral scents could alleviate the detrimental effects of IPA exposure in susceptible bees. The results from this study should lead to a better understanding of honey bee task allocation under predatory stress, and may help finding practical solutions to limit the consequences of such stress during beekeeping practices.

VETERINARY APIARY SUPPORT WITHIN THE NABIG-PROJECT: INTEGRATING COLONY ASSESSMENTS, PATHOGEN DIAGNOSTICS, AND SAMPLE ARCHIVING TO ENHANCE HONEY BEE HEALTH

P 232

Elmar E. Ebner[1,2,3], Vinzenz Loimayer[4,5], Anita Winkler[5,6]

[1]Vetmeduni, PhD Student (Veterinary virology), Vienna, Austria
[2]HAUP, Bachelor student for Agrarian and Environmental Pedagogics, Vienna, Austria
[3]Practical Veterinarian, Debanttal, Austria
[4]Practical Veterinarian, specialized in honey bee health, Ternberg, Austria
[5]Austrian Veterinary Chamber, Vienna, Austria
[6]Practical Veterinarian, specialized in honey bee health, Vienna, Austria

Abstract

The **NaBiG** (Sustainable Bee Health)-project establishes a veterinary-led framework for structured health supervision of apiaries, combining standardized colony assessments with comprehensive laboratory diagnostics. The aim is to improve honey bee health by enabling early detection of pathological processes, enhancing beekeepers' management practices, and distinguishing physiological stress from disease-related burdens. Veterinary field visits include anamnesis, systematic inspections of colony strength, brood development, hygienic behaviour, environmental pressures and visible signs for pathogens. All findings are documented using a uniform assessment protocol to ensure reproducible and comparable data across apiaries.

Collected samples—including adult bees, brood and hive debris-are subjected to an extensive diagnostic panel targeting key pathogens of economic and ecological relevance. Viral diagnostics focus on high-impact agents such as Deformed Wing Virus (DWV-A/B), Acute Bee Paralysis Virus (ABPV), Chronic Bee Paralysis Virus (CBPV), Sacbrood Virus (SBV), and Black Queen Cell Virus (BQCV). Microsporidian infection with *Nosema ceranae* is quantified to assess colony-level energetic stress, while bacterial pathogens are monitored to support early recognition of foulbrood. Parasitic burdens, especially The load of *Varroa destructor*, is documented both visually and via KI-supported. Three viral pathogens (ABPV,DWV,SBV) were monitored with on hive- lateral flow test, others in lab with qPCR.

A core element of NaBiG is the long-term archiving of all collected samples under controlled storage conditions. This biobank enables retrospective analyses, the development of new diagnostic assays, and the investigation of emerging pathogens or shifts in genomic caused pathogen virulence. It further allows longitudinal comparisons to distinguish normal physiological fluctuations—from pathological stress responses driven by infections or parasite pressure.

Initial observations indicate that structured veterinary involvement substantially improves diagnostic accuracy and provides beekeepers with actionable insights. The NaBiG approach establishes a sustainable, data-driven model for long-term honey bee health management.

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AUTUMN *VARROA* CONTROL AND PATHOGEN STATUS IN AN 200 COLONY URBAN SETUP IN VIENNA

Elmar E. Ebner[1,2], Matthias Kopetzky[3,4], Simon Pree[5]

[1]Practical Veterinarian, Vienna, Austria
[2]Vetmeduni- PhD student for Veterinary Virology, Vienna, Austria
[3]Wiener Bezirksimkerei, Vienna, Austria
[4]Biene Österreich, Vienna, Austria
[5]Institute of Forest Ecology, BOKU University, Vienna, Austria

Abstract

Effective autumn treatment is critical for controlling *Varroa destructor*, pathogen status and ensuring colony survival during winter. Urban beekeeping environments, such as Vienna, present unique disease-ecology dynamics due to high colony density, diverse foraging landscapes and seasonal lack in food. This study evaluated the efficacy of different standard autumn *Varroa* management strategies in a Viennese apiary and assessed the associated pathogen load in honey bee colonies.

Varroa control was assessed in 200 colonies distributed across 35 apiaries within five organic acid based treatment groups In parallel, molecular diagnostics were used to quantify key honey bee pathogens, including DWV-B, *Nosema ceranae*, BQCV and CBPV.

Results showed a low initial Varroa load, high reinvasion pressure and a substantial reduction in *Varroa* mite loads following treatments, with post-treatment infestation levels mostly remaining below critical winter thresholds. At the same time, the comparison of the five treatment groups indicated that some treatment approaches may negatively affect bee health and contribute to colony losses. A pronounced apiary-site effect was observed, highlighting the strong influence of local conditions on treatment outcomes, colony performance, and pathogen dynamics. DWV-B was the most prevalent viral agent detected and was correlated with pre-treatment *Varroa* levels. *N. ceranae* was present at varying levels, while BQCV and CBPV appeared sporadically at high titers. Overall, the pathogen profile suggests that, despite effective mite suppression, viral pressure persists and reflects the typical pathogen landscape of Central European urban apiaries. This study highlights the importance of timely autumn *Varroa* management, careful selection of an adequate treatment strategy and continuous pathogen monitoring in urban beekeeping. The findings provide practical insights for maintaining colony health in highly populated beekeeping regions such as Vienna, while also emphasizing that both local site conditions and potential treatment-related side effects must be considered in management decisions.

ESTABLISHMENT OF SUSTAINABLE ANTIBODY PRODUCTION IN DIATOMS FOR IMMUNOASSAYS DETECTING THE CAUSATIVE AGENT OF AMERICAN FOULBROOD AND ITS MAIN ERIC-GENOTYPES

P 234

Sandra Ehrenberg[1], Lukas Rüttinger[2], Stas Hans[3], Alina Eilers[3], Marc O. Schäfer[1], Robert Kammerer[1]

[1]Friedrich-Loeffler Institute, Greifswald, Germany
[2]Senova, Weimar, Germany
[3]Phaeosynt, Hannover, Germany

Abstract

American foulbrood (AFB) is the most devastating bacterial brood disease of honey bees. AFB causes colony and economic losses worldwide and is a notifiable disease in many countries. *Paenibacillus larvae*, the causative agent of AFB, can be distinguished into ERIC-genotypes. The two mainly occurring ERIC-genotypes ERIC I & ERIC II show different levels in virulence and thus containment strategies could differ accordingly. Disease diagnosis is usually conducted via visual inspection. If a colony is suspicious, the causative agent has to be confirmed in the laboratory, which often is very time-consuming. For fast AFB diagnostic tools immunoassays like ELISAs and lateral flow assays (LFAs) using specific monoclonal antibodies (mAb) are needed. While antibody production is usually hybridoma based and often needs animal-derived products like FCS, we transformed mAb production from hybridoma to diatoms for mAb production. Diatoms can serve as cheap and sustainable protein factories, needing only low amounts of nutrition and light source for photosynthesis.

We sequenced the variable regions of the previously developed hybridoma-derived mAbs, introduced them into diatoms, and subsequently cultivated and selected mAb–producing diatom strains. Furthermore, diatom-derived mAbs were compared to hybridoma-derived mAbs. We conducted SDS-Page, Western Blot and ELISAs for comparison of hybridoma and diatom-derived mAbs. So far, four diatom-derived mAbs have been transferred and tested. Three of these diatom mAbs show similar reactivity compared to hybridoma-derived mAbs and will be used in an LFA. Here, we present the transfer of mAb production to a sustainable and cost-effective approach for antibody production that achieves functionality comparable to conventional hybridoma-derived mAbs. In addition, mAb production using diatoms represents an inexpensive platform that can make ELISAs and LFAs more affordable for diverse stakeholders, broaden their applicability, and therefore LFAs will be widely available for rapid on-site diagnosis, which will help to prevent the spread of AFB.

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BUSINESS AS USUAL VS. AN INNOVATIVE APPROACH - COMPARING COLONY PERFORMANCE UNDER DIFFERENT *VARROA* MANAGEMENT CONCEPTS ACROSS EUROPE AND BEYOND

Martin S. Gabel[1], Marin Kovačić[2], Cecilia Costa[3], Janez Prešern[4], Josipa Štavalj[5], Zlatko Puškadija[5], Jesús Yániz[6], Matías Casalongue[6], Pilar Santolaria[6], Raffaele Dall'Olio[7], Jerzy Wilde[8], Dylan Elen[9], Małgorzata Bieńkowska[10], Dariusz Gerula[10], Victoria Soroker[11], Angelina F. Osabutey[11], Robert Brodschneider[12], Georg Schaunitzer[12], Fani Hatjina[13], Leonidas Charistos[13], Aleksandar Uzunov[14], Magdalena Jovanovska[14], Sergio Albertazzi[3], Egoitz Galartza[15], Melanie Parejo[15], Antonios Tsagkarakis[16], Maria Bouga[16], Athanassios Antonopoulos[16], Mirjana Jankulovska[14], Gennaro Di Prisco[17], Viviana Valenzano[17], Marina D. Meixner[1]

[1]LLH Bieneninstitut Kirchhain, Kirchhain, Germany. [2]Croatia, Croatia, Croatia. [3]CREA Research Centre for Agriculture and Environment, Bologna, Italy. [4]Agricultural Institute of Slovenia, Ljubljana, Slovenia. [5]Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia. [6]EPSH, University of Zaragoza, Huesca, Spain. [7]BeeSources, Bologna, Italy. [8]Warmia and Mazury University in Olsztyn, Olsztyn, Poland. [9]Taskforce Research ZwarteBij.org, Gavere, Belgium. [10]The National Institute of Horticultural Research, Skierniewice, Poland. [11]Agricultural Research Organization, The Volcany Institute, Rishon LeZion, Israel. [12]University of Graz, Graz, Austria. [13]ELGO DIMITRA, Institute of Animal Science, Nea Moudania, Greece. [14]Ss. Cyril and Methodius University in Skopje, Faculty for Agricultural Science and Food, Skopje, North Macedonia. [15]University of the Basque Country (UPV/EHU), Leioa-Bilbao, Spain. [16]Agricultural University of Athens, Athens, Greece. [17]CNR-Institute for Sustainable Plant Protection, Naples, Italy.

Abstract

Sustainable hive management practices need to be tailored to the long-term needs of beekeeping and beekeeping-related services in the respective environment. They thus not only differ from region to region but also need to be adapted to changing conditions like shifted brood rearing rhythms, newly invading parasites and climate change on regular basis. To achieve sustainability on a broad scale, modern hive management needs to adapt natural mechanisms for both, the production of bee products and the provision of ecosystem services. In this context, the mite V. destructor remains a key challenge for beekeeping and honey bee health worldwide. The BEEGUARDS project investigates effects of an innovative yet nature-based *Varroa*-control concept on performance and resilience of colonies in comparison to conventional management standards. To assess this approach, 17 institutions across 12 countries started a large-scale field experiment involving nearly 500 colonies in summer 2024. The conventional approach reflects the treatment most commonly applied in each respective region, whereas the innovative approach is characterised by an induced summer brood interruption with subsequent oxalic acid application and a threshold-based strategy for the eventual winter treatment. Colony development and mite population are continuously monitored. Over all, colonies from both management groups performed comparable in terms of brood and bee population. However, *Varroa* infestation dynamics differed with higher levels in the innovative group early in the season up to summer treatment. In turn, conventionally managed colonies showed higher infestation loads after summer treatments in late summer and autumn.To complement this field data, half of the test colonies was equipped with digital scales, in-hive thermal sensors and bee counters, forming a Pan-European Digital Apiary and enabling new modeling approaches.

CALISTRIP BIOX – EFFICACY AND SAFETY OF A NEW FORMULATION OF OXALIC ACID

P 236

Claudia Garrido[1], Antonio Nanetti[2]

[1]BeeSafe, Bielefeld, Germany
[2]CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Oxalic acid is a widely used varroacide with high efficacy on dispersing mites. It was developed to be applied first by spraying, which is rather time consuming. Therefore, the application by trickling and sublimation was developed to ease the workflow in practical beekeeping. More recently, some strip formulations appeared intended for a long-term release of oxalic acid, to avoid the need for queen caging during the brood rearing period. However, these strips were not registered in the EU. Nonetheless, the demand of European beekeepers to have access to an easy-to-use strip application remained.

With Calistrip Biox the first strip formulation of oxalic acid complying with the strict requirements of EU regulations was recently registered. In a first study, we tested the efficacy of this product and its safety for honey bee colonies in Northern Italy. Calistrip Biox was applied in autumn 2025 in low or no brood conditions.

The efficacy of Calistrip Biox was satisfactorily high. As safety features, the daily worker mortality rate as well as the colony strength were assessed. No adverse effects of the strip application were observed at both individual bee and colony level.

Therefore, our preliminary results show that Calistrip Biox is a valid additional option for treating varroosis with oxalic acid under the conditions of the trial.

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RESPONSES FROM US COMMERCIAL BEEKEEPERS TO INCREASED PERCEPTION OF AMITRAZ RESISTANCE BY *VARROA*

Agostina Giacobino[1], Dan Aurell[1], Frank Rinkevich[2], Cissy Ballen[1], Geoffrey R. Williams[1]

[1]Auburn University, Auburn, United States
[2]USDA ARS, Baton Rouge, United States

Abstract

Amitraz-based products are the most frequently applied treatments for the control of *Varroa destructor* by commercial beekeepers in the United States; however, several studies identified amitraz-resistant mite populations. Here we assessed the extent of amitraz-based product use by commercial beekeepers, their perceptions of its effectiveness, and their efforts to overcome potential amitraz efficacy issues. We analyzed 76 responses from beekeepers who participated in the US Beekeeping Survey during 2024 and 2025 and conducted in-depth interviews with 25 additional beekeepers that focused on management practices over the preceding ten years. The US Beekeeping Survey revealed >90% of respondents using amitraz to control *Varroa* mites (median year of first use was 2008); 54% of these beekeepers reported decreased effectiveness of amitraz over time (median first observation of reduced effectiveness was in 2019). Meanwhile, the in-depth interviews revealed that 23 beekeepers (92%) had used amitraz in the past year. Over the preceding 10 years, 44% of the interviewees reported increasing their annual frequency of amitraz-based product use, whereas 24% increased their dosage per application. During the same 10 year period, the maximum number of non-amitraz active ingredients used per year was 2 (range 0–4), but decreased to 1 (range 0–3) in the most recent year. Similarly, over the past 10 years the maximum number of non-chemical practices applied per year was 2, compared with 1 (range 0–4) in the most recent year. Results indicate that many US commercial beekeepers responded to perceived amitraz resistance by increasing frequency and dosage of amitraz-based products, and reducing number of non-amitraz treatments and non-chemical practices.

SMALL STEPS TOWARD FINDING THE JUVENOID RECEPTOR IN *VARROA DESTRUCTOR*

P 238

Alexandru-Ioan Giurgiu, Daniel S. Dezmirean, Adriana Aurori

University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Cluj-Napoca, Romania

Abstract

The parasitic mite *Varroa destructor* remains one of the most destructive threats to *Apis mellifera* colonies worldwide. The mite's infestation success largely depends to its tightly synchronization of reproductive cycle with that of the host larvae within the capped cells. Although host-derived hormones, such as 20-hydroxyecdysone and juvenile hormone (JH), have been suspected as being implicated in triggering *Varroa* reproduction, the molecular mechanisms underlying hormone perception by the mite remain poorly understood.

The results of a RNAseq analysis led us to the idea that volatiles from host may have juvenile hormone-like (juvenoid) effect in *Varroa* with methyl farnesoate as a possible candidate. However, a critical gap remains in the understanding of how *Varroa* detects and transduces JH-related signals. In particular, database annotations (e.g., UniProt) reveal the presence of several putative variants of the *Varroa* methoprene-tolerant protein (*Met*), which have been automatically annotated but not functionally characterized. As *Met* is widely recognized as the intracellular receptor mediating JH signaling in insects there is a known divergence of JH signaling pathways in Acari and so, the learnings from insects may not apply to the latest. The fact that candidate *Met* proteins exist in *Varroa* requires the identification of the right one, if there is any functional. Subsequently, fundamental questions regarding ligand specificity are waiting to be answered.

Here, we analyzed RNA-seq data from *Varroa* mites infesting drone and worker larvae at 8 and 30 h post-capping, revealing the expression of putative *Met* genes that may help clarify the identity of the juvenoid receptor in *Varroa*.

In perspective, elucidating the identity and functionality of *Varroa* JH receptor(s) could be essential for understanding how host-derived hormonal cues regulate mite reproduction and may provide new molecular targets for disrupting its reproductive cycle.

P 239 THE BEST OF BOTH WORLDS TO IMPROVE *VARROA DESTRUCTOR* RESISTANCE SELECTION

Matthieu Guichard[1], Vincent Dietemann[2,3], Markus Neuditschko[4], Benjamin Dainat[1]

[1]Swiss Bee Research Centre, Agroscope, Bern, Switzerland
[2]Swiss Bee Research Centre, Agroscope, Bern, Switzerland
[3]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland
[4]Animal GenoPhenomics, Agroscope, Posieux, Switzerland

Abstract

Infestation by *Varroa destructor* remains the biggest threat to the health of honey bees of European origin. Attempts to breed resistant lines have started several decades ago and follow two general approaches: the use of modern animal breeding techniques involving animal models and breeding values based on one or a few resistance phenotypes and, at the other end of the spectrum, relying on natural selection of unidentified locally adapted resistance traits. To date, neither of these approaches produced broadly available commercial stock which beekeepers can keep and maintain without varroacidal treatments. We propose a paradigm shift by combining these diametrically opposed approaches to give nature more scope to select local resistance traits while using modern breeding and beekeeping techniques to foster genetic progress and prevent large-scale die-offs, which are inherently linked to survival selection. In addition, we propose to better consider the so far neglected co-evolutionary processes between host, parasite, associated pathogens, and microbiome. We will also present Plan-B-omics, a four-year proof-of-principle project which will test such approach by relying on metagenomics to include beneficial microbes and pathogens towards a co-selection process for increased honey bee colony health and survival.

ISOLATION OF KEY HEMOLYMPH PROTEINS IN HONEYBEES FOR FUNCTIONAL AND PHYSIOLOGICAL STUDIES

P 240

Eva Hrtánková, Silvie Dostálková, Jiří Danihlík, Marek Petřivalský

Department of Biochemistry, Palacký University, Olomouc, Czechia

Abstract

Proteins present in honeybee hemolymph play an essential role in energy storage, molecular transport, immune defense, nutrition, and antioxidant protection. In worker bee hemolymph, the predominant proteins are apolipophorin (ApoLp), vitellogenin (Vg), hexamerin (Hex) and transferrin (Tra). Vg is a 180 kDa multifunctional phospholipoglycoprotein that is important for longevity and nutrition and can be proteolytically cleaved into 150 kDa and 40 kDa fragments. ApoLp plays a key role in lipid transport. Three ApoLp components are present in honeybees: two integral forms, ApoLp-I (240 kDa) and ApoLp-II (80 kDa), and one exchangeable unit, ApoLp-III (18 kDa). Hex is a storage protein that functions mainly during the larva–pupa transition. Four Hex types have been identified in the honeybees (Hex 70a, Hex 70b, Hex 70c, Hex 110); however, only Hex70a (81 kDa) is abundantly present in adult worker bees. Tra (80 kDa) is a major protein involved in iron transport. Despite the existing knowledge, the precise mechanisms by which these proteins influence specific physiological processes in honeybees remain insufficiently understood. The isolation of protein standards for detailed studies is therefore an essential missing step. In this study, various ion-exchange chromatography matrices were evaluated using low-pressure liquid chromatography to determine optimal conditions for the isolation of individual proteins. For Vg, both weak and strong anion exchangers proved to be the most effective. ApoLp was successfully isolated using cation-exchange chromatography, with higher efficiency observed for the weak cation exchanger. Hex was obtained in a partially enriched form together with Tra using weak anion-exchange chromatography. The isolated protein standards will be utilized in subsequent studies to develop more precise analytical methods for investigating the distribution and physiological roles of these proteins in honeybees.

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INTRASPECIFIC VARIATION AND ENVIRONMENTAL STRESSORS INFLUENCE NUTRITIONAL AVAILABILITY FOR BEES IN *HELIANTHUS ANNUUS*

Salena Husband[1,2], Julia Kehmstedt[3], Carlos Martel[4], Dragana Miladinović[5], Vladimir Miklič[5], Silvio Erler[1,2]

[1]Julius Kühn Institute, Institute for Bee Protection, Braunschweig, Germany
[2]Zoological Institute, Technische Universität Braunschweig, Braunschweig, Germany
[3]Julius Kühn Institute, Institute for Bee Protection, Berlin, Germany
[4]Royal Botanic Gardens, Kew, United Kingdom
[5]Institute of Field and Vegetable Crops (IFVCNS), National Institute of the Republic of Serbia, Novi Sad, Serbia

Abstract

Floral resource diversity and availability are critical for bee health and reproductive success. Drivers of global environmental change, such as frequent drought and heat events, trigger adaptation strategies from farmers to adjust regional crop selection. These are a few of many factors changing the spatiotemporal nutritional landscapes for bees. Sunflower, *Helianthus annuus* L., is one crop that is increasing and expanding from its typical production due to its high tolerance to heat and drought stressors. Sunflowers are known to attract a large diversity of managed and wild bees; however, the macro and micronutrients of their pollen and nectar have been shown to vary considerably. This nutrient variability might differently impact neighboring bee communities' health as different bee species may differ in their nutritional needs. Therefore, our study aimed to investigate two facets of sunflower nutritional variance for bees for a subset of nutrients (e.g., free amino acids, free fatty acids, and sterols in pollen; and sugars in nectar). 1) This included plant genotype variability between hybrids with pollinator attractive traits in a field trial, and 2) drought stress induced variability between drought resistant genotypes in a controlled greenhouse setting. Hybrids with pollinator attractive traits showed genotype-specific nutrient profiles across individual and total essential amino acids, fatty acids, and nectar sugars. Under artificial drought treatments, nutrients varied in their response. Fatty acids remained stable in all genotypes and treatments; however amino acids showed genotype x environmental impacts. The drought resistant genotype showed a significant increase in free amino acids in the most extreme drought condition. By understanding how floral resource nutrients dynamically change, strategic crop selection creates an opportunity to match nutritional availability with nutritional demand to improve bee health and resilience.

FROM EGG TO ADULT – PRACTICAL TIPS FOR REARING *OSMIA BICORNIS* IN LABORATORY CONDITIONS

P 242

Jacek Jachuła[1], Aleksandra Splitt[1], Mikołaj Borański[1], Zuzanna M. Filipiak[2]

[1]The National Institute of Horticultural Research, Skierniewice, Poland
[2]Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Krakow, Poland

Abstract

Rearing solitary bees *in vitro* is necessary for experiments investigating the effects of e.g. diet, environmental stressors. Under laboratory conditions, individual bees can be observed throughout development enabling a wide range of experimental studies. However, even under standardised temperature, humidity, and feeding regimes, the physical properties of rearing substrates can still influence developmental outcomes.

As a model organism for laboratory rearing of solitary bees, *Osmia bicornis* can be used. Under laboratory conditions it is crucial to provide conditions that allow for proper cocoon spinning, including space and container material roughness.

The suitability of 2 ml Eppendorf tubes and 48-well cell culture plates for *Osmia* rearing was tested in two independent laboratories: The National Institute of Horticultural Research, Puławy and Jagiellonian University, Krakow. In each laboratory both types of containers with: 1) originally smooth surface, 2) originally smooth surface with added piece of paper, 3) matte surface (roughened with a drill), were tested. Two independent teams transferred eggs, predicted to develop into females and males, into the containers with their original pollen loads, creating twelve groups in total. Daily inspections recorded the start and end of cocoon formation. Four referees evaluated the cocoon quality. After hibernation, the specimens were incubated at an elevated temperature to assess emergence success. Then, the intertegular span was measured.

The overall evaluation was conducted to point out the strong and weak points of each rearing method. The preliminary results and practical tips to achieve successful development of bees in laboratory conditions will be presented in the poster.

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EXPOSURE REGIME AND INTRASPECIFIC VARIABILITY SHAPE INSECTICIDE SENSITIVITY IN HONEY BEES

Bhanu Janam[1], Abdulrahim T. Alkassab[2], Jens Pistorius[2], Sergio Angeli[1,3]

[1]Faculty of Agricultural, Environmental, and Food Sciences, Free University of Bozen-Bolzano, Bolzano, Italy
[2]Institute for Bee Protection, Federal Research Centre for Cultivated Plants, Julius Kühn-Institut, Braunschweig, Germany
[3]Competence Centre for Plant Health, Free University of Bozen-Bolzano, Bolzano, Italy

Abstract

Pollinator health is increasingly challenged by pesticide exposure, raising concerns for bee survival. Honey bees (*Apis mellifera* L.) are important pollinators widely used in ecotoxicological risk assessments. However, the high genetic diversity across subspecies and hybrids is underrepresented in current regulatory frameworks, potentially overlooking variability in responses. In this study, we investigated genotype-specific responses of honey bees to three commonly used commercial insecticide formulations. Acute and chronic oral toxicity tests were conducted following OECD (213 and 245) guidelines using flupyradifurone (Sivanto® Prime), lambda-cyhalothrin (Karate Zeon®), and cyantraniliprole (Minecto™ One) across five European honey bee genotypes, namely *A. m. mellifera*, *A. m. carnica*, *A. m. ligustica*, *A. m.* Buckfast, and a local population from Braunschweig (Germany). Acute toxicity was evaluated using median lethal dose (LD_{50}) estimates and survival analyses (Kaplan–Meier and Cox proportional hazards models), alongside observations of behavioural abnormalities. Chronic exposure further revealed time-dependent toxicity dynamics, including Time-Reinforced Toxicity (TRT) and Fast-Acting Toxicity (FAT). Across both exposure regimes, pronounced genotype-specific differences in sensitivity were observed. A. m. mellifera exhibited higher tolerance to lambda-cyhalothrin and cyantraniliprole in both acute and chronic conditions, whereas *A. m. carnica* showed consistently higher susceptibility under chronic exposure. Notably, genotype rankings in sensitivity shifted between acute and chronic conditions. *A. m.* Buckfast appeared more tolerant to flupyradifurone in acute assays, but under chronic exposure this compound showed clear Time-Reinforced Toxicity. Overall, our findings demonstrate that pesticide sensitivity in honey bees is strongly shaped by genetic background and exposure regime. Integrating acute and chronic endpoints across diverse genotypes provides a more realistic understanding of pesticide risks. Importantly, our findings highlight the need to incorporate intraspecific variability into ecological risk assessment frameworks.

METATRANSCRIPTOMIC VIROME ANALYSIS REVEALS NOVEL VIRUSES IN *TROPILAELAPS MERCEDESAE*

P 244

So-yoon Jang, Minhyeok Kwon, Eui-Joon Kil

Gyeongkuk National University, Andong, Korea, Republic of Korea

Abstract

Tropilaelaps mercedesae is a significant ectoparasitic mite in Asian apiculture and has emerged as a major threat to honey bee health due to its rapid reproductive rate and brood-parasitizing behavior. Despite its ecological and economic importance, the composition and genetic diversity of the virome associated with *T. mercedesae* remain poorly understood. In this study, a metatranscriptomic approach was employed to characterize the virome of *T. mercedesae* collected from Andong, Gyeongsangbuk-do, Republic of Korea. Total RNA was subjected to high-throughput sequencing (RNA-seq), followed by a comprehensive bioinformatics pipeline including quality filtering, host sequence removal, and de novo assembly. Viral contigs were identified through homology-based searches (tBLASTx and BLASTn), open reading frame (ORF) prediction, and conserved domain analysis. All detected viruses were further validated by RT-PCR. Our analysis identified four previously reported honey bee-associated viruses and one known *T. mercedesae*-associated virus. In addition, five putative novel RNA viruses exhibiting low sequence similarity to known viral taxa were discovered, suggesting the presence of previously uncharacterized viral diversity within this mite species. These findings provide foundational insights into the virome composition of *T. mercedesae* and establish a framework for future investigations into the diversity, evolution, and potential pathogenic roles of mite-associated viruses in honey bee health.

P 245 TEMPORAL VARIATION IN HIVE ODOUR: DYNAMICS OF VOLATILE ORGANIC COMPOUNDS IN *APIS MELLIFERA* COLONIES

Louise Jeandroz, Marie Carrière, Yoann Lepape, Maxime Verdier, Fanny Mondet

INRAE Bees and Environment, Avignon, France

Abstract

Chemical communication plays a key role in homeostasis maintenance within *Apis mellifera* colonies. While the composition and concentration of pheromones are known to vary with colony state, worker activity, brood quantity, and, developmental stage, most studies have focused on individual bee or brood profiles. However, little is known about whether such changes can be observed at the whole-hive level.

This study investigates the temporal dynamics of volatile organic compounds (VOCs) emitted by honey bee colonies under natural conditions. To capture these dynamics, VOCs were collected using Tenax cartridges and analyzed by gas chromatography-mass spectrometry at different times of day and over several months. In parallel, colony status (health, bee population, brood quantity) and environmental conditions (temperature, ...) were monitored. Additionally, to assess the influence of the brood stage on VOC emissions, samples were collected directly from frames containing brood larvae to emerging bees.

The results provide a comprehensive characterisation of the VOC composition, concentration, and temporal dynamics within honey bee hives. The work lays a preliminary foundation for future research in bee chemical ecology and identifies potential applications of VOC emissions as indicators of hive status.

COMPREHENSIVE ANALYSIS OF ENVIRONMENTAL SAMPLES AS A TOOL FOR ASSESSING THE EXPOSURE OF WILD POLLINATING INSECTS TO PESTICIDES – PRELIMINARY RESULTS

P 246

Tomasz Kiljanek[1], Milena Goliszek[1], Olga Burek[1], Matti Leponiemi[2], Mark Brown[2], Reet Karise[3], Pilar De la Rúa[4]

[1]Department of Chemical Research of Food and Feed, National Veterinary Research Institute (PIWET), Pulawy, Poland
[2]Department of Zoology, University of Cambridge, Cambridge, United Kingdom
[3]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia
[4]Dpto. Zoología y Antropología Física, Fac. de Veterinaria, Universidad de Murcia, Murcia, Spain

Abstract

It can be assumed that wild pollinators are exposed to pesticide residues in their environment. However, whilst the sources of honeybee exposure to pesticide residues are the subject of systematic research, enabling us to understand this issue increasingly well, this knowledge does not fully translate to the exposure of wild pollinating insects. This is because the behaviour of honeybees differs from that of hundreds of species of wild pollinating insects. The way to characterise the exposure of wild pollinators is through a comprehensive assessment of environmental contamination. The results of such an analysis will create a database, the elements of which we can then use to piece together a picture tailored to the lifestyle and behaviour of a given wild pollinator species.

For this purpose, as part of the WildPosh project, dedicated methods have been developed to determine the residues of 150 different pesticides, including fungicides, herbicides, insecticides, acaricides and plant growth regulators, in soil, plant, water and pollen samples. The range of compounds studied focuses on pesticides currently used in plant protection in the EU and the UK, and on the compounds most commonly found in pollen collected by honeybees, bumblebees and mason bees. This approach will allow for the most up-to-date and realistic assessment of the exposure of wild pollinating insects to pesticides in their environment.

These methods have so far been used to analyse samples collected near selected wheat and rapeseed fields in Spain and Estonia.

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SAFETY AND EFFICACY OF REPEATED OXALIC ACID DRIBBLE TREATMENT ON HONEY BEE HEALTH AND QUEEN QUALITY

Serhii Kondratiuk, Alvaro De la Mora, Ryan Merkl, Oleksii Obshta, Midhun S. Jose, Marina C. Bezerra da Silva, Elemir Simko, Sarah Wood

Western College of Veterinary Medicine, Saskatoon, Canada

Abstract

The parasitic mite *Varroa destructor* is a major threat to western honey bee colonies, reproducing in capped brood cells and feeding on adult and pupal fat bodies. Oxalic acid dribble is widely used to control infestations due to its simplicity and low cost; however, the effects of repeated applications on colony health remain unclear.

In this study, we evaluated the efficacy and potential toxicity of repeated applications of 3.5% (Canadian standard) and 4.2% (Italian standard) oxalic acid dihydrate solutions prepared in sugar syrup. Sixty nucleus colonies were treated during the summer with 5 mL per interframe of bees every four days (maximum 50 mL per hive). After four applications, 30 colonies continued treatments up to eight total applications, while queens from the remaining colonies were transferred to untreated colonies to assess acceptance, and queenless colonies were monitored for replacement ability. Queen quality (sperm viability and total sperm count) was assessed in September for both newly mated and treated queens.

Honey production decreased by approximately 11–12 kg following repeated applications. Both the number of applications and the oxalic acid concentration significantly affected honey production. Colony population was not significantly affected. Relative to controls, bee mortality increased by an average of 181 (high dose) and 103 (low dose) bees. Treated colonies produced smaller queen cells, while no differences were observed in queen quality parameters. Varroa infestation levels were maintained at approximately 1%.

These results indicate that, despite reduced honey production, slightly increased mortality, and smaller queen cell size, repeated oxalic acid dribble treatments are suitable for use in nucleus colonies and splits under the tested conditions. This project is still in progress to further assess oxalic acid efficacy, including overwinter survival of experimental colonies. Future studies will explore repeated applications during winter.

RESOLVING TAXONOMIC CONFUSION OF LAKE SINAI VIRUS IN HONEY BEES: TOWARD STANDARDIZED CLASSIFICATION CRITERIA

P 248

Minhyeok Kwon, Eui-Joon Kil

Gyeongkuk National University, Andong, Korea, Republic of Korea

Abstract

Since its initial discovery, Lake Sinai virus (LSV) has been widely reported across diverse geographic regions and is considered a potential contributor to honey bee colony decline, including Colony Collapse Disorder (CCD). With the rapid expansion of high-throughput sequencing and bioinformatics approaches, a substantial number of LSV-related sequences have been deposited in public databases. However, the absence of standardized taxonomic criteria has resulted in inconsistent and often ambiguous classification of LSV sequences. In this study, we aimed to establish a robust and reproducible classification framework for LSV. A total of 211 near-complete genome sequences (≥5,000 bp) were retrieved from the NCBI GenBank database. After rigorous quality control, including the removal of sequences with analytical inconsistencies and the exclusion of recombinant genomes, 191 sequences were retained for downstream analysis. Phylogenetic reconstruction based on full-length genome sequences revealed that LSV can be consistently divided into seven distinct clades, supported by a nucleotide sequence identity threshold of ≥75%. Furthermore, detailed analysis of the RNA-dependent RNA polymerase (RdRp) region enabled the identification of 13 distinct genotypes within these clades, providing additional resolution for intra-clade diversity. Collectively, this study proposes a standardized classification criterion for LSV, resolving current taxonomic inconsistencies and offering a foundational framework for future investigations into the evolutionary dynamics, pathogenicity, and epidemiological significance of this virus.

P 249 IMPACT OF ORAL PESTICIDE EXPOSURE ON THE SURVIVAL AND THE IMMUNE SYSTEM OF THE RED MASON BEE (*OSMIA BICORNIS*)

Lena Ladewig[1], Gennaro Di Prisco[2], Cecilia Costa[3], Annely Brandt[1]

[1]LLH-Bee Institute Kirchhain, Kirchhain, Germany
[2]IPSP-CNR Institute for Sustainable Plant Protection, Naples, Italy
[3]CREA Research Centre for Agriculture and Environment, Bologna, Italy

Abstract

Solitary bees are important pollinators, but both species diversity and population density are threatened by a wide range of environmental stressors. One major stress factor in modern agricultural landscapes is the exposure to plant protection products (PPP). Therefore, we investigated the effects of PPP exposure on the survival and immune responses in male and female red mason bees (*Osmia bicornis*) under laboratory conditions, as a task of the BEE-GUARDS project. For this purpose, the systemic butenolide insecticide Flupyradifurone (4.8 mg/kg) and the triazole fungicide Tebuconazole (0.14 mg/kg) were used. Since Tebuconazole belongs to the group of ergosterol biosynthesis inhibitors, which can impair detoxification processes in insects and thus inhibit the degradation of insecticides in bees, a combination of both active ingredients (Tebuconazole + Flupyradifurone, T+F) was tested in addition. Survival assays showed that oral exposure to Flupyradifurone and to the combined treatment (T+F) significantly reduced the lifespan of male red mason bees. Additionally, we investigated the physiological parameters linked to the immune system of *O. bicornis*. We found that, in both males and females, the number of hemocytes was significantly reduced in the PPP-exposed groups compared to the control group. Moreover, we will present functional analyses of the immune system, including measurements of melanisation and encapsulation responses to a foreign object. The antibacterial activity of the hemolymph will be demonstrated using inhibition zone assays.

Taken together, these findings demonstrate that oral exposure to Flupyradifurone, Tebuconazole, and their combination significantly impairs both survival and key immune parameters in *O. bicornis*, highlighting the need to incorporate solitary bee species into pesticide risk assessment frameworks to better protect wild pollinators in agricultural landscapes.

COMPARISON OF *VARROA DESTRUCTOR* MORPHOMETRIC CHARACTERISTICS BETWEEN RESISTANT AND SUSCEPTIBLE MITE TO ACARICIDES FROM GREECE AND FRANCE

P 250

Karatosidi Lavrentia, Alexandros Papachristoforou

Laboratory of Applied Apiculture, School of Agriculture, University of Thessaly, Larissa, Greece

Abstract

Varroa destructor has become the most damaging parasite of the Western honey bee *Apis mellifera* worldwide, and colony losses in untreated apiaries remain high. Effective control of Varroa infestations is increasingly challenged by the mite's ability to develop resistance to commonly used acaricides. While the physiological and biochemical mechanisms underlying acaricide resistance are relatively well studied, potential behavioural or morphological adaptations remain largely unexplored.

The aim of this study was to investigate whether resistant and susceptible *V. destructor* populations differ in body size and shape, potentially indicating a morphological component to resistance against two widely used acaricides: amitrazand tau-fluvalinate. Mites were collected from twelve apiaries -six in Greece and six in France- representing apiaries with documented differences in resistance profiles.

Using a stereomicroscope equipped with a digital imaging system, we photographed the ventral side of each mite and performed geometric morphometric analyses based on 14 body landmarks. Body shape variation was assessed through Procrustes superimposition, principal component analysis (PCA), and canonical variate analysis (CVA). Additionally, dorsal body length and width were measured to evaluate size differences across resistance groups.

Preliminary findings indicate detectable variation in morphometric traits between resistant and susceptible mites, suggesting potential plasticity associated with acaricide exposure or selection pressure. Full statistical analyses and final conclusions will be presented at the conference.

P 251 AGE-DEPENDENT CHRONIC ORAL TOXICITY OF THE INSECTICIDE BROFLANILIDE IN HONEY BEE WORKERS (*APIS MELLIFERA*)

Tevž Levstek, Gordana Glavan

Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Abstract

Broflanilide is a novel broad-spectrum insecticide increasingly used in agriculture to control pest insects on a variety of crops. It acts as an allosteric antagonist of the ionotropic γ-aminobutyric acid receptor. Due to its high acute toxicity to honey bees (*Apis mellifera*), broflanilide may pose a risk to non-target pollinators. However, its chronic toxicity and physiological effects in honey bees remain poorly understood. Because broflanilide is highly lipophilic, it may accumulate in hive matrices and contribute to prolonged exposure.

In this study, we investigated the effects of chronic oral exposure to broflanilide on honey bee mortality and selected physiological biomarkers. Mortality and LC_{50} values were assessed during a 10-day oral exposure experiment conducted according to the OECD Test Guideline 245. Standard tests were performed with 2-day-old worker bees, while additional experiments were conducted with hive bees and forager bees to evaluate age-dependent differences in sensitivity. In all groups, the activities of two biochemical biomarkers – acetylcholinesterase (AChE) and glutathione S-transferase (GST) – were measured after 10 days of exposure. AChE was assessed as a biomarker of nervous system function, whereas GST served as an indicator of detoxification processes and oxidative stress responses.

Mortality increased in a dose-dependent manner in 2-day-old bees (LC_{50} = 0.036 µg/mL). A dose–response relationship was also observed in hive bees (LC_{50} = 0.022 µg/mL), although with wider confidence intervals, whereas no clear dose–response relationship was detected in forager bees within the tested concentration range. In all age groups, AChE and GST activities did not differ significantly between treated and control bees. These results indicate age-dependent differences in sensitivity to chronic oral exposure to broflanilide, while the tested concentrations did not affect the examined biochemical biomarkers.

MOLECULAR, STRUCTURAL, AND EVOLUTIONARY INSIGHTS INTO OCTβ2R MUTATIONS ASSOCIATED WITH AMITRAZ RESISTANCE IN *VARROA DESTRUCTOR*

P 252

Daniela Lima[1,2], Maíra Costa[1], Fernanda Li[1], José A. Rufino[3], Raquel Martín-Hernández[4], Mariano Higes[4], Maria Alice Pinto[1], Dora Henriques[1]

[1]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal
[2]Pontificia Universidade de Minas Gerais, 30140-108, Belo Horizonte, Brazil
[3]CeDRI, SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[4]Centro de Investigación Apícola y Agroambiental (CIAPA). IRIAF. Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Marchamalo, Spain

Abstract

Amitraz resistance in the parasitic mite *Varroa destructor* has been associated with point mutations in the octopamine receptor gene Octβ2R (N87S, Y215H, F290L, and Y337F). To better understand the functional implications of these variants, we combined population genetics with *in silico* structural modelling and molecular docking.

First, we genotyped a 1064 bp fragment of Octβ2R in 83 mites collected from 14 Portuguese apiaries across Portugal, which were managed under organic (oxalic acid) or conventional (amitraz) systems, including three apiaries with no amitraz treatment for at least the last five years. Results showed a widespread frequency of the resistance-associated F290L allele across all sampled populations (98.3%). No other known mutations were detected in the sequenced fragment.

To investigate the specific mechanistic impact of these variants on the protein, structural analyses were conducted. The three transmembrane mutations showed relatively high RMSD values (1.68–2.01 Å) compared to the wild-type AlphaFold model (TM-scores 0.92–0.94), with no major structural damage detected and modest stability effects according to Rosie ΔΔG. SIFT analysis predicted functional impact for three of the four mutations. This work shows that the structural data help to understand the mutation's impact on Iberian Varroa populations, providing a plausible mechanistic explanation for its current high prevalence.

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W DIFFERENT COMBINATIONS OF GABA RECEPTORS MIGHT EXPLAIN SENSIBILITY TO PESTICIDES: FROM ELECTROPHYSIOLOGY TO BEHAVIOUR

Maxime Linard[1], Michel Boissac[1], Luciana Kollman de Barros[1], Coline Monchanin[2], Sandro Andreu[1], Louis Closson[1], Thierry Cens[1], Pierre Charnet[1], Matthieu Rousset[1]

[1]Institut des Biomolécules Max Mousseron, Montpellier, France
[2]Centre de Biologie Intégrative de Toulouse, Toulouse, France

Abstract

Honey bees are essential for ecosystem functioning and agricultural productivity. However, their populations are increasingly threatened by exposure to pesticides, many of which target neuronal ion channels. Gamma-aminobutyric acid (GABA) receptors are key components of inhibitory neurotransmission in insects and represent potential targets of neurotoxic compounds including phenylpyrazole, lindane or abamectins. Recently, by combining cryo-electron microscopy of the overexpressed honeybee RDL subunit with structure-function analyses, we demonstrated that honey bee GABA receptors are organized as pentameric receptor consistent with the canonical architecture of ligand-gated ion channels.

However, beside RDL, the honey bee genome encodes several other GABA receptor subunits whose combinatorial assembly is expected to generate receptor types with specific biophysical and pharmacological properties. Our work aims to characterize the different *Apis mellifera* GABA receptor subunits in order to better understand their fundamental physiological roles and their involvement in pesticide sensitivity at chronic and sublethal doses.

Through electrophysiological characterizations and transcriptomal analyses, we investigate the expression patterns, pharmacological properties, and potential assembly of GABA receptor subunits. Ultimately, we plan to rely our systematic approach with behavioral and anatomical assessment of cognitive impairments after pesticides intoxications. By identifying the receptor combinations that mediate inhibitory signalling in the bee nervous system, this work will provide new insights into the mechanisms underlying pesticide-induced neurotoxicity.

DETERMINATION OF THE MEDIAN INFECTIVE DOSE (ID50) OF THE MICROSPORIDIUM *NOSEMA (VAIRIMORPHA) CERANAE* IN DIFFERENT SUBSPECIES OF *APIS MELLIFERA*

P 254

Raquel Martín-Hernández[1], Soledad Sagastume[1], Giovanni Cilia[2], Noureddine Adjlane[3], Banan Al-Shagour[4], Mustafa Necati Muz[5], Dilek Muz[5], Marion Zammit Mangion[6], Antonio Nanetti[2], Mariano Higes[1]

[1]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Centro de Investigación Apícola y Agroambiental de Marchamalo, (CIAPA), Marchamalo, Spain
[2]CREA Research Centre for Agriculture and Environment, Bologna, Italy
[3]Department of Agronomy, Faculty of Sciences, University of Boumerdes, Bourmedes, Algeria.
[4]NARC-National Agriculture Research Center, Amman, Jordan
[5]Department of Parasitology, Faculty of Veterinary Medicine, University of Namik Kemal, Tekirdag, Turkey
[6]Department of Physiology and Biochemistry, University of Malta, Msida, Malta

Abstract

Nosema (*Vairimorpha*) *ceranae* is a microsporidian, obligate parasite of the epithelial cells of the ventricle of *Apis mellifera*, which disrupts various physiological, immunological and behavioral aspects of the host, as well as colony survival. Although its significance as a pathogen is well documented, its infectivity in *A. mellifera* has not been described, which limits the interpretation of its effects on different bee populations. In this context, the aim of the study was to determine the mean infectious dose (ID50) of *N. ceranae* in different Mediterranean subspecies of *Apis mellifera*. To this end, *N. ceranae* spores obtained from naturally infected bees from experimental apiaries (CIAPA, Spain) were purified and sent to six participating laboratories. Subsequently, adult bees (5 days old) were inoculated, with each bee individually administered 2 µL of a solution in distilled water, at 7 different doses (from 0-100,000 spores/bee). Each dose was administered to 100 bees. The same procedure was carried out with *A.m. iberiensis*, *A.m. ligustica*, *A.m. syriaca*, *A.m. anatoliaca*, *A.m. ruttneri*, *A.m. intermissa* and *A.m. sahariensis*. From day 0 until day 10 post-infection (p.i.), 10 bees were collected per concentration. More than 4000 bees were analysed by qPCR to determine the infection and the *N. ceranae* load. The highest number of infected bees was detected at the highest doses used for infection. The ID_{50} was determined in those days p.i. in which more than 50% of bees were detected positive to *N. ceranae*. Different ID_{50} were observed from day 3 to 9 post-infection, showing the different susceptibility of each subspecies, with *A.m. ligustica* showing the lowest sensitivity to the infection. On the contrary, mainly *A.m. iberiensis* but also *A.m. ruttneri* showed the lowest ID_{50} values.
These results are under the project 2011-MEDIBEES, which is part of the PRIMA programme supported by the European Union.

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VESPA VELUTINA PRIMARY NEST DETECTION BY TELEMETRY

Gioia Mei[1], Walter Massagli[2], Antonio Felicioli[1]

[1]University of Pisa, Pisa, Italy
[2]Toscana Miele Association, Lucca, Italy

Abstract

Within the life cycle of *Vespa velutina* knowledge on the overwinter surviving rate and fertility of the new queens is still lacking.

On April 2025 twelve overwintered *V. velutina* queens were captured during their nectar foraging activity on Camelia flowers. Each queen was tagged, fed and released.

Six out of the twelve queens returned successfully to their primary nest. These queens were captured again and their tags recovered.

Four out of the six detected nests were underground, one was under plain air in *Rubus* sp bush and one in a building eaves. All the nests were at their initial stage with only three to five cells with eggs. The building eaves nest was surprisingly inhabited by two queens the tagged one and another one.

All the seven queens were dissected in order to check their ovaries status.

All the six queens show mature eggs in their ovaries while the additional queen of the building eaves nest did not show eggs in its ovaries.

This very preliminary and anecdotal investigation suggest that a large part of primary nests could be hidden from the perception of operator and that starting nesting activities of *V. velutina* show similarities with nesting activities of *Vespa orientalis*. Moreover the occurrence of overwintered queens without mature eggs in their ovaries could indicate that not all of them are successfully fertilised and that some of them could behave as helper in nesting care.

MONITORING THE INVASION AND THE EXPANSION OF *VESPA ORIENTALIS* IN THE SLOVENIAN REGION OF PRIKORSKA

P 256

Blanka Mekinda[1], Aleš Bozovičar[2], Smon Golob[2], Eftychia Evangelopoulou[3], Themistoklis Giannoulis[4], Alexandros Papachristoforou[3]

[1]Slovenian Research and Innovation Agency, Ljubljana, Slovenia
[2]Beekeepers' Association of Slovenia, Lukovica, Slovenia
[3]Laboratory of Applied Apiculture, Department of Animal Science, University of Thessaly, Larissa, Greece
[4]Laboratory of Biology, Genetics and Bio-informatics, Department of Animal Science, University of Thessaly, Larissa, Greece

Abstract

The Oriental hornet *Vespa orientalis* (Linnaeus, 1771; Hymenoptera: Vespidae: Vespinae) is widely distributed across some of the warmest regions of the world. It is native to parts of southern Europe, North Africa, and Southwest Asia, but in recent years its geographic range has expanded, facilitated by increasingly favorable climatic conditions. *V. orientalis* is a generalist predator that targets a broad spectrum of insects, including key pollinators such as honey bees and solitary bees. Its establishment in new regions therefore poses significant risks to apiculture, biodiversity, and urban environments.

During inspections conducted in September 2025, five nests of this non-native species were discovered in the old city center of Koper, in the western Slovenian region of Primorska. Specimens collected were subjected to DNA analysis and morphological examination, both of which confirmed their identification as *Vespa orientalis*. Among the nineteen individuals captured, four were identified as foundresses (future queens), fourteen as workers, and one as a male.

Following the initial detection, an additional 21 nests of *V. orientalis* were observed in towns and villages located 8–30 km from Koper, indicating that the species is now established in the Primorska region. Monitoring of this invasive species is continuing throughout 2026, and detailed observations and findings will be presented at the 11th European Conference of Apidologie.

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BEHAVIOURAL RESISTANCE IN *VARROA DESTRUCTOR*: FIRST EVIDENCE IN RESPONSE TO ACARICIDE?

Théa Missud[1,2], Max Watkins[3], Paulo D. Mielgo[3], Alexandros Papachristoforou[4], Karine Monceau[2], Benjamin Poirot[1]

[1]Apinov, Scientific Beekeeping and Training Center, Lagord, France
[2]Centre d'Etudes Biologiques de Chizé, UMR-CNRS 7372, Villiers-en-Bois, France
[3]Vita (Europe) Limited, Hampshire, United Kingdom
[4]Laboratory of Applied Apiculture, Department of Animal Science, University of Thessaly, Larissa, Greece

Abstract

The ectoparasite *Varroa destructor* (Mesostigmata: Varroidae) poses a major threat to honey bee colonies due to its role as a viral vector and its capacity to develop resistance to treatments. While physiological resistance and target-site mutations are well documented, behavioural mechanisms remain largely unexplored. This study investigated the potential existence of behavioural resistance in *V. destructor*, defined as a modification of behaviour in response to an acaricidal molecule. Using Y-maze olfactometric assays, we evaluated mite responses to three acaricides—amitraz, tau-fluvalinate, and oxalic acid—across five French populations with distinct treatment histories. Forty groups of five mites were exposed to "acaricide/solvent" pairs, and choices were recorded after 18 hours. Results revealed marked heterogeneity among populations. Two population exhibited significant avoidance of tau-fluvalinate, providing the first case of avoidance behaviour in *V. destructor*, but no coinciding phenotypic resistance. No avoidance was detected for amitraz an oxalic acid. These findings suggest that behavioural resistance may exists in France but is not systematic. Identifying this phenomenon opens new perspectives on the parasite's adaptation to treatments and underscores the need to integrate behavioural aspects into control strategies.

HIVEMAP - HONEYBEE (*APIS MELLIFERA*) INTEGRATED SUBSPECIES VERIFICATION MICROBIOME ANALYSIS AND PATHOGEN DETECTION PROTOCOL

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Bartłomiej Molasy[1], Michał Kolasa[2], Andrzej Oleksa[3], Łukasz Neumann[4], Mateusz Krajewski[4]

[1]Jagiellonian University, Kraków, Poland
[2]National Research Institute of Animal Production, Balice, Poland
[3]Kazimierz Wielki University, Bydgoszcz, Poland
[4]University of Technology, University of Technology, Poland

Abstract

Western honey bees (*Apis mellifera*) are exposed to numerous stressors, including a wide range of pathogens, fluctuations in the availability and quality of floral resources, and various pressures associated with human activity. The large-scale movement of managed colonies over long distances has accelerated the spread of pathogens and facilitated the widespread introduction of non-native subspecies, leading to increasing hybridization with local honey bee populations.

Assessing the health status of honey bee colonies and determining their subspecies identity remains a complex challenge that is still insufficiently addressed. Beekeepers often lack adequate tools, and dedicated diagnostic services are not widely accessible for them. This limitation has important consequences for everyday beekeeping practices, honey bee welfare, the conservation of genetic diversity, the monitoring of epizootic dynamics, and the planning of preventive interventions or treatments.

To address this gap, we present HIVEMAP (Honeybee Integrated subspecies VErification, Microbiome Analysis and Pathogen detection) - a set of modern, high-throughput, relatively cost-effective interdisciplinary tools. HIVEMAP integrates geometric morphometrics supported by artificial intelligence algorithms with molecular approaches. It enables rapid subspecies identification, qualitative and quantitative analysis of gut microbiota composition, and pathogen detection. The molecular component is based on simultaneous multiplication and sequencing of hosts', bacterial and pathogen marker genes. Moreover, the inclusion of artificial plasmids enables estimation of absolute abundances, while a dedicated bioinformatic pipeline ensures effective decontamination and robust data analysis.

Here, we present initial results, including validation of the applied methods and directions for further development. In the future, HIVEMAP may support large-scale monitoring of honey bee health, contribute to the conservation of local genetic resources, and enable more precise planning of preventive and treatment strategies.

P 259 DIAGNOSTIC PERFORMANCE OF DIFFERENT SAMPLE MATRICES FOR CHRONIC BEE PARALYSIS

Linde Morawetz[1], Michael Opitz[1], Hemma Köglberger[1], Kerstin Seitz[2], Adi Steinrigl[3]

[1]AGES – Austrian Agency for Health and Food Safety, Vienna, Austria
[2]Austrian Chamber of Agriculture, Vienna, Austria
[3]AGES – Austrian Agency for Health and Food Safety, Mödling, Austria

Abstract

Beekeepers in Europe face a range of diseases, pests, and predators that they must control to keep their honeybee colonies healthy. Of these, Chronic Bee Paralysis (CBP) and its symptoms are poorly known among beekeepers and effective countermeasures are still lacking or are only based on anecdotal evidence. This viral disease affects adult bees and leads to severe colony disorders. Over the past decade, European researchers and practitioners have reported an increase in both occurrence and severity of CBP. The Austrian Citizen Science project CS-DeChrEase aims to evaluate countermeasures and to increase the knowledge of CBP among Austrian beekeepers. In this ongoing study, we assess the feasibility of different sampling procedures and their diagnostic value. Three sample matrices were collected from diseased colonies at various stages of their recovery process: (1) bees from the flightboard, (2) bees from a comb and (3) a swab from the flight board. Initial results show significant differences in virus loads between the three sample matrices, with the highest loads detected in flight board bee samples and the lowest in flightboard swab samples (n=60, p=0.008). Furthermore, colonies showing clinical symptoms exhibited higher virus loads than colonies without symptoms. At the same time, virus loads obtained from the two flightboard–based sample matrices were correlated (r=0.45). Based on an increased sample size, these data will enable the development of viral load thresholds for CBP diagnosis for all three sample matrices, thereby increasing the practical value of lab results for advisory work. In addition, our findings suggest, that non-invasive swab samples can provide meaningful diagnostic information. Such a non-invasive standard procedure would be particularly useful for assessing disease severity and monitoring colony recovery while avoiding the need for living bees and thereby being cost- and time efficient.

Project funded by the Forschungsförderungsgesellschaft Niederösterreich.

THE V-ITA PROJECT: A NATIONWIDE STUDY FOR THE IDENTIFICATION AND PREVENTION OF ACARICIDE RESISTANCE IN *VARROA DESTRUCTOR* IN ITALY

P 260

Arrigo Moro[1], Michela Bertola[1], Anna Granato[1], Claudia Casarotto[1], Mirko Ruzza[1], Elena Biasibetti[2], Matteo Frasnelli[3], Marco Pietropaoli[4], Stefano Gavaudan[5], Luciano Ricchiuti[6], Anna Cerrone[7], Leonardo Marino[8], Antonio M. Salvaggio[9], Antonio Pintore[10], Riccardo Terriaca[11], Giuseppe Cefalo[12], Raffaele Cirone[13], Franco Mutinelli[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy. [2]Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Torino, Italy. [3]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy. [4]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Roma, Italy. [5]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy. [6]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy. [7]Istituto Zooprofilattico del Mezzogiorno, Portici, Italy. [8]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy. [9]Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy. [10]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy. [11]Miele in Cooperativa, Arezzo, Italy. [12]UNAAPI – Unione Nazionale Associazioni Apicoltori Italiani, Firenze, Italy. [13]FAI – Federazione Apicoltori Italiani, Roma, Italy.

Abstract

The ectoparasitic mite *Varroa destructor* is the major threat to the health and welfare of honey bee colonies. This parasite compels beekeepers worldwide to apply regular chemical treatments in order to maintain their colonies alive. Synthetic acaricides have been widely used to control its infestation, but their effectiveness is declining due to the mite's ability to develop resistance. Consequently, these compounds are being replaced by softer alternatives. Under ongoing climate change, however, the opportunities to apply such alternatives are expected to become more and more limited as their efficacy depends on long winter brood breaks, which are reducing, and milder summer temperatures, which are on the rise. Therefore, limiting the development of resistance to synthetic compounds in *V. destructor* populations represents a key strategy for mitigating future managed colony losses and ensuring the sustainability of the sector.

We will present the V-ITA project, whose aims are to investigate the occurrence of acaricide resistance in *Varroa* mites in Italy and to support beekeepers' decision-making in treatment selection. Using a population genetic approach, we will identify possible patterns of acaricide-induced selection in Italian mites by analysing a large number of samples from throughout the peninsula. Exposure assays on live mites collected from suspected resistant hotspots will then be used to confirm resistant populations. The results will be integrated into a web platform to assist Italian beekeepers in selecting the most appropriate treatment to apply based on local climatic conditions and local resistance patterns. V-ITA represents the first nationwide study of *V. destructor* resistance, and provides a model for developing cost-effective approaches to detect and monitor resistance at national scale, ultimately guiding beekeepers toward improved practices for the sustainability of the sector.

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A FIRST APPROACH TO THE CORRELATION OF THE PATHOGEN LOAD AFFECTING *BOMBUS PAULOENSIS* WITH THE LAND USE IN BUENOS AIRES

Anabella R. Nicolli[1], Gregorio Fernandez de Landa[2], Pablo D. Ravainera[2], Daniele Alberoni[3], Mateo Fernandez de Landa[2], Francisco Zumpano[4], Constanza Brasesco[2], Francisco J. Reynaldi[5], Silvina Quintana[2], Romina Petrigh[2], Diana Di Gioia[3], Leonardo Galetto[6], Martin J. Eguaras[2], Matias D. Maggi[2]

[1]Instituto de Investigaciones Biológicas (IIB), Mar del Plata, Argentina
[2]Instituto de investigaciones en producción sanidad y ambiente (IPROSAM), Mar del Plata, Argentina
[3]Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy
[4]Instituto de investigaciones marinas y costeras (IIMyC), Mar del Plata, Argentina
[5]Instituto multidisciplinario de biologia vegetal, Cordoba, Argentina
[6]Centro de microbiología básica y aplicada (CEMIBA), La Plata, Argentina

Abstract

Land-use change is a major driver of pollinator decline and may reshape host-pathogen dynamics by altering community composition and increasing contact between managed and wild bees. We assessed whether land-use intensity influences the prevalence and infection intensity of common bee pathogens (*Nosema ceranae* and *Crithidia bombi*) in wild populations of *Bombus pauloensis*, a key native pollinator in southern South America. Sampling was conducted during spring and summer across four sites in Buenos Aires province (Argentina) representing contrasting levels of anthropization: a protected reserve, an agricultural field, a plant nursery, and an urban area. Land-use intensity was characterized using NDVI within a 3 km landscape buffer. Pathogen detection and quantification were performed using qPCR. *Nosema ceranae* and *C. bombi* were widely distributed across all sites, with no significant differences in infection intensity among land use categories. Despite contrasting landscape contexts, pathogen circulation appeared homogenized at the regional scale. The ubiquitous presence of managed honeybees across sites suggests potential spillover and host shifts contributing to pathogen maintenance in wild bumble bee populations. Our results indicate that pathogen pressure in B. pauloensis may not be strongly structured by local habitat differences but instead influenced by broader landscape connectivity and multi-host transmission dynamics. These findings underscore the need to integrate land-use change and pathogen ecology when assessing pollinator health in human-modified ecosystems.

ONE HEALTH WITH BEES: INTEGRATING MICROBIOTA, ECOSYSTEM MONITORING, AND BEE PRODUCTS IN A GLOBAL ONE HEALTH FRAMEWORK

P 262

Aslı Özkırım[1], Terd Disayathanoowat[2,3], Wantida Chaiyana[4,5], Rajnibhas S. Samakradhamrongthai[4,6], Aygün Schiesser[7], Hao Zheng[8], Daniele Alberoni[9], María Shantal Rodríguez-Flores[10], Simone Cutajar[11,12]

[1]Department of Biology, Faculty of Science, Hacettepe University, Ankara, Türkiye, ÇANKAYA, Turkey. [2]Research Center of Deep Technology in Beekeeping and Bee Products for Sustainable Development Goals (SMART BEE SDGs), Chiang Mai University, Chiang Mai, Thailand. [3]Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. [4]Research Center of Deep Technology in Beekeeping and Bee Products for Sustainable Development Goals (SMART BEE SDGs), Chiang Mai University, Chiang Mai, Thailand. [5]Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand. [6]Division of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand. [7]Department of Biology, Faculty of Science, Hacettepe University, Ankara, Turkey. [8]College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China. [9]Agro-Environmental Microbiology Area, Department of Agricultural and Food Sciences, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy. [10]Department of Vegetal Biology and Soil Sciences, Faculty of Sciences, University of Vigo, Ourense, Spain. [11]Agro-Environmental Microbiology Area, Department of Agricultural and Food Sciences, *Alma Mater Studiorum* Università di Bologna, Bologna, Italy. [12]Institute of Earth Systems, University of Malta, Msida, Malta.

Abstract

The One Health framework recognises the interdependence of environmental, animal, and human health and has become a central priority for global efforts addressing sustainability and public health. Within this context, honey bees (*Apis mellifera*) represent a unique biological interface linking these domains, and are increasingly used in biomonitoring programmes.

Honey bees function as continuous ecosystem samplers, collecting biological and chemical signals across diverse landscapes through their foraging activity. This makes them sensitive bioindicators of environmental conditions, including biodiversity loss, climate variability, pesticide exposure, antibiotic resistance, and pathogen distribution. Simultaneously, as key pollinators, they play a fundamental role in global food security, influencing both crop yield and nutritional quality.

A key component of bee health and resilience is the microbiota, which contributes to immunity, pathogen resistance, detoxification, and metabolic homeostasis. Alterations in microbial communities are increasingly associated with colony losses and reduced capacity to cope with environmental stressors. Moreover, microbiota-driven processes also contribute, to varying extents, to the biochemical composition and bioactivity of certain bee products, particularly fermented products such as bee bread and, to a lesser degree, honey. Bee-derived products can therefore be considered as functional outputs shaped by interactions among environmental conditions, floral resources, and bee physiology, with some having documented antimicrobial, immunomodulatory, and therapeutic properties. In this sense, bees not only act as indicators of ecosystem health but may also provide biologically active compounds of potential relevance to the One Health perspective.

Here, we propose an integrative perspective, "One Health with Bees", that connects ecosystem monitoring, microbiota dynamics, and the bioactivity of bee-derived products. This perspective outlines a research pathway in which honey bees may be investigated both as sentinel organisms and as potential mediators of environment–health interactions.

P 263 SEASONAL VIRUS DYNAMICS IN COLONIES OF THE WESTERN HONEY BEE *APIS MELLIFERA*

Delphine Panziera[1,2], Vera I.D. Ros[3], Rene van der Vlugt[1,3]

[1]Biointeractions and Plant Health, Wageningen University & Research, Wageningen, Netherlands.
[2]National reference laboratory for bees' notifiable diseases, Wageningen Bioveterinary Research, Lelystad, Netherlands
[3]Laboratory of Virology, Wageningen University and Research, Wageningen, Netherlands

Abstract

Colonies of the western honey bee (*Apis mellifera*) are susceptible to a wide range of predators, parasites and pathogens threatening their health and often their survival. Among these, viruses are ubiquitous but their impact is largely unknown, with the exception of a few well-studied species. In this study, we monitored monthly the presence and loads of 10 viral species by RT-qPCR in 45colonies of *Apis mellifera* in 3 geographically distinct apiaries, from the beginning of summer, when new queens were introduced, until winter. The development of the colonies (measured via the Liebefeld method) as well as the infestation rates of the ectoparasitic mite *Varroa destructor* were also monitored monthly, enabling the investigation of associations between viral species' prevalence and colony dynamics. A special focus is given to several recently discovered viruses such as Apis rhabdovirus-1 (*Rhabdoviridae*), Bee Macula-like virus (*Tymoviridae*), Hubei Partiti like virus 34 (*Partitiviridae*) and *Varroa* orthomyxovirus 1 (*Orthomyxoviridae*). This study gives insight into the ecology and impacts of a range of viruses detected in honey bee colonies.

EFFECTS OF A PROTEIN DIETARY SUPPLEMENT ON WORKER IMMUNITY AND THE SURVIVAL OF MASS-OVERWINTERED *APIS MELLIFERA* QUEENS

P 264

Camila Perera Perez[1], Andrée Rousseau[1,2], Pierre Giovenazzo[1]

[1]Laval University, Quebec, Canada
[2]CRSAD, Quebec, Canada

Abstract

Honey bees (*Apis mellifera*) are essential to Canadian agriculture through pollination and honey production. Each year, colonies face harsh winters, nutritional deficiencies, and pathogen pressure, resulting in average winter losses of 27% since 2010. To compensate, Canadian beekeepers rely on over 300,000 imported queens annually, a practice that risks introducing parasites and diseases, compromises queens reproductive quality through transport stress, and undermines local adaptation and selective breeding programs.

It is therefore crucial to develop solutions that provide locally produced queens early in spring. Our research team has developed a promising methodology for the mass overwintering of queens in "queen banks", produced locally and selected for their performance under our specific environmental and beekeeping conditions. Each bank consists of 20 queens placed within a colony of thousands of nurse bees. However, optimizing nutrition during this critical period remains poorly understood.

This study evaluates the effect of protein supplementation on winter bee immunity and queen reproductive quality during queen bank overwintering. Twenty queen banks were established in August 2025. Prior to wintering, half received sucrose syrup alone, while the other half also received a commercially available protein supplement (UltraBee). Worker bee health is assessed four times (September, November 2025, February, and April 2026) by measuring hypopharyngeal gland acini size, hemocyte counts, total hemolymph protein concentration, and abdominal fat content. In parallel, proteomic analyses are conducted on the hemolymph samples. Queen reproductive quality, including sperm viability and total sperm count, is evaluated before and after overwintering.

We expect that banks supplemented with protein will show higher queen survival rates and improved immunity parameters in worker bees. This research aims to generate practical recommendations for improving overwintering conditions and supporting local queen production, while advancing our understanding of winter bee immunity under nutritional stress.

P 265 CONTROL STRATEGIES FOR VARROOSIS: EFFICACY OF OXALIC ACID AND AMITRAZ IN SLOW-RELEASE STRIPS UNDER FIELD CONDITIONS

Antonio Pérez-Pérez, Soledad Sagastume, Raquel Martín-Hernández, Mariano Higes

Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Centro de Investigación Apícola y Agroambiental de Marchamalo, (CIAPA), Laboratorio de Patología Apícola, Marchamalo, España, Marchamalo, Spain

Abstract

The parasitic mite *Varroa destructor* is one of the main health issues affecting *Apis mellifera* colonies, being the etiological agent of varroosis, a disease that causes significant losses globally, particularly in the northern hemisphere, including Spain. This parasite affects both developmental stages and adult bees, feeding on hemolymph and fat tissue, which leads to the progressive weakening of the host. Additionally, *V. destructor* serves as a vector for various viruses, amplifying its impact on colonies, reducing their survival capacity, and causing devastating effects in the absence of proper control.

Despite the promotion of integrated pest management (IPM), chemical treatment remains essential in professional beekeeping, provided that established protocols are followed. Improper application of these treatments, such as failure to comply with recommended dosages or the use of unauthorized products, exerts strong selective pressure on mite populations, favoring the emergence of resistant *Varroa* strains and decreasing long-term effectiveness. In this context, the rotation of acaricides is a recommended strategy to mitigate this issue.

In this study, we compared two treatment regimens for varroosis control: (1) an integrated approach using slow-release strips of oxalic acid (Calistrip® Biox) in spring and amitraz (Apitraz®) in autumn, and (2) a conventional treatment using only amitraz (Apitraz®) in autumn. The results showed a 96.7% reduction in mite load in colonies treated with oxalic acid in spring, compared to a 175.5% increase in untreated colonies. In autumn, the reduction was 98.7% in group 1 and 97.5% in group 2. The colonies in group 1 exhibited greater vitality due to better parasite control. Furthermore, the integrated regimen showed higher efficacy, reducing the risk of resistance and ensuring sustainable long-term control.

FIRST HISTOPATHOLOGICAL OBSERVATIONS IN PFAS EXPOSED WORKERS AND DRONES OF *APIS MELLIFERA*

P 266

Karen Power[1], Arrigo Moro[2], Laura Zulian[2], Greta Foiani[2], Anna Granato[2], Gionata De Vico[1], Franco Mutinelli[2], Michela Bertola[2]

[1]University of Naples Federico II, Department of Biology, Naples, Italy
[2]Istituto Zooprofilattico Sperimentale delle Venezie, NRL for honey bee health, Legnaro, Italy

Abstract

Per- and Polyfluoroalkyl substances (PFAS) are a broad class of fluorinated synthetic compounds whose carbon–fluorine backbone makes them highly resistant to degradation, leading to long persistence in environmental and biological systems. Exposure to PFAS has been found to affect survival, reproduction, growth, and behaviour of a wide range of animals. Particularly, PFAS can directly affect the endocrine and the reproductive system impairing metabolism and fertility, and alter the regulation of different set of genes affecting the immune system. To date very little is known on the impacts of PFAS on *Apis mellifera* health, particularly with regard to tissue alterations. In this preliminary study, honey bee workers and drones that survived to PFAS exposure (PFOA, PFOS and PFBA), in an acute oral toxicity test conducted at the IZSVe insectary in August 2025 were analysed by routine histological techniques. Microscopic examination of workers challenged with PFAS revealed a high number of fat-body associated oenocytes presenting accumulation of brown-yellowish granules in their cytoplasm (PFOS 83, 21%; PFOA 78%; PFBA 97,65%) and degeneration of the fat-body, probably due to detoxification attempts. In drones, histological findings included degeneration and increased spaces between the testes seminiferous tubules, confirming targeting of PFAS on the reproductive system also in *Apis mellifera*. Although based on a limited number of samples, our findings pave the way to further investigations on the possible ecopathological impacts of PFAS on the health of *Apis mellifera*.

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THE POLLEN-FEEDING THRESHOLD FOR IAPV INFECTION IN BUMBLE BEES

Durga Prasad[1], Yitzhak T. Sade[1,2], Elad Bonda[1], Sigal Popovsky[1], Hagai Y. Shpigler[1]

[1]Department of Entomology, Institute of Plant Protection, Agricultural Research Organization, Rishon LeZion, Israel
[2]The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University in Jerusalem, Rehovot, Israel

Abstract

Bumble bees (*Bombus terrestris*) are important managed pollinators in modern agriculture. The commercial bumble bee industry produces millions of colonies annually. In rearing facilities, colonies are fed pollen collected by honey bees (*Apis mellifera*), which may introduce pathogens into production systems. Among these pathogens, Israeli Acute Paralysis Virus (IAPV) is a widespread RNA virus that infects multiple bee species. Understanding the infection threshold following dietary exposure is therefore important for improving biosecurity in commercial bumble bee production.

In this study, we investigated the infection threshold of IAPV transmitted through pollen using two experimental systems: Founder queens were fed sterilized pollen cakes containing increasing IAPV concentrations (~10^3, 10^5, 10^7 and 10^9 viral particles g^{-1}). After five weeks, viral loads in the digestive system, fat body, and ovaries of the queens were quantified using RT-qPCR to evaluate systemic infection. IAPV level was also quantified in the fat body and gut of workers grown on the infected pollen. In a second experiment, twelve worker bees were fed pollen cakes containing ~10^3, 10^5, 10^7 and 10^9 viral particles g^{-1}, and viral loads in the digestive system and fat body were measured after one and five weeks in order to study the timeline of the infection.

Both queens and workers showed a minimum infection threshold of ~10^5 while lower levels were not infective regardless of time. At ~10^5 concentration, infection occurred in approximately 50%, 30%, and 20% of queen gut, ovary, and fat body samples, respectively. In workers, ~50% of gut samples and ~20% of fat body samples were infected. In higher concentration treatments (10^7 & 10^9) all bees showed systemic infection.

These findings highlight the importance of pollen sterilization and pathogen monitoring to reduce viral transmission risks in commercial bumble bee production systems.

HEMP FOR BOOST BEE IMMUNITY AND VARROASIS ERADICATION

P 268

Aneta A. Ptaszyńska

BeeLab UMCS, Department of Cell Biology, Institute of Biological Sciences, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Lublin, Poland. Beemmunity Unlimited Sp. z o.o., Poland, Jarosław, Poland

Abstract

Protecting honey bees from *Varroa destructor* is critical because this ectoparasitic mite severely compromises bee immunity. The global spread of this parasite is not only a challenge for beekeepers, but also a major scientific and agricultural imperative with far-reaching implications for food security and ecosystem stability.

The two-year research included laboratory and field experiments. In these experiments, several parameters were determined: the LD50 of the preparation, the life span of honeybees in each group (observed in a group of 400 individuals), the activity of phenoloxidase (PO) and lysozyme in the haemolymph of honeybees, and the expression levels of genes related to immunity (abacin, hymenoptecin, defensin, cecropin). Oxalic acid was used as a control.

A significant reduction in the population of *Varroa destructo*r mites was observed throughout the treatment period. The study also showed that the preparation can be used to prevent and limit *Varroa* infestation in bee colonies. The hemp preparation had a statistically significant effect on the expression of PO and lysozyme, and it also increased the expression of abacin and defensins. Furthermore, no differences were observed between the experimental and control colonies in terms of odour, taste, honey yield, bee bread, propolis and pollen pellets. After application of the preparation, the colonies successfully overwintered and quickly regained their strength in spring. The main advantage of the preparation is its composition, as it contains ingredients of natural origin. It can be used in both traditional and organic beekeeping. Furthermore, tests have shown no residues of the product in honey samples, which bodes well for its potential use in the production of commercial honey.

This research work was conducted as part of the B090/0045/2.1 project – "Development of an innovative miticide for honey bee colonies". Results are protected by patents: PL.235761, EPC.3562495 (owner: Beemmunity Unlimited Sp. z .o.o.).

P 269 THE PRELIMINARY STUDY: TWO PATHWAYS OF THE HOST IMMUNE RESPONSE TO MICROSPORIDIAN PARASITES

Aneta A. Ptaszyńska[1], Tomasz M. Karpiński[1,2], Paweł Żukowski[1], Marek Gancarz[3,4,5]

[1]BeeLab UMCS, Department of Cell Biology, Institute of Biological Sciences, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Lublin, Poland
[2]Chair and Department of Medical Microbiology, Poznań University of Medical Sciences, Poznań, Poland
[3]Faculty of Production and Power Engineering, University of Agriculture in Krakow, Kraków, Poland
[4]Institute of Agrophysics Polish Academy of Sciences, Lublin, Poland
[5]Center for Innovation and Research on Prohealthy and Safe Food, University of Agriculture in Kraków, Kraków, Poland

Abstract

Honeybees (*Apis mellifera* L.) and greater wax moths (*Galleria mellonella* L.) demonstrate contrasting responses to infection. While honeybees exhibit heightened individual susceptibility, this is compensated for by colony-level immunity through hygienic behaviours. In contrast, wax moths develop robust individual immune responses. Genomic comparisons reveal that honeybees possess around a third fewer genes related to the immune system than other insects, reflecting evolutionary trade-offs favouring social defence mechanisms over individual immune capacity.
Preliminary studies have shown that microsporidiosis impairs the honeybee immune response by reducing levels of antibacterial polypeptides, while significantly increasing lysozyme and phenoloxidase levels in *G. mellonella*. Other studies have demonstrated that microsporidiosis disrupts the hypopharyngeal glands of *A. mellifera*, causing necrotic lesions in the intestines and leading to dysbiosis of the microbiota. Preliminary studies have also indicated that nosemosis reduces the survivability of bee larvae. It is unclear whether this is due to changes in royal jelly secretion (as the hypopharyngeal glands, which produce royal jelly, are affected by nosemosis), the altered antimicrobial properties of the honey subsequently fed to worker larvae or shifts in microbiota composition and biofilm formation. Furthermore, *N. ceranae* infection alters the honeybee microbiota. No comparable data on such changes exist for *G. mellonella*.
This study was supported by the grant Opus 29, no. 2025/57/B/NZ6/04290 "Two pathways of the host immune response to microsporidian parasites and microsporidiosis co-infections".

CANNABIS EXTRACTS AS PROTECTIVE AND THERAPEUTIC AGENTS FOR HONEYBEES: A STUDY ON THEIR EFFECTS AGAINST PESTICIDES AND *NOSEMA* SPP. INFECTION

P 270

Aneta A. Ptaszyńska[1], Jakub Gębalski[2], Daniel Załuski[2]

[1]BeeLab UMCS, Department of Cell Biology, Institute of Biological Sciences, Faculty of Biology and Biotechnology, Lublin, Poland
[2]Department of Pharmaceutical Botany and Pharmacognosy, Ludwik Rydygier Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland

Abstract

This study investigates the protective and therapeutic properties of *Cannabis* extracts on honeybees (*Apis mellifera*) exposed to neonicotinoid pesticides and infected with *Nosema* spp. *Cannabis* plants contain approximately 500 different compounds, including over 80 cannabinoids, which may have beneficial effects on honeybee health. The aim of this research was to evaluate whether ethanolic and aqueous extracts could mitigate the harmful effects of pesticides and reduce *Nosema* spp. spore loads in infected honeybees.

Plant material from *Cannabis sativa* subsp. *sativa* L. and *Cannabis sativa* var. *ruderalis* Janisch. was processed by either ethanolic or hot water extraction, then filtered and lyophilised. The extracts were incorporated into bee diets at various concentrations for three experimental designs: pesticide protection (feeding extracts for 6 days prior to neonicotinoid exposure), *Nosema* spp. infection treatment (feeding extracts to artificially infected bees), and toxicity assessment (monitoring survival of healthy bees fed extracts for 17 days).

The study showed that *Cannabis* extracts significantly improved honeybee survival when exposed to neonicotinoid pesticides. The most pronounced protective effect was observed in the acetamiprid group, which had the lowest mortality rate among the test groups.

In *Nosema*-infected bees, treatment with *Cannabis* extracts resulted in a significant reduction in spore counts. While untreated infected bees developed up to 750,000 *Nosema* spores per bee, the treated groups showed significantly lower infection levels, down to around 52,000 spores per bee.

In addition, *Cannabis* extracts were non-toxic to honeybees, with survival rates in extract-fed groups (ca. 22-24.5 bees/group) comparable to control groups (25 bees) after 17 days.

These results suggest that *Cannabis* extracts are a promising natural approach to protect honey bees from neonicotinoid pesticides and to treat *Nosema* spp. infections, potentially offering new tools for addressing bee health issues.

Results are protected by patents: PL. 241381, PL. 241380.

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AMERICAN FOULBROOD IN FINNISH HONEY BEE HIVES IN 2020-2025

Emilia Pulkkinen, Henry Kuronen

Finnish Food Authority, Kuopio, Finland

Abstract

American foulbrood (AFB) can have significant impacts on bee colonies and apiaries, including the loss of bee hives and economic consequences for the beekeepers. AFB, caused by the spore-forming bacterium *Paenibacillus larvae*, is one of most important brood diseases affecting honey bees (*Apis mellifera*). The spores of *P. larvae* germinate in honey bee larvae, leading to larval death within 7-12 days at the pre-pupal stage. The dead larvae become heavily infested with *P. larvae* spores, facilitating the spread of the disease. Ultimately, AFB weakens the hive and can lead to the collapse of the colony.

The annual number of Finnish professional beekeepers has remained between 60-80 beekeepers. In total, there were more than 76 000 honey-producing bee hives in the country in 2025, including professional and hobbyist beekeeping operations. In Finland, AFB is a notifiable animal disease, requiring that confirmed or suspected cases have to bee reported to official authorities. The detection of AFB and other honey bee diseases is centralized at the Finnish Food Authority. AFB is diagnosed through bacterial cultivation from symptomatic brood combs, adult bees, or honey samples by using YGP (yeast extract, glucose, and pyruvate) agar plates and nalidixic acid (3mg/l) enriched YGP agar plates. Honey samples are heat-treated at 100 °C for 10 minutes prior to cultivation. Plates are incubated 9 days at 35 °C at normal atmosphere. The colonies typical for *P. larvae* are further confirmed by using MALDI-TOF (Bruker) and gram-staining, if necessary.

Between 2020-2025, *P. larvae* was isolated from 440 samples, and the annual number of affected apiaries ranged between 12-51. AFB was found widely across Finland, except the northernmost Lapland region. The most common positive sample was honey, as most of the AFB testing in Finland is conducted as voluntary, precautionary screening prior to the appearance of any clinical symptoms.

INNOBEE: INNOVATIVE BIOTECHNICAL AND PATHOGEN-ORIENTED APPROACHES FOR SUSTAINABLE HONEY BEE HEALTH MANAGEMENT

P 272

Zlatko Puškadija[1], Marin Kovačić[1], Josipa Štavalj[1], Karolina Tucak[1], Dragana Tekić Novaković[2], Tamaš Petrović[3], Dalibor Todorović[3], Sava Leđanac[3], Vladimir Polaček[3], Ivana Đurica[4], Gabriella Haas[4], Gordana Jakovljević[4], Tamara Davidović[4], Ivan Pihler[2]

[1]Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia
[2]University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia
[3]Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia
[4]Fund for European Affairs and Development of the Autonomous Province of Vojvodina Novi Sad, Novi Sad, Serbia

Abstract

The INNOBEE project addresses major challenges to beekeeping sustainability in the cross-border region of Croatia and Serbia, focusing on *Varroa destructor*, associated viral pathogens, and the need for innovative and sustainable management strategies. By combining applied research with active beekeeper participation, the project aims to improve honey bee colony health, resilience, and productivity while reducing dependence on conventional chemical treatments.

A core component of the project is a pilot study evaluating a biotechnical approach based on summer brood interruption followed by oxalic acid treatment at queen release. Colony performance is assessed through repeated measurements of *Varroa* infestation, colony strength, honey production, and queen survival. In parallel, regular sampling of bees will be conducted to detect the presence and dynamics of key honey bee viruses and *Nosema* spp. using molecular diagnostic methods. Additionally, monitoring of the invasive hornet *Vespa velutina* is performed at apiary level to assess its presence and potential impact on colony health.

The study is implemented in close collaboration with beekeepers, ensuring fast application and facilitating knowledge transfer. By integrating innovative *Varroa* control with pathogen and pest monitoring, the project aims to deliver evidence-based guidelines for sustainable colony management and to strengthen the resilience of the beekeeping sector in the region in conditions of increasingly intense climate change.

P 273 WHAT SECRETS DOES DNA METAGENOMIC ANALYSIS OF HONEY REVEAL?

Risto Raimets, Reet Karise

Estonian University of Life Sciences, Tartu, Estonia

Abstract

Honeybees (*Apis mellifera* L.) face constant pressure from various pathogens and pests, many of which are difficult to identify through standard visual checks. This study highlights DNA metagenomics (ISO 17025 certificate) as a potential diagnostic tool for assessing colony general health. Researchers analyzed two types of Estonian honey: pooled honeycomb samples taken directly from hives (2021-2022) and retail honey jars provided by beekeepers (2022-2024). The analysis revealed that honey from jars contained very low levels of pathogen DNA. This is likely due to the "dilution effect," where mixing honey from numerous colonies masks the presence of stressors. Conversely, samples taken directly from the hives showed higher pathogen concentrations. Notably, pollen mold (*Bettsia alvei*) exceeded 1% of the total genetic sequences in several samples, peaking at 5.13%, while one sample showed a significant presence of *Paenibacillus larvae* (2.59%). While DNA from *Varroa* mites and wax moths was detected only in trace amounts, the study concludes that current DNA metagenomic analysis offers broader insights for beekeepers and alternative analysis methods are also recommended to use to get more precise overview of colony health status. In terms of using DNA metagenomics, testing honey from individual colonies is more effective than analyzing blended or pooled samples.

SYNERGISTIC EFFECT OF ABAMECTIN AND DIFENOCONAZOLE ON ACUTE ORAL TOXICITY IN STINGLESS BEES *SCAPTOTRIGONA POSTICA*

P 274

Gabriela Reani Rodrigues Garcia[1], Rafaela Garcia da Silva[1], Joyce Oliveira Costa[1], Janete Brigante Casteli[2], Eny M. Vieira[1]

[1]USP-IQSC, São Carlos, Brazil
[2]USP-EESC, São Carlos, Brazil

Abstract

Bees are insects of great importance for biodiversity and agricultural production, as they play a fundamental role in pollination, fruit formation, and the production of honey, wax, propolis, and pollen. Brazil hosts one of the greatest diversities of bee species, among which the tribe *Meliponini*, composed of stingless bees, stands out. Among these, the species commonly known as Mandaguari (*Scaptotrigona postica*) is considered a highly efficient pollinator, as its colonies can exceed 8,000 individuals. This study aimed to evaluate the acute oral toxicity of abamectin and difenoconazole, individually and in combination, in *S. postica*. In the acute oral toxicity assays, bees were exposed to analytical standard solutions of abamectin within the concentration range of 5.0×10^{-1} to 2.5 ng μL^{-1}. For difenoconazole, the evaluated range was 1.5×10^{3} to 2.0×10^{4} ng μL^{-1}. Combined exposure was performed within the range of 9.0×10^{-2} to 5.0 ng μL^{-1}.Mortality was monitored throughout the experimental period to determine toxicological endpoints. Abamectin showed an LD_{50} of 0.0068 ± 0.00018 µg bee^{-1} (72 h), whereas difenoconazole presented an LD_{50} of 95.48 ± 26.59 µg bee^{-1} (48 h). Combined exposure resulted in LD_{50} values of 0.00563 ± 0.00072 µg bee^{-1} (72 h) and 0.00791 ± 0.00093 µg bee^{-1} (48 h), indicating increased toxicity and a possible synergistic effect. The protein profile of Mandaguari bees and the interference of the contaminant in insect metabolism were analyzed by biochemical electrophoresis in polyacrylamide gel, where some alterations were observed in exposed individuals, suggesting possible responses related to detoxification and tolerance.

P 275 CONTROLLING INVASIVE ASIAN HORNET (*VESPA VELUTINA NIGRITHORAX*) NESTS USING ACTIVATED CHARCOAL

Carolin Rein, Berenike Breuer, Kirsten S. Traynor

State Institute of Bee Research, Stuttgart, Germany

Abstract

The invasive Asian hornet (*Vespa velutina nigrithorax*) poses a significant threat to biodiversity and apiculture due to its predation of insects, particularly honey bees. Consequently, effective nest removal strategies are essential to mitigate its impact. Secondary nests are often located high in treetops and thus difficult to access. The application of activated charcoal via a telescopic lance represents a practical and environmentally friendly alternative to insecticides or costly mechanical removal.

In this study, 19 secondary nests were treated with activated charcoal using a telescopic lance, while only air was blown through the lance into 4 nests that survived as a control. All were reassessed for hornet activity after one week. In addition, a laboratory experiment was conducted in which 250 adult hornets and 250 larvae were exposed to five charcoal concentrations (0, 3, 30, 300, and 600 mg), with mortality recorded at six time points over 48 hours.

In the field, 63.2% of treated nests showed no continued activity after one week, indicating successful inactivation. Higher application rates (>0.5 kg per nest) increased efficacy to 85.7%. In the laboratory tests, adult hornets exhibited a clear concentration-dependent increase in mortality. While only 1 out of 50 individuals died in the control group, all adults exposed to 300 mg and 600 mg died within 48 hours. In contrast, larval mortality was not affected by charcoal exposure ($p = 0.59$). These results suggest that higher charcoal doses enhance nest inactivation, likely because newly emerging adults are exposed to residual charcoal within the combs. Nevertheless, mechanical destruction of the combs immediately after treatment remains advisable to prevent potential reactivation. Overall, this method provides a cost-effective and field-applicable tool for authorities and beekeepers to control *V. velutina* in hard-to-access locations.

Funding provided by the Ministry of Food, Rural Affairs and Consumer Protection (MLR), Baden-Württemberg.

VOLATILE CHEMICAL SIGNATURES OF HONEY BEE COLONIES UNDER COMBINED STRESS FROM *VESPA VELUTINA*, *VARROA DESTRUCTOR*, AND WINTER ENVIRONMENTAL CONDITIONS

P 276

María Shantal Rodríguez-Flores[1,2], Alberto Castro Parada[1,3], Ana Diéguez-Antón[1,2], Olga Escuredo[1,2], María Carmen Seijo Coello[1,2]

[1]Departamento de Biología Vegetal y Ciencias del Suelo, Facultad de Ciencias, Universidade de Vigo, Ourense, Spain
[2]Instituto de Agroecoloxía e Alimentación (IAA), Universidade de Vigo, Campus Auga, 32004 Ourense, Spain, Ourense, Spain
[3]Centro de Investigación Mariña, Universidad de Vigo, Vigo, Spain

Abstract

The survival of honey bee (*Apis mellifera*) colonies depends on interactions between biotic stressors and environmental conditions. The present study investigated the volatile organic compound (VOC) profiles of colonies exposed to predation pressure from the invasive hornet *Vespa velutina* and varying levels of *Varroa destructor*, and evaluated their relationship with overwintering survival.

Volatile sampling was performed in ten colonies across two apiaries using solid-phase microextraction (SPME) fibres (DVB/CAR/PDMS; 45–60 min). VOCs were analysed by GC-MS with PARADISe deconvolution, and compound identification was curated to remove analytical artefacts. *V. velutina* pressure was assessed using camera traps, colony strength was evaluated three times prior to winter (up to October), and *Varroa* infestation was quantified using the *Varroa* Check method.

The colonies exhibited a wide range of *Varroa* infestation levels (0-16.85%), including values that exceeded the critical threshold of 7%, which was associated with winter collapse. VOC profiles were dominated by fatty acids, phenolic compounds, and long-chain hydrocarbons, indicating metabolic stress, microbial activity, and altered colony structure. Unsaturated ketones such as 2-buten-1-one further suggested lipid oxidation under stress.

Environmental conditions included high external humidity (95-100%) and low ambient temperatures. Collapsed colonies contained dead bees despite adequate food reserves. Pre-winter assessments revealed that surviving colonies had higher brood levels and more homogeneous structure, whereas colonies that later collapsed showed high proportions of empty comb (>65%).

VOC profiles reflect chronic physiological stress and colony disorganisation rather than acute alarm signalling. Colony collapse appears to result from the combined effects of pre-winter stressors and adverse environmental conditions, highlighting the importance of integrating chemical, biological, and environmental data into multi-stressor scenarios.

Acknowledgements: We thank the beekeepers for access to their apiaries.

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P 277 ASSESSING SUBLETHAL EFFECTS OF PESTICIDE MIXTURES ON BUMBLEBEE VISUOMOTOR BEHAVIOUR USING A GAP-CROSSING ASSAY

Silvia Rönnau, Lina Herbertsson, David O'Carroll, Elisa Rigosi

Lund University, Lund, Sweden

Abstract

When bumblebees forage in flowering crops, they are exposed to cocktails of pesticides while performing highly coordinated visuomotor behaviours to navigate complex environments and avoid obstacles. Exposure to neuroactive insecticides may negatively affect the neural circuits underlying these behaviours. Moreover, bees encounter additional pesticides in crop fields, including fungicides. Although fungicides are generally considered to have low toxicity for insects, some can interfere with detoxification pathways, potentially producing synergistic effects when combined with insecticides. Whether and how such combined exposure influences visuomotor control in bees remains largely unknown.

To address this question, we developed a naturalistic yet highly controlled behavioural assay in which homing bumblebees must fly through a narrow gap. This task requires precise visual detection of gap edges and background texture, lateral scanning manoeuvres, and a characteristic angled turn to avoid wing collisions. By manipulating the visual salience of the background, we can simulate changes in light conditions as bees experience in nature.

Bumblebees were exposed to a commonly used insecticides, a fungicide, or their combination under sublethal, field-realistic conditions and their flight trajectories were recorded. At the concentrations tested, exposure did not affect the proportion of bees returning to the colony. Ongoing analyses are quantifying more subtle changes in visuomotor coordination, including lateral scanning manoeuvres, gaze and body orientation, time spent near the obstacle, and general flight kinematics (e.g., speed and angular velocity).

By integrating ecologically relevant exposure scenarios with mechanistic behavioural metrics, this approach provides a sensitive method for assessing how sublethal pesticide exposure may affect flight control in bumblebees and potentially other social flying insects.

WITHDRAWN

FALL *VARROA DESTRUCTOR* INFESTATION REDUCES WINTER SURVIVAL BUT NOT REPRODUCTIVE QUALITY OF BANKED HONEY BEE (*APIS MELLIFERA*) QUEENS

P 278

Andrée Rousseau[1], Mireille Levesque[2], Laurence Plamondon[1], Pierre Giovenazzo[3]

[1]Centre de Recherche en Sciences Animales de Deschambault, Deschambault, Canada
[2]Université Laval, Québec, Canada
[3]Unversité Laval, Québec, Canada

Abstract

In northern temperate regions, limited availability of honey bee (*Apis mellifera*) queens in early spring constrains beekeeping operations. Long-term queen banking offers a potential solution, but the biological factors influencing queen survival and quality during extended winter storage remain poorly understood, particularly the role of *Varroa destructor* infestation in bank colonies. We evaluated the effects of fall *Varroa destructor* infestation on the survival, quality, and performance of queens banked from September 2024 to May 2025 and compared banked queens with commercially imported queens from California. A total of 560 locally reared queens were banked in 14 queen banking colonies with either low or high fall *Varroa* infestation levels. Queen survival was monitored and queen morphometrics, reproductive traits, and pathogen loads were measured during bamking. In spring, queen introduction success was assessed followed by brood production, honey yield, and colony health. Queen survival during banking remained high and was not influenced by fall *Varroa* infestation, whereas higher infestation reduced survival during winter. Surviving queens from both bank groups displayed similar body size, weight, and reproductive characteristics. Imported queens showed reduced morphometric traits, lower sperm viability, and detectable viral infections. While colony establishment was successful across groups, colonies headed by banked queens produced more honey than those headed by imported queens. These findings indicate that long-term queen banking can maintain high-quality queens in northern climates when fall *Varroa* infestation is effectively managed.

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SEASONAL PARASITE DYNAMICS IN WILD-LIVING AND MANAGED HONEY BEE COLONIES ACROSS SWITZERLAND

Benjamin Rutschmann[1], Martin Hasselmann[2], Patrick L. Kohl[2]

[1]Agroscope, Zürich, Switzerland
[2]University of Hohenheim, Stuttgart, Germany

Abstract

Parasites and pathogens pose a major threat to honey bee health, with significant implications for both apiculture and pollinator conservation. However, there are limited empirical data on parasite burden in wild-living honeybee colonies , particularly across seasons. Consequently, their role in disease dynamics remains unclear; they may either exhibit reduced parasite burden compared to managed colonies due to natural life-history processes such as swarming and brood interruption, or act as reservoirs of parasites that are transmitted to managed colonies and other bees. To address these questions, we investigated occurrence and burden of parasites in wild-living and managed honey bee colonies across Switzerland, using wild-living colonies identified through a nationwide citizen-science monitoring initiative.

We collected more than 230 samples from colonies at three time points in 2025 (April, July, and September), thereby capturing seasonal variation in parasite dynamics. Our study included wild-living colonies nesting in trees, buildings, and nest boxes, comprising both recently founded and established colonies with known life histories prior to sampling, as well as managed colonies differing in management intensity. Total RNA was extracted from pools of 20 worker bees per sample, and the colony-level abundance of multiple microparasite taxa (including Tracheal mites, *Nosema* spp., pathogenic bacteria and several viruses) was quantified using an established array of diagnostic assays for a high-throughput qPCR platform.

This study will provide one of the most detailed assessments of honeybee parasite dynamics in wild-living and managed colonies in Europe to date, contributing to a better understanding of the role of wild-living honeybees in disease ecology, apiculture, and pollinator conservation.

EVALUATING THE EFFICACY OF COMMONLY USED MITICIDES AGAINST *VARROA DESTRUCTOR* IN THE TROPICAL ENVIRONMENT OF RÉUNION ISLAND

P 280

Abalhassani Saïd, Margot Camoin

Groupement de Défense Sanitaire de la Réunion, La Plaine des Cafres, France

Abstract

Varroa destructor was first detected in Réunion island in 2017, since then it represents a major sanitary challenge for beekeepers. This is especially due to the island tropical environment where the absence of a marked winter leads to continuous brood production and persistent development of varroa populations. Treatments for varroa are usually applied twice a year, between Brazilian pepper tree and Litchi tree honeyflows.

Between 2023 and 2025, several field trials have been conducted to assess the efficacy and potential side effects of different authorized miticides. The experiments involved more than 150 hives distributed in several apiaries located in the southern and western parts of the Island. The experimental protocol relied on repeated sugar washes to assess the number of mites per 100 bees, weekly monitoring of mite falls on sticky boards and application of an oxalic acid control treatment to estimate residual mite populations and calculate the efficacy. Additionally, colony strength (brood surface and adult bee population size) and potential side effects were monitored.

Formic Pro® and Apilife Var®, based on formic acid and thymol evaporation, showed high mean efficacies of respectively 96.7% and 95.3%. Delayed application of the full dosage or lower dosage were tested to limit transient side effects such as absconding or broken brood pattern. Varromed® used while brood was present in the colony, needed successive dribbling to reach sufficient mean efficacy (82.0% after 4 applications, 99.3% after 5 applications). The newly authorized Calistrip Biox®, providing extended oxalic acid release, achieved a high mean efficacy (95.2%) only when a artificial brood break was created thanks to queen caging.

Treatments authorized in conventional beekeeping, Apivar®, Apistan® and Bayvarol® still demonstrated a high and consistent mean efficacy exceeding 98%, which contrasts to the efficacies measured in European countries.

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NUTRITIONAL TRANSCRIPTOMIC REVEALS NEGATIVE IMPACT OF VIRAL DISEASE IN HONEY BEE

Roberta Santopietro[1], Lena Ladewig[2], Josipa Štavalj[3], Viviana Valenzano[1,4], Flavia V. Chianese[1], Marin Kovačić[3], Annely Brandt[2], Gennaro Di Prisco[1]

[1]CNR-IPSP, The Italian National Research Council Institute for Sustainable Plant Protection, Portici, Napoli, Italy
[2]LLH-Bee Institute Kirchhain, Erlenstr, Kirchhain, Germany
[3]University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia
[4]Department of Biology, University of Napoli Federico II, Napoli, Italy

Abstract

Honey bees can be infected by at least 19 viruses, most of which have a single-stranded RNA genome. Among these, the globally spread Deformed Wing Virus (DWV), is one of the main pathogens associated with colony collapse. Stress factors like lower temperature, *Varroa destructor*, and poor nutrition contribute to reduced emergence rates and lifespan, increased susceptibility to infections, impaired brood development, and weakened immune function, and in turn promoting viral replication.

In this study, a cage experiment was conducted to evaluate the effect of different dietary regimens, characterized at nutritional point of view on individual immune response. This was assessed by quantifying DWV viral load and analyzing the transcriptome. Newly emerged worker bees were reared under controlled conditions for 10 days and divided into three experimental groups, each receiving a specific pollen-based diet based on content of protein and fat (poor and rich). DWV viral loads were quantified with qRT-PCR approach, while RNA-Seq analysis was performed to study the transcriptome changes and gene ontology.

As part of the BEE-GUARDS project, this study explores the immune function, with particular emphasis on how variations in nutritional quality influence gene expression and immune modulation.

FROM HIVE TO HONEY: ANTIMICROBIAL RESISTANCE IN *PAENIBACILLUS LARVAE* AND ONE HEALTH

P 282

Violeta Santrac[1], Ivana Tlak Gajger[2], Kazimir Matovic[3], Maxim Chernik[4], Elmar E. Ebner[5], Giovanni Formato[6], Xesus Feas[7]

[1]Public Institution Veterinary Institute of the Republic of Srpska "Dr. Vaso Butozan" Republic of Srpska, Banja Luka, Bosnia and Herzegovina
[2]Faculty of Veterinary Medicine University of Zagreb, Zagreb, Croatia
[3]Veterinary Specialized Institute "Kraljevo", Kraljevo, Serbia
[4]Belarusian State Technological University, Minsk, Belarus
[5]Department for Biomedical Research and Pathobiology, Institute for Infectiology and Virology, University for Veterinary Medicine Vienna, Vienna, Austria
[6]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Roma, Italy
[7]Academy of Veterinary Sciences of Galicia, Galicia, Spain

Abstract

Paenibacillus larvae, a Gram-positive, spore-forming bacterium, is the etiological agent of American foulbrood disease in honeybees (*Apis mellifera*) and is globally distributed. The widespread use of metaphylactic antibiotic treatments in apiculture, aimed at suppressing clinical symptoms, represents a poor management strategy. While temporarily effective, such practices fail to eradicate the pathogen, which persists in contaminated environments due to its highly resistant spores. Prolonged and repeated antibiotic use within hives promotes the selection of resistant strains, contributing to the emergence of antimicrobial resistance (AMR) in *P. larvae* and its presence in honey. Although *P. larvae* is not considered a zoonotic pathogen, its spores may pose a potential risk under specific conditions, such as medical applications involving honey or in individuals with compromised barriers or altered microbiota. Furthermore, the presence of antibiotic resistance genes represents an additional concern, as these may contribute to the broader public health threat of antimicrobial resistance.

This communication summarizes current evidence and emerging concerns regarding AMR in *P. larvae* within the One Health framework.

Despite increasing evidence of AMR in *P. larvae*, this issue remains largely overlooked by both the medical community and consumers. This communication highlights the urgent need for interdisciplinary research to better understand resistance mechanisms, assess potential human health implications, and evaluate the role of honey as a non-clinical reservoir of AMR within the One Health framework.

The global trade of honey, together with heterogeneous and sometimes unregulated antibiotic use in beekeeping, may facilitate the dissemination of resistant bacteria and resistance genes. In this context, *P. larvae* may act as a relevant vector of AMR, underscoring the need for improved surveillance and responsible management practices in apiculture.

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A HEALTHY FUTURE WITH FLOURISHING DIVERSITY – A PROJECT FOR PEOPLE, ANIMALS, AND NATURE

Marc O. Schäfer[1], Wolfgang Kesselheim[2]

[1]Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany
[2]University of Greifswald, Greifswald, Germany

Abstract

Our project is set against the backdrop of the steady decline in biodiversity, especially among insects. In the project, we will collaborate with citizens to transform a conventionally farmed field into a biotope for insects and flowering plants. On the one hand, protection of pollinators and increasing their biodiversity are particularly important to us. On the other hand, we want to explore the impact of a biodiversity-friendly environment on people: Does spending time in such an environment have a positive effect on people's well-being? What effect does it have on their concept of "biodiversity"?

In our project, we are testing how a field that has been converted from conventional to biodiversity-friendly farming in collaboration with citizens can be developed and permanently established as a new, practical learning opportunity for the One Health region of Western Pomerania. On the farmland we are transforming, we will accompany and support people throughout the entire transformation process. During this time, a continuous exchange of knowledge will take place in both directions: The participating citizens will learn how to transform farmland into an area relevant to biodiversity and have sensory experiences about its connection to human well-being. And we will document this and learn how participants connect their personal well-being with the biodiversity they experience.

Participants will learn the basics of biodiversity measurement. For example, we will work together to record and identify flowering plants and their pollinators. We will also run workshops for schools and kindergartens to highlight the importance of biodiversity areas for human and environmental health. Citizens can actively participate in developing recreational and experiential trails as learning spaces, support the project by sponsoring flowering plants, etc. In return, we will learn how to inspire people about these topics and how to engage them in building sustainable, socially relevant structures.

UTILIZING THE ONE HEALTH PERSPECTIVE TO SYNERGISTICALLY PREPARE A TRANSNATIONAL BORDER REGION FOR INVASIVE PESTS SUCH AS *VESPA VELUTINA*

P 284

Dirk L. P. Schorkopf

AGES - Austrian Agency for Health and Food Safety, Vienna, Austria

Abstract

Here, I will share the first results, observations and lessons learned from the ongoing EU-Interreg project "ZUSIMLANA", which utilizes One Health approaches to prepare the Austro-Hungarian border region against invasive species of major ecological and economical importance, such as by the invasive yellow-legged hornet *Vespa velutina (Vv)*. This invasive pest may be particularly suitable for One Health approaches for the following reasons: Today, *Vv* is well known not only to affect animal health (e.g. honey bees), but also agriculture and human health, with likely significant and long-lasting impacts to the European environment. Adopting a holistic, cross-sectoral strategy to address *Vv* and similar emerging threats should enable faster, more efficient, socially more accepted and economically less disruptive management solutions.

P 285 DETOXIFICATION IS ESSENTIAL TO XENOBIOTIC TOLERANCE IN HONEY BEES BUT A POOR DIET MAY COMPROMISE THIS FUNCTION

Elisa Seffin, Davide Frizzera, Virginia Zanni, Desiderato Annoscia, Francesco Nazzi

Department of Agricultural, Food, Environmental and Animal Sciences (Di4A), University of Udine, Udine, Italy

Abstract

Honey bees (*Apis mellifera* L.) play a crucial role in ecosystems by providing essential pollination services, thus significantly contributing to crop production and plant biodiversity.

Over the past decades, a worrying decline in honey bee colonies has been reported in many countries. Colony losses are due to the interaction of multiple stressors, including parasites and pathogens, climate change, landscape degradation and exposure to xenobiotics, such as agrochemicals present in the environment or acaricides used by beekeepers, but also compounds naturally present in nectar and pollen.

Pollen is an essential nutritional resource for bees, promoting bee health and influencing the detoxification of pesticides; however, the existence of a direct link between pollen nutrition and detoxification hasn't yet been demonstrated through a manipulative experiment.

To assess how pollen affects detoxification and how this latter influences the survival of contaminated bees, we carried out an experiment under controlled laboratory conditions in which bees were fed with pollen and/or a xenobiotic; some bees were also treated with piperonyl butoxide (PBO), a P450 inhibitor. As a model xenobiotic, we considered the toxic alkaloid nicotine, which can be found in the nectar and pollen of some plant species and has a notable chemical affinity with some insecticides. We assessed both the survival of treated and control bees and the expression of a set of genes involved in xenobiotic detoxification.

We found that pollen is essential to sustain the detoxification capacity of contaminated bees.

In sum, we showed that the essential detoxification function in honey bees, which allows bee to survive in a chemically challenging world, can be impaired by poor nutrition. In this way, we further contributed to the appreciation of this biological system's complexity: an essential step towards the elucidation of the causes of the pollinator crisis and the identification of effective remedies.

BRIDGING THE GAP: ESTABLISHING *IN VIVO* LARVAL BIOASSAYS WITH SOLITARY BEES FOR TESTING OF BIOPESTICIDES

P 286

Marie Christine Seidel, Silvio Erler, Jens Pistorius, Abdulrahim T. Alkassab

Julius Kuehn-Institute, Braunschweig, Germany

Abstract

Regulatory frameworks for plant protection products and toxicity testing primarily rely on honey bee-based guidance. However, solitary bees, representing the majority of wild bees, exhibit distinct life histories and substance sensitivities, making it essential to development species-specific testing protocols. While semi-field trials provide realistic data, they are quite resource-intensive. This study focuses on method development and optimization of an *in vivo* larvae testing protocol, using the solitary mason bee *Osmia cornuta* as a model organism, for first strep screenings in ecotoxicology.

The larvae rearing methodology was refined through a series of comparative laboratory setups to identify the most effective conditions. Several diets using either honey bee or *Osmia* collected pollen were tested, while also evaluating the effects of different temperature settings and well-plate orientations. Larvae were exposed to various biopesticides in the diet, and larval mortality, developmental duration, successful pupation and emergence were evaluated. Preliminary results from the optimization phase showed a high efficiency of the selected setup, achieving pupation rates exceeding 70%. No differences between the pollen used on the pupation rates was found. A slight difference in developmental duration was observed between the different well-plate orientations. Preliminary results indicate that a setup using honey bee pollen and horizontal oriented well-plates was most optimal for both bee development and handling procedures. Further results on the potential impacts of the tested biopesticides are still under evaluation. This method development contributes to the establishment of standardized, cost-effective, and reliable *in vivo* protocols, enhancing environmental risk assessment and toxicity testing for solitary bees.

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A EUROPEAN ROADMAP FOR THE MANAGEMENT OF INVASIVE ASIAN HORNETS: POLICY GAPS, MULTI-SECTORAL IMPACTS, AND RECOMMENDATIONS FOR ACTION

Sebastian Spiewok[1], Cindy Adolphe[2], Noa Simon Delso[2]

[1]Deutscher Imkerbund, Wachtberg, Germany
[2]BeeLife European Beekeeping Coordination, Brussels, Belgium

Abstract

Invasive social vespids - notably *Vespa velutina* and the neo-invasive *Vespa orientalis* - represent a structurally underestimated, cross-sectoral threat to European biodiversity, agriculture, beekeeping, and public health. Despite the provisions of EU Regulation 1143/2014 on invasive alien species, management of these hornets across Member States remains fragmented, underfunded, and reactive. This contribution presents a policy brief developed by BeeLife European Beekeeping Coordination, a civil society organisation working at the intersection of pollinator protection and environmental health advocacy.

We analyse the current epidemiological situation of both species across Europe, documenting their rapid range expansion, facilitated by climate change and highly opportunistic behaviour. We assess their multi-sectoral impacts: predation on a wide diversity of insect taxa, negative impact on pollinator-plant interactions, increased winter colony losses (cited as an important driver of 2023–2024 losses by 22% of French beekeepers), direct economic damage to beekeeping and fruit/wine production, and emerging allergological risks for humans in heavily infested areas.

We further identify the structural failures underpinning inadequate control: inter-ministerial jurisdictional conflicts, the absence of coordinated EU-wide data sharing, a shortage of approved biocides, and the systematic under-resourcing of monitoring and nest-removal operations. Finally, we present a tiered set of evidence-based recommendations spanning EU, national, and regional/local governance levels, covering surveillance protocols, stakeholder coordination, IT-supported reporting platforms, research priorities (including selective traps, Trojan methods, and novel food integration), and funding reform.

Our roadmap calls for a paradigm shift: from beekeeping-centric, reactive nest removal to a professionalised, multi-sectoral, One Health-framed response that fully implements the existing regulatory framework while closing its critical gaps.

LABORATORY AND FIELD ASSESSMENT OF CALISTRIP®: CARDIAC IMPACTS ON *VARROA DESTRUCTOR* AND EFFICACY IN HEAVILY INFESTED COLONIES

P 288

Gregory Tziovaras, Lavrentia Karatosidi, Alexandros Papachristoforou

Laboratory of Applied Apiculture, Department of Animal Science, University of Thessaly, Larissa, Greece

Abstract

Calistrip® is an acaricidal strip formulation containing 6.44 g of oxalic acid. It provides prolonged activity and has previously been reported as highly effective against *Varroa destructor* when applied in broodless colonies. The present study evaluates the efficacy of Calistrip® in field conditions when applied to heavily infested colonies containing brood. In addition, a novel laboratory bioassay was applied to assess the impact of the product on the cardiac activity of *Varroa* mites. In laboratory assays, Calistrip® induced an acute collapse of cardiac function in *Varroa destructor*, reducing both the frequency and duration of cardiac pulses. This disruption resulted in the death of all examined mites within 7–12 minutes. Field trials were carried out in autumn 2025 in the area of Ioannina, northwestern Greece. Calistrip® was applied in a group of eight colonies naturally infested by *V. destructor*. Daily mite mortality was recorded using screened bottom boards, and potential effects on honey bee mortality were evaluated with Gary traps. Colony strength – measured as adult population and brood area – was also monitored. After 28 days of application, residual mite populations were assessed using amitraz (Apivar® strips), and the efficacy of Calistrip® was compared to that of a control group consisting of four untreated colonies. Calistrip® achieved an efficacy of 77.37%, which differed significantly from the natural mite fall observed in control colonies and is considered satisfactory under conditions involving brood and high infestation levels. No significant differences in colony strength or honey bee mortality were detected between treated and control colonies. Overall, Calistrip® appears to be a promising new "green" product for the control of *Varroa destructor*.

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SWEET AND SMART: HOW HONEY BEES ENRICH THEIR OWN FOOD

Petra Urajová[1], Václav Krištůfek[2], Alena Krejčí[3,4]

[1]Centre Algatech, Institute of Microbiology, Czech Academy of Sciences, Třeboň, Czechia
[2]Biology Centre, Institute of Soil Biology and Biochemistry, Czech Academy of Sciences, České Budějovice, Czechia
[3]University of South Bohemia, Faculty of Science, České Budějovice, Czechia
[4]Biology Centre, Institute of Entomology, Czech Academy of Sciences, České Budějovice, Czechia

Abstract

The natural phenolic compound p-coumaric acid supports honey bee health by enhancing detoxification, immunity and longevity. It also possesses antioxidant, anti-inflammatory and anticancer properties relevant to human health. While commonly detected in honey and pollen, it is absent from nectar and sucrose-based supplements typically used in beekeeping. Concerns have been raised whether supplemental saccharide feeding deprives bees of this essential phytochemical. Moreover, the origin of p-coumaric acid in honey remained an enigma. In this study, we quantified *p*-coumaric acid in various bee-derived products and in supplementary sucrose syrup before and after feeding to bees, using HPLC-HRMS analysis. Although fresh sucrose syrup contained negligible amounts of *p*-coumaric acid, syrup stored in capped combs exhibited levels comparable to or higher than those in honey. We identified propolis in combs as the primary source of *p*-coumaric acid, diffusing into both honey and sucrose syrup during storage. Our findings demonstrate that supplemental feeding with sucrose syrup does not diminish the bees' access to this key phytochemical and underscore the importance of a propolis rich environment in bee health management.

HONEY BEE CONTRIBUTION TO THE TRANSMISSION OF VIRUSES THROUGH FLOWERS

P 290

Algirdas Valys[1], Živilė Strazdaitė-Žielienė[1], Vilija Lapinskaitė[1], Saulius Serva[2], Elena Servienė[1]

[1]State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania
[2]Vilnius University Life Sciences Center, Vilnius, Lithuania

Abstract

Pathogenic RNA viruses play a key role in honey bee health at the individual and colony level. Spreading of honey bee viruses in natural and agricultural environments is multifaceted, occurring both vertically and horizontally. One possible, yet so far scarcely investigated route of inter- and intraspecies viral transmission is by flowers acting as viral exchange platforms.

During this study, the presence of seven honey bee-infecting viral species was examined using endpoint RT-PCR and sequencing, while the viral load in bees was assessed by qRT-PCR. *Apis mellifera* worker bee samples were collected in apiaries during the flowering periods of dandelions (*Taraxacum officinale*) and linden trees (*Tilia cordata*), along with flower samples of those plants growing near the same apiaries during a two-year period. Viral particles were concentrated from flowers utilising a centrifugation and filtration-based approach. Additionally, samples of deceased overwintering *A. mellifera* were investigated for the presence of viruses to assert a greater understanding of viral spread in the country.

Sacbrood virus, black queen cell virus, and deformed wing virus were found to be the most prevalent amongst all samples – both insect and floral. Tested plant species differed in their variety of harboured viral species, with some of the differences being explained by viral load measurements in corresponding honey bee samples. Phylogenetic analysis of amplified viral sequences provided results either affirming or negating the importance of managed *A. mellifera* being the chief contributor of deposited viruses on investigated flowers, depending on the viral species.

The results of this study assist in furthering fundamental understanding of insect virus spread through shared floral resources.

This study has received funding from the Research Council of Lithuania (LMTLT), agreement No. S-MIP-24-55.

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CHALLENGES IN CONTROL OF VETERINARY DRUG RESIDUES IN HONEY

Ivana Varenina[1], Nina Bilandžić[1], Jelena Kaurinović[1], Božica S. Kolanović[1], Andreia Freitas[2], Ines Varga[1]

[1]Croatian Veterinary Institute, Zagreb, Croatia
[2]Instituto Nacional de Investigação Agrária e Veterinária, IP (INIAV), Vila do Conde, Portugal

Abstract

The importance of bee colonies for a sustainable ecosystem is indisputable, while honey is one of the most nutritious foods produced by animals, providing significant health benefits.

For the prevention and treatment of bee diseases, various antimicrobial substances are used, including streptomycin, sulphonamides, lincomycin, erythromycin, tetracyclines, fluoroquinolones, and other banned substances. Depending on the drug and dose, residues can persist for up to ten months and may cause allergic reactions or toxic effects on consumer health.

To ensure the high quality of honey and bee products, it is necessary to comply with European Regulation (EU) 2017/625 regarding the control of certain substances and residues in live animals, animal products, and animal feed. The Croatian national monitoring programme includes control of honey for the presence of banned substances such as chloramphenicol, nitrofurans, and dapsone, as well as antibiotics authorised for use in other animals, including aminoglycosides, macrolides, quinolones, tetracyclines, and sulphonamides.

Due to the varying chemical properties of antimicrobial substances and the complexity of the honey matrix and its chemical interactions with residues, the sample preparation procedure is adapted for each group of antibiotics. Extraction procedures involve different chemical methods: chloramphenicol requires specific Molecularly Imprinted Polymers (MIP); nitrofurans require hydrolysis and derivatisation of the metabolites present; aminoglycosides, due to their high polarity, require the use of weak anion exchange SPE columns; while for other antibiotics, a multimethod is used with the application of QUECHERS salts, allowing high sample throughput and reduced solvent use. For precise detection and high selectivity of antimicrobial residues, liquid chromatography coupled with mass spectrometry (UHPLC-MS/MS) is used as the analytical technique.

VIRAL MODULATION OF THE GLUTAMATE-GLUTAMINE CYCLE: A MOLECULAR MECHANISM FOR COGNITIVE IMPAIRMENT IN DWV-A INFECTED HONEY BEES

P 292

Marisol Vargas[1], Felipe Becerra[1], Diego Silva[1,2], Danthe Villa[1], Yazmin Fuentes[1], Carolina Gomez[2]

[1]University of Concepcion, Chillan, Chile
[2]University of Oviedo, Oviedo, Spain

Abstract

Deformed Wing Virus (DWV-A) is a key pathogen in the global decline of *Apis mellifera*. This virus causes a reduction in olfactory reception and sensitivity in honey bees with high viral loads, leading to behavioral alterations. Although its tropism for the central nervous system is well-documented, the molecular mechanisms underlying functional impairment remain under investigation. This study explores how viral replication in the brain and antennae alters the glutamatergic system, the primary excitatory neurotransmitter system in insects, and how this dysregulation impacts behavioral responses. We utilized worker bees experimentally inoculated with DWV-A to evaluate their olfactory discrimination capacity using a Y-tube olfactometer against floral volatiles (blueberry, cherry, and cherry with 1,4-cineole). Subsequently, through qPCR and correlation analysis, we quantified the expression of eight genes critical for brain glutamatergic signaling and homeostasis. Our results demonstrate that increasing viral loads correlate negatively with the individuals' choice capacity, without behavioral differences between the stimuli evaluated. At the molecular level, correlation analysis revealed a distinct transcriptional pattern linked to viral load; we observed a significant upregulation of glial support genes, specifically GlnS (Glutamine Synthetase) and Eaat-2 (Excitatory Amino Acid Transporter 2). This viral-load-dependent response suggests that DWV-A induces a state of metabolic stress and neurotoxic environment, altering the glutamate recycling cycle. In conclusion, high DWV-A viral load in head of honey bees alters the expression of neurochemical genes, and a reduction of olfactory sensitivity, which could causes problems with pollination activities.

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'RAPID BROOD DECAPPING' TO ASSESS THE RECAPPING BEHAVIOUR IN HONEY BEE COLONIES

Adrien von Virag[1], Hannah Bratschi[1], Victor Rossier[1], Markus Neuditschko[2], Vincent Dietemann[1,3], Benjamin Dainat[1]

[1]Swiss Bee Research Centre, Agroscope, Bern, Switzerland
[2]Animal GenoPhenomics, Agroscope, Posieux, Switzerland
[3]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

Abstract

Recapping is the ability of honey bee workers to uncap and recap brood cells infested with *Varroa destructor* mites. This behaviour has recently attracted attention as a possible selection trait in breeding programs for *V. destructor* resistance due to its high expression rate in honey bee populations naturally resistant to the mite. Assessing the recapping ability of colonies is however time-consuming because each cap must be disconnected from the cell walls using tweezers so that its underside can be examined under a binocular microscope to check the integrity of the larval silk layer. Recently, the 'rapid brood decapping' method, which uses wax strips, was developed to efficiently diagnose *Tropilaelaps mercedesae* infestations. Here, we tested whether this method could also be used to assess the recapping ability of colonies. Wax strip brands were compared to select the most effective and practical. The reliability of the method was compared to that of cell uncapping with tweezers. We also tested the method's efficiency under various conditions, such as on combs of different ages and shapes, and at different ambient temperatures. Additionally, the fate of the uncapped brood was investigated to evaluate the side effects of the method. We present the pros and cons of the 'rapid brood decapping' method for investigating the biological basis of the recapping behaviour and selecting colonies for breeding.

ARTIFICIAL LIGHT AT NIGHT AS A CHRONIC STRESSOR IN HONEY BEES: EFFECTS ON SURVIVAL AND COGNITIVE BIAS

P 294

Julia J. Wincenciak, Karolina Kuszewska

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie, Kraków, Poland

Abstract

Artificial light at night (ALAN) is an increasingly common feature of human-altered environments and is now regarded as an important factor disrupting natural circadian light–dark rhythms. Although its impact on vertebrate physiology and behavior has been extensively studied, relatively little is known about its long-term effects on invertebrates, particularly with respect to lifespan and cognitive processes. The present study aimed to evaluate how different light conditions influence survival, learning performance, and cognitive bias in worker honey bees (*Apis mellifera*).

Forager workers derived from four independent colonies were subjected to five distinct light regimes, ranging from continuous illumination (L:D 100:0), through intermediate cycles (L:D 75:25, 50:50, 25:75), to complete darkness (L:D 0:100). Part of the cohort was used to determine lifespan under controlled cage conditions, while the remaining individuals were assessed for learning efficiency and responses indicative of cognitive bias.

Our findings demonstrate that prolonged exposure to elevated light levels (L:D 100:0 and 75:25) has detrimental effects on both survival and cognitive functioning. Bees maintained under these conditions showed shortened lifespan, impaired learning ability, and a greater tendency toward negative cognitive bias compared to those kept in darkness (L:D 0:100) or under more balanced light–dark cycles (L:D 25:75 and 50:50). These results support the hypothesis that ALAN functions as a chronic environmental stressor, potentially compromising individual performance and, consequently, colony-level stability.

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DETERMINING THE EFFECTS OF APPLICATION TEMPERATURE ON OXALIC ACID APPLIED VIA VAPORIZATION FOR THE CONTROL OF *VARROA DESTRUCTOR*

Marie T. Yanchak, Cameron J. Jack

University of Florida, Gainesville, United States

Abstract

Oxalic acid (OA) is a widely used miticide for controlling the ectoparasite *Varroa destructor* in honey bee (*Apis mellifera*) colonies, yet limited guidance exists regarding optimal vaporization temperature and application duration. This study evaluated (i) the time required to fully vaporize 4 g of OA at different temperatures and (ii) the effects of vaporization temperature on colony strength and *V. destructor* control under field conditions. In Experiment 1, repeated vaporization trials demonstrated a temperature-dependent reduction in vaporization time, requiring approximately 161 s at 180°C, 107 s at 230°C, and 37 s at 280°C. In Experiment 2, 48 colonies were assigned to one of four groups: OA vaporization treatments at 180°C, 230°C, or 280°C, or to an untreated control, with four applications at 7-day intervals. Colony strength parameters were unaffected by treatment temperature, although temporal declines were observed across all groups. *Varroa destructor* infestation measured by alcohol washes showed a significant temperature x time interaction, with colonies treated at 230°C exhibiting a significant reduction in mite infestation relative to pretreatment levels, while untreated colonies showed increased infestation. *Varroa destructor* infestation measured by brood examination was not significantly affected by any treatment. Repeated OA applications increased 72-hour mite fall during early treatment periods across all OA-treated groups. Despite OA's known thermal decomposition point, vaporization at 230°C did not reduce treatment efficacy, suggesting that decomposition was minimal or offset by dose and exposure duration.

EVALUATION OF SEVERAL OXALIC ACID BASED TREATMENTS FOR *VARROA DESTRUCTOR* (ACARI:MESOSTIGMATA) INFESTATION IN MANAGED *APIS MELLIFERA* COLONIES

P 296

Marie T. Yanchak, Kaylin G. Kleckner, Cameron J. Jack

University of Florida, Gainesville, United States

Abstract

Managed honey bee (*Apis mellifera*) colonies in the United States continue to experience high losses, largely driven by the ectoparasitic mite *Varroa destructor*. Beekeepers need new and effective treatments to rotate in integrated pest management programs. Api-Bioxal™ RTU Beehive Solution (OA RTU) is a glycerin-based oxalic acid (OA) formulation designed to extend OA exposure within colonies that was recently registered and approved for use in the United States. This study evaluated OA RTU as a new ready-to-use OA trickle for the control of *V. destructor* infestations and investigated the impacts of OA application methods on colony health. Fifty-five colonies were treated with OA RTU, OA vaporization, OA sugar syrup trickle, Apivar®, or an untreated control. Mite infestation was assessed via sticky board traps, alcohol washes, and a novel rapid brood uncapping technique to quickly assess *V. destructor* infestation of immature honey bees. All treatments significantly reduced brood mite infestation, whereas other OA methods and Apivar® did not significantly affect brood-level infestation. Adult bee mite infestation declined over time across treated colonies, and all miticides increased 72-hour mite fall at different timepoints throughout the study, relative to untreated colonies. None of the treatments evaluated adversely affected colony strength as measured through visual estimation of bee population, brood area, and honey and pollen storage area. These findings demonstrate that extended-exposure OA formulations may provide additional benefits for managing *V. destructor*, particularly within brood cells. This work offers practical guidance for optimizing OA-based *V. destructor* treatments for beekeepers to implement into their larger integrated *Varroa* management programs.

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COMPARATIVE EVALUATION OF EXTENDED-RELEASE OXALIC ACID TREATMENTS FOR *VARROA DESTRUCTOR* CONTROL IN HONEY BEE (*APIS MELLIFERA*) COLONIES

Marie Yanchak[1] and Cameron J. Jack[1]

[1]Entomology and Nematology Department, University of Florida

Abstract

The ectoparasitic mite *Varroa destructor* remains the primary driver of colony losses in managed honey bee (*Apis mellifera*) populations, necessitating effective and practical control strategies. Oxalic acid (OA) is widely used due to its efficacy and lack of documented resistance; however, commonly used application methods (vaporization and trickle) require repeated treatments and may be labor-intensive or inconsistent under varying environmental conditions. Extended-release OA offer a promising alternative, but limitations related to cost and performance variability persist. This study evaluated the efficacy of a novel extended-release formulation, compared to the currently available extended-release product and an untreated control. Forty colonies were equalized for strength and initial *V. destructor* infestation and assigned to one of four treatment groups (n = 10). Treatments were applied according to manufacturer guidelines. *Varroa destructor* infestation was quantified using alcohol washes of adult bees, brood examinations, and 72-hour sticky board mite fall counts. Measurements were conducted pre-treatment and at 3, 6, and 9 weeks post-application. Colony strength parameters, including colony weight and adult bee population, were assessed concurrently. This research provides critical data on the performance of a novel OA delivery system and its potential to improve integrated pest management strategies for beekeepers. Findings will help inform the adoption of more efficient, accessible, and effective *V. destructor* control tools, contributing to the sustainability of apiculture.

ASSESSING ENVIRONMENTAL HEALTH IN TRENTO THROUGH APOIDEA DIVERSITY AND RESIDUE ANALYSIS OF PESTICIDES AND HEAVY METALS IN HONEY BEE POLLEN

P 298

Marzia Zagallo[1], Sergio Angeli[1], Maria V. Zucchelli[2]

[1]Free University of Bozen-Bolzano, Faculty of Agricultural, Environmental and Food Sciences, Italy, Bozen, Italy
[2]Muse - Museo delle Scienze di Trento, Trento, Italy

Abstract

Pollinators are essential for plant reproduction, biodiversity maintenance, ecosystems health and most crop plants rely on them. However, pollinators face many threats due to habitat loss and fragmentation, climate change and increasing pesticides exposure. The project aligns with the objectives of the EU Biodiversity Strategy for 2030 and the EU Pollinators Initiative, addressing the urgent need to reverse pollinator decline. This study investigates the correlation between the presence of the key pollinators Apoidea (Hymenoptera) and environmental health conditions in Trento municipality (Italy). In Trento around 1000 Apoidea individuals, representing six families (Apidae, Halictidae, Andrenidae, Megachilidae, Colletidae and Melittidae) and genera including *Ceratina, Halictus, Lasioglossum, Osmia, Megachile, Rhodanthidium, Anthidium, Anthophora*, and *Bombus* (e.g. *B. pascuorum, B. terrestris, B. argillaceus, B. vestalis*), have been collected so far using pan traps and net transects. Furthermore, visual transects allowed the observation of 1,306 individuals, which were classified into size-based categories defined in this study: 45% small bees, 14% large bees, and 18% bumblebees (*Bombus* spp.). Additionally, pollen samples from local bees were collected and analysed to detect agrochemicals and heavy metal residues. A total of 32 pesticides active ingredients were found, including 5 insecticides (chlorantraniliprole, acetamiprid, carbaryl, etofenprox, pirimicarb), 24 fungicides (e.g. folpet, captan, dithiocarbamates, tolylfluanid, fludioxonil), and 3 herbicides (glyphosate, metamitron, pendimethalin). This research contributes to a deeper understanding of environmental health conditions and assess exposure to agrochemicals and other pollutants.

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ACCESS TO SHARED FOOD RESOURCES IS KEY TO THE DIVISION OF LABOUR IN HONEY BEES

Virginia Zanni[1], Davide Frizzera[1], Elisa Seffin[1], Agnese Giacometti[1], Alessia Losinno[2], Giacomo Ortis[2,3], Desiderato Annoscia[1], Francesco Nazzi[1]

[1]Department of Agricultural, Food, Environmental and Animal Sciences (Di4A), University of Udine, Udine, Italy
[2]Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padua, Padua, Italy
[3]Department of Biotechnology, University of Verona, Verona, Italy

Abstract

Division of labor underlies the extraordinary success of eusocial insects and represent a milestone of colony organization in honey bees (*Apis mellifera*, L.). Age-related division of labor is specific of workers and implies that young bees are involved in nursing while older individuals collect food to meet the colony demands. The proximate causes of temporal polyethism have been investigated but the exact role of endogenous and exogenous factors hasn't yet been defined.

To reconstruct the chain of factors affecting temporal polyethism and disentangle the role of endogenous and exogenous stimuli, we excluded newly emerged bees from the social stimuli, under different diets, for different times. We compared their behavior and the expression of some genetic markers with that of coetaneous bees exposed all the time to the social environment.

Our study highlighted a key role of endogenous factors in determining the acceptance of workers in the brood area and their access to a nutrient diet that in turn influences their aptitude to nursing. The displacement of the aging bees from the brood area by their younger nestmates, is likely at the root of the transition to foraging as old workers can only rely on a poor diet and are obliged to perform riskier outside activities. The emerging picture is consistent with a predominant influence of endogenous factors on nursing, while spatial factors likely play a major role in determining the transition to foraging.

Our results support the view that temporal and reproductive division of labour are dictated by nutrition as affected by dominance interactions among nestmates. This model is consistent with an evolutionary trajectory implying a double decoupling, disjoining three functions primitively performed in cycles by the same solitary bee: oviposition, nursing and foraging.

NEST TEMPERATURE OF BEE COLONIES – USEFUL INFORMATION FOR BEEKEEPERS AND RESEARCHERS

P 300

Lucija Žvokelj[1], Miha Finžgar[2], Samo Simončič[2], Maj Smerkol[3], Metka Pislak Ocepek[4], Barbara Hočevar[4], Monika Kozar[4], Anton Gradišek[3]

[1]Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia
[2]Senso4s d.o.o., Trzin, Slovenia
[3]Jožef Stefan Institute, Ljubljana, Slovenia
[4]Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia

Abstract

Social thermoregulation is essential for honey bee colonies to survive the winter. Monitoring nest temperature during winter may provide indicators of the health and survival status of honey bee colonies. We hypothesised that nest temperature, recorded at 10-minute intervals throughout the season, can also indicate the health status of bee colonies, which will be clinically observed later. From late summer 2025, twenty colonies in four different climate locations were monitored with a smart system that continuously records beehive mass, temperature, humidity, air pressure, and VOC concentration inside and outside the hive. Colonies were continuously evaluated for colony strength, food reserves, and varroa infestation levels. Temperature sensors were installed immediately below the upper frame and on the lower frame of the hive. We ensured that the comb with sensors was in the middle of the nest, which was easier to achieve during the season; however, during winter, we succeeded in recording from the middle of the nest in eight colonies in different locations throughout the winter until spring. Thermal amplitude proved to be a useful tool for detecting colony winter survival. This can help beekeepers remove and clean hives before the active season for bees. Colonies with less ability to maintain optimal brood temperatures are also more prone to brood disease, and our work contributes to a more detailed understanding of the relationship between pathogens, pests, and brood nest temperature.

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SAFETY AND EFFICACY OF DIFFERENT OXALIC ACID TREATMENTS

Lucija Žvokelj[1], Janko Božič[2], Gordana Glavan[2], Barbara Hočevar[1], Monika Kozar[1], Rene Rus[1], Metka Pislak Ocepek[1], Suzana Skerbiš[1]

[1]Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia
[2]Biotechnical Faculty, Ljubljana, Slovenia

Abstract

Varroa destructor remains one of the main threats to honeybees. Researchers, together with beekeepers, are making substantial efforts to improve mechanical and chemical methods to control *Varroa* mites. Oxalic acid (OA) is widely used as an acaricide, and concentrations of up to 4.6% are generally well tolerated by adult bees. When brood is present, application is typically combined with queen caging or administered using newly approved products that release OA slowly via impregnated strips. However, relatively little is known about the physiological effects of OA on honeybees. To address this gap, we investigated the influence of two different varroa control strategies on: i) varroa infestation level, ii) colony strength before wintering and in the following spring, and iii) acetylcholinesterase (AChE) and glutathione S-transferase (GST) activity in nurse bees. The study was conducted on 30 colonies across two apiaries. In the first group, we caged queens and used a single OA spray application during the broodless period; in the second group, colonies retained brood throughout the summer and were treated with OA strips. House bees were sampled i) before caging the queen, ii) just before applying OA treatment, iii) 48 hours after treatment, and iv) 30 days after treatment to measure AChE and GST activity levels. Our results show that OA slow-release strips were less effective at reducing varroa infestation compared to queen caging with subsequent OA spraying. Additionally, colonies treated with strips exhibited elevated GST activity in colonies treated with strips, indicating a stronger stress response relative to the queen caging group. Based on these findings, long term treatment with high doses of oxalic acid dihydrate should be applied with caution.

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