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Factors determining variation in colour morph frequencies in invasive *Harmonia axyridis* populations

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Honek A., Brown P.M.J., Martinkova Z., Skuhrovec J., Brabec M., Burgio G., et al. (2020). Factors determining variation in colour morph frequencies in invasive *Harmonia axyridis* populations. *BIOLOGICAL INVASIONS*, 22(6), 2049-2062 [10.1007/s10530-020-02238-0].

Availability:

This version is available at: <https://hdl.handle.net/11585/766923> since: 2020-07-23

Published:

DOI: <http://doi.org/10.1007/s10530-020-02238-0>

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(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Evans E. W., Fournier M., Grez A. A., Kulfan J., Lami F., Lucas E., Lumbierres B., Masetti A., Mogilevich T., Orlova-Bienkowskaja M., Phillips W. M., Pons X., Strobach J., Viglasova S., Zach P., Zaviezo T. *“Factors determining variation in colour morph frequencies in invasive Harmonia axyridis populations”*

which has been published in final form in BIOLOGICAL INVASIONS 2020, vol. 22(6), p. 2049-2062

The final published version is available online at:

<https://doi.org/10.1007/s10530-020-02238-0>

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2 Factors determining variation in colour morph frequencies 3 in invasive *Harmonia axyridis* populations

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10 Received: 21 May 2019 / Accepted: 27 February 2020
11 © Springer Nature Switzerland AG 2020

12 **Abstract** The Harlequin ladybird *Harmonia axyr-*
13 *idis* Pallas, native to eastern Asia, is an invasive, non-
14 native species that has recently achieved an almost
15 worldwide distribution. A conspicuous feature of this
16 species is colour polymorphism of the elytra. In its
17 native area, the populations consist of a recessive non-
18 melanic morph, several dominant melanic morphs and
19 small numbers of other (rare) morphs. The morph
20 proportions in native populations have been inten-
21 sively studied and vary with geographic area, climate

and time. In contrast, colour polymorphism in invaded 22
regions has been little studied. We examine and try to 23
account for the morph frequencies observed across the 24
different invaded regions. In America, monomorphic 25
populations consist of the non-melanic morphs while 26
European populations contain also melanic morphs. In 27
particular geographic areas of Europe, the average 28
percentage of the non-melanic morphs varied between 29
78 and 99%. It was highest in the lowlands of northern 30
Italy and central and northern Europe and decreased in 31
the Alps and western (Spain, UK) and eastern 32
(southeast Russia) margins of the recently invaded 33
area. In central Europe the frequency of the non- 34
melanic morphs decreased over the course of the year 35

A1 **Electronic supplementary material** The online version of
A2 this article (<https://doi.org/10.1007/s10530-020-02238-0>) con-
A3 tains supplementary material, which is available to authorized
users.

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36 but increased over the years from 2010 to 2018. The
 37 local differences might thus arise through gradual
 38 change of the morph composition of the founder
 39 invasive, non-native population. However, the varia-
 40 tion in non-melanic morph frequency was not corre-
 41 lated with climatic characteristics that might affect
 42 coccinellid polymorphism. The observed rate of
 43 change in morph proportions in our data was too
 44 small to explain the diversification of what was
 45 supposedly a uniform invasive, non-native population
 46 at the point of introduction.

47 **Keywords** Polymorphism · Alien species ·
 48 Distribution · Variation · Climate · Selection

49 Introduction

50 The Harlequin ladybird *Harmonia axyridis* Pallas
 51 (Coleoptera: Coccinellidae) is native to the east
 52 Palearctic and Oriental regions (Kovar 2007; Orlova-
 53 Bienkowskaja et al. 2015). Its recent spread into
 54 several continents where it is non-native has been well
 55 studied (Roy et al. 2016). In its native range, *H.*
 56 *axyridis* is an abundant and efficient predator of aphids
 57 (Kuznetsov 1975). Due to its qualities as a biological
 58 control agent, many attempts were made to introduce

H. axyridis in intensive agriculture areas outside of its
 59 native range. Several early introduction attempts of *H.*
 60 *axyridis* in Europe (Kuznetsov 1987; Coutanceau
 61 2006) and North America (McClure 1987) were
 62 unsuccessful. In contrast, later unintended introduc-
 63 tions resulted in the spread of the species in North
 64 America from the late 1980s (Chapin and Brou 1991;
 65 Tedders and Schaefer 1994; LaMana and Miller 1996)
 66 and subsequently in South America (Martins et al.
 67 2009; Grez et al. 2010), Europe (Adriaens et al. 2003;
 68 Cuppen et al. 2004; Brown et al. 2008), Africa (Stals
 69 2010; Nedvěd and Háva 2016), western Asia (Biran-
 70 vand et al. 2019) and New Zealand ([https://www.mpi.
 71 govt.nz/document-vault/12261](https://www.mpi.govt.nz/document-vault/12261)). Thus, *H. axyridis* has
 72 now spread to all continents except Antarctica (Ca-
 73 macho-Cervantes et al. 2017). The invasion into Eur-
 74 ope was evidently derived from biological control
 75 introductions mixing with an invasive population from
 76 eastern North America (Lombaert et al. 2010).
 77

In recently colonized areas, *H. axyridis* is regarded
 78 as an efficient aphid natural enemy (Riddick 2017),
 79 but also an unwelcome competitor and predator of
 80 other members of the aphidophagous guild (Brown
 81 et al. 2015; Kenis et al. 2017; Masetti et al. 2018;
 82 Zaviezo et al. 2019). The abundance and distribution
 83 of *H. axyridis* has increased dramatically whilst the
 84 numbers of several native species have decreased,
 85

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86 with *H. axyridis* implicated in the declines. This has
87 helped increase interest in this species, with more than
88 1300 references since 1990 published on Web of
89 Science concerning the life cycle, predation beha-
90 viour, distribution, and other elements of the ecology
91 and genetics of *H. axyridis* (Roy et al. 2016).

92 Among the most intensively studied aspects of *H.*
93 *axyridis*' biology is its conspicuous colour polymor-
94 phism, and the genetics, distribution, ecological and
95 evolutionary factors influencing it. Interest in this
96 issue has a long history. Patterns of variation of *H.*
97 *axyridis*, its genetic determination and factors that
98 determine morph distribution have been studied since
99 the 1920s (Dobzhansky 1924; Komai 1956; Timofe-
100 eff-Ressovsky and Svirezhev 1967; Komai and Chino
101 1969) and continue today (Seo et al. 2007; Wang et al.
102 2009, 2011).

103 In its native area the species has over 200 described
104 colour morphs, grouped into 15 classes (Tan and Li
105 1934; Hosino 1940; Tan 1946). The individuals are
106 classified according to the colour pattern of their
107 elytra. Four major morph groups, light-coloured non-
108 melanic *succinea* and dark-coloured melanic *axyridis*,
109 *spectabilis* and *conspicua* (Fig. 1) are among the most
110 frequent in the species' native area, where there is
111 extensive variation in morph proportions among local
112 populations (Gautier et al. 2019). *Succinea* morphs
113 have elytra with yellow to red ground colour and on
114 each elytron up to nine black spots organised in four
115 transversal rows. Pale coloration of the dorsal side
116 leads these morphs to be referred to as the "non-
117 melanic morphs". The ground colour of the three other
118 morphs mentioned is black. These morphs are distin-
119 guished by the number of red spots, i.e. one (*conspicua*
120 morph), two (*spectabilis* morph) or six (*axyridis*
121 morph) on each elytron (see Gautier et al. 2019).
122 The mostly black dorsal side leads these morphs to be
123 classified and further referred to as "melanic morphs".
124 Elytral colour pattern in *H. axyridis* is determined by a
125 multiple-allelic series, with melanic morphs

126 dominating non-melanic morphs in the order of 126
127 dominance *conspicua* > *spectabilis* > *axyridis* > *suc* 127
128 *cinea* (Tan and Li 1934; Tan 1946). In the native (and 128
129 to an extent, introduced) ranges, there are a number of 129
130 other morphs found in low proportions, the genetics of 130
131 which have not been well studied (e.g. Hosino 1940; 131
132 Komai 1956; for a review see Sloggett and Honek 132
133 2012). Morph identity is determined by genetic 133
134 factors. Specifically the morph is determined by 134
135 mosaic dominance, which itself is shaped by both 135
136 the dominance relationships between colour morph 136
137 alleles and the expression of a transcription factor 137
138 (pannier); this determines the formation of melanic 138
139 elements on the elytra (Gautier et al. 2019). A large 139
140 inversion in the cis-regulatory regions of this tran- 140
141 scription factor exists between colour morphs and is 141
142 thought to underly the maintenance of so much 142
143 variation within populations (Gautier et al. 2019). 143
144 An additional factor of phenotypic variation within a 144
145 morph is temperature during pre-imaginal develop- 145
146 ment, which modifies the degree of melanisation. In 146
147 the non-melanic morphs low temperature increases the 147
148 size and number of black spots, while its effect on the 148
149 size of red spots in melanic morphs is below the limit 149
150 of resolution (Michie et al. 2010). 150

151 The morph frequencies in the native area of *H.* 151
152 *axyridis* differ among three geographic regions: the 152
153 insular region (Japan) is characterized by a mixture of 153
154 non-melanic and melanic morphs (Komai et al. 1950; 154
155 Komai 1956; Noriyuki and Osawa 2015), the east 155
156 continental region (China, Korea and the Russian Far 156
157 East) is characterized by a high frequency of the non- 157
158 melanic morphs, while the central Siberian region is 158
159 dominated by the *axyridis* morph (Dobzhansky 1924; 159
160 Komai et al. 1950; Komai and Chino 1969; Kholin 160
161 1988, 1990; Vorontsov and Blehman 2001; Zakharov 161
162 and Blehman 2001; Korsun 2004; Blehman 2009). 162
163 This coarse pattern of morph distribution slightly 163
164 varies among localities and in time. Likely causes of 164
165 this variation are differences in local climate (Purse 165
166 et al. 2015) and a complex of biotic factors that 166
167 manifest in variation of morph frequencies among host 167
168 plants (Komai and Chino 1969). Temporal variation in 168
169 morph frequency was observed several times (Komai 169
170 et al. 1950; Komai and Chino 1969). Seasonal trends 170
171 include an increase in the proportion of non-melanic 171
172 morphs in the growing season, and vice versa during 172
173 the winter (Osawa and Nishida 1992; Wang et al. 173
174 2009). While in the short term (a few years) the 174

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175 difference was not significant (Kholin 1990), after
176 many years there were significant changes in morph
177 frequency (Komai et al. 1950; Komai and Chino 1969;
178 Bogdanov and Gagalchij 1986).

179 Polymorphism in native populations is balanced by
180 seasonal variation in mating preferences (Osawa and
181 Nishida 1992; Ueno et al. 1998): in populations in
182 Japan (Kyoto), females breeding in the spring pre-
183 ferred mating with non-melanic males and this prefer-
184 ence led to a c. 4% increase in the proportion of the
185 non-melanic morphs in the summer generation
186 (Osawa and Nishida 1992). In summer, females
187 showed no preference for males of a particular morph
188 and consequently the frequency of melanic morph
189 progeny in the autumn generation was relatively
190 higher (Osawa and Nishida 1992). Seasonal changes
191 in mating preferences also influenced morph frequen-
192 cies in east continental Asia (Beijing, China) where
193 the percentage of non-melanic morphs increased over
194 the growing season to c. 85% because of high mating
195 activity of the non-melanic morphs. This decreased
196 during the winter to c. 50% (Wang et al. 2009).

197 In contrast to the well studied variation of *H.*
198 *axyridis* colour polymorphism in its native range, the
199 pattern of variation in morph frequency in recently
200 colonized areas has been studied only to a limited

201 extent (Adriaens et al. 2008; Burgio et al. 2008; Pons
202 et al. 2015; Jovicic et al. 2016). Thus here colour
203 polymorphism in *H. axyridis* populations of recently
204 invaded areas is investigated. Increased melanisation
205 may confer fitness advantages in particular climatic
206 conditions and/or at some times of year, potentially
207 leading to differences in the relative survival of *H.*
208 *axyridis* morphs. For example, heavily melanised
209 morphs may have a thermal advantage in cooler
210 conditions, since they are more able to absorb thermal
211 radiation (Brakefield and Willmer 1985). The differ-
212 ing phenology of host plants may have an affect on
213 phenotypic variation in *H. axyridis* morphs. For
214 example, a 2 weeks difference was observed in leafing
215 between *Acer* and *Tilia* in the Czech Republic (Honek
216 et al. 2019). This differing host plant phenology,
217 causing variation in micro-habitats, could potentially
218 lead to the differential success of *H. axyridis* morphs
219 between two host plants. Overall, the study of
220 variation in frequency of colour morphs in invasive,
221 non-native populations is important since (pheno-
222 typic) plasticity is a factor that may confer an
223 advantage to an invasive species (Briolat et al. 2019).

224 Data on *H. axyridis* morphs were collected from the
225 invaded range to: (1) investigate macro-geographic
226 variation and seasonal and annual trends in morph

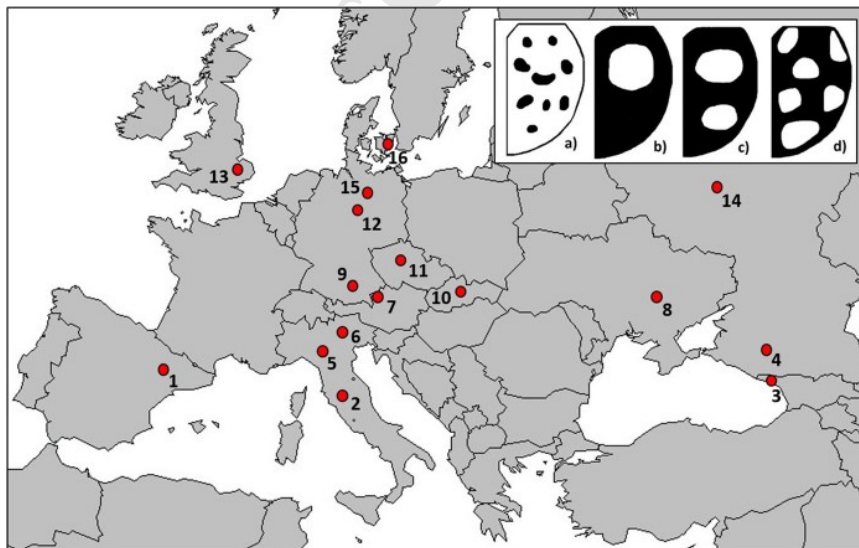


Fig. 1 The distribution of sampling localities of *H. axyridis* in Europe. The points indicate centres of particular areas where populations included in this study were collected, the areas are labelled serial numbers in the same order as in Table 1. 1 Spain, 2

Italy 1, 3 Georgia, 4 Russia 1, 5 Italy 2, 6 Italy 3, 7 Austria, 8 Ukraine, 9 Germany 1, 10 Slovakia, 11 Czech Republic, 12 Germany 2, 13 UK, 14 Russia 2, 15 Germany 3, 16 Denmark. Insert: morphs of *H. axyridis*: a—*succinea*, b—*conspicua*, c—*spectabilis*, d—*axyridis*

Table 1 The distribution of colour morphs on main hostplant types, from 2010–2018

Hostplant	Total	<i>succinea</i> N (%)	<i>conspicua</i> N (%)	<i>spectabilis</i> N (%)	<i>axyridis</i> N (%)	Σ melanic N (%)
Crop	82	74 (90.2)	0 (0.0)	8 (9.8)	0 (0.0)	8 (9.8)
Herb	1870	1669 (89.3)	29 (1.6)	168 (9.0)	3 (0.2)	200 (10.7)
Tree	23,619	21,026 (89.0)	537 (2.3)	2031 (8.6)	25 (0.1)	2594 (11.0)
Total	25,571	22,769 (89.5)	566 (1.3)	2207 (9.1)	28 (0.1)	2802 (10.5)

227 variation; (2) investigate micro-geographic and tem-
 228 poral variation in morph frequency in relation to host
 229 plant and temperature. For the first hypothesis data
 230 was collected from throughout the invaded range in
 231 Europe and America, whilst for the second hypothesis
 232 a more detailed dataset was available, collected from
 233 one part of the European range (Czech Republic).

234 **Materials and methods**

235 Localities

236 **AO4** Samples of invasive populations of *H. axyridis* adults
 237 were collected in 19 areas of North and South America
 238 and Europe (Table S1 in Supplementary Material,
 239 Fig. 2), between 2007 and 2018. The data, from
 240 sampling of coccinellid communities including *H.*
 241 *axyridis*, were mostly collected from small geographic
 242 areas determined by the different research pro-
 243 grammes of the participating authors. As a conse-
 244 quence, the intensively searched and investigated
 245 areas were surrounded by large unexplored areas.
 246 Although the geographic pattern of collection sites and
 247 ladybird data accumulated in this way is irregular, the
 248 large total area covered by this sampling is likely to

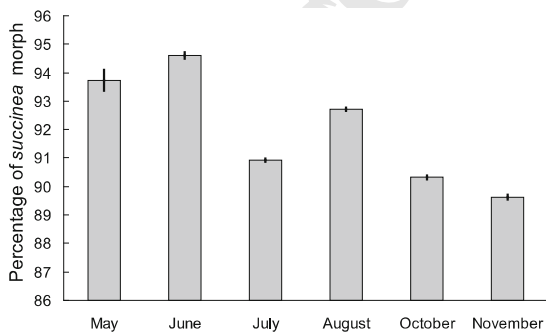


Fig. 2 Seasonal trend in percentage of *succinea* morph on trees. The figure shows mean ± 95% CI calculated using angular transformed data

provide a clear insight into the general patterns of geographic variation of colour polymorphism of *H. axyridis*.

Sampling procedure

Harmonia axyridis populations were collected from trees, low growing herbaceous vegetation and crop stands. The sampling was performed by sweeping with an entomology net or beating the branches above sampling trays, during daylight hours, on dry days with low winds. More than 20 people participated in these sampling activities at 19 sites in 14 countries. It was impractical to compare differences in their sampling efficiency, but all participants were skilled entomologists with relevant fieldwork experience. This ensured that the composition of samples corresponded to the composition of natural populations and that colour morphs were determined correctly. Data on populations assembled at overwintering sites (buildings and shelters) were also included in this study where available (Czech Republic, Italy 2, Slovakia and Spain).

Data analysis

Macro-geographic variation in morph frequency was tested using the data of all sampling sites and samples collected at particular geographic areas. The “areas” are clusters of sampling sites situated close to each other (within maximum tens of km apart). The geographic areas are denoted by the political name of the country and a serial number where more than one cluster was sampled within a country (Table S1 in Supplementary Material). To assess variability in the morph frequency a logistic regression framework was used. The prevalence of the non-melanic *succinea* morph (among all morphs present) was used as the response variable in our models. To assess variability of the *succinea* proportion among geographic

285 locations, a random country effect was used and fitted
286 to the resulting model as a logistic generalized
287 additive model (GAM) (Wood 2006). The geographic
288 trends were further investigated in a GAM logistic
289 model, allowing for a spatial trend with smooth
290 additive latitude and longitude components.

291 Before investigating macro-geographic variation of
292 morph frequency we needed to estimate the extent of
293 micro-geographic and temporal variation. To investi-
294 gate the factors of small-scale variation in morph
295 frequency, micro-geographic variation in morph fre-
296 quency and variation associated with host plants were
297 checked. Micro-geographic and temporal variation in
298 morph frequency was tested using the extensive data
299 of the Czech Republic. In this analysis, samples
300 of ≥ 5 individuals of *H. axyridis* were used. Micro-
301 geographic variation was investigated using data
302 collected in stands of *Tilia* spp. at seven sites located
303 along a 5 km longitudinal transect, between 50.0813N
304 14.2610E and 50.0936N 14.3331E. These data were
305 not biased by seasonal variation in morph frequency
306 because coccinellids were sampled at regular bi-
307 weekly intervals through the growing season (May to
308 October) of 2011–2016. Variation in morph frequency
309 among host plants was established using cumulative
310 data from trees (*Acer*, *Betula*, *Cerassus*, *Prunus*, *Salix*,
311 *Tilia*), herbs (low growing herbaceous vegetation,
312 *Artemisia*, *Tripleurospermum*, *Urtica*) and crops
313 (*Avena*, *Hordeum*, *Medicago*) sampled over the period
314 of 2010–2018. Seasonal variation in the frequency of
315 morphs was analysed using data collected on trees
316 (*Acer*, *Betula*, *Tilia*) in 2011–2018. The data were
317 sampled in each of the years in weekly (2017–2018) or
318 bi-weekly (2011–2016) intervals from May to Octo-
319 ber. Annual variation in morph frequency was tested
320 using cumulative data of all sampling sessions from
321 May to October 2011–2018.

322 Differences in morph frequencies between sam-
323 pling sites and host plants and between seasonal and
324 annual trends in morph proportions were tested using
325 ANOVA, with the frequency of the *succinea* morph as
326 the response variable and sampling site, host plant,
327 month or year as factors. As the test of normality of
328 distribution of morph percentage failed, in all analy-
329 ses, the Kruskal–Wallis one-way Analysis of Variance
330 on ranks was used. The trends in change of percentage
331 of the non-melanic morphs were tested using linear

332 regression, with the percentage of the *succinea* morphs
333 as the response variable and time (month, year) as the
334 explanatory variable. The frequency of recessive
335 *succinea* alleles was calculated using the Hardy–
336 Weinberg law as the square root of frequency of the
337 *succinea* morphs. The calculations were made using
338 the SigmaStat 3.5 software package (Systat Software
339 2006). Note that at best this provides only an
340 approximate estimate, reliant on the invasive popula-
341 tions being in Hardy–Weinberg equilibrium, which in
342 reality they are likely not to be.

343 The relationship between the frequency of the non-
344 melanic morphs and climate at the place of origin of
345 European populations was established using data of
346 meteorology stations situated as close as possible to
347 the centres of the geographic areas listed (Table S1 in
348 Supplementary Material), using areas where $N > 10$.
349 Climate data were obtained from the University of
350 Indiana (<https://webapp1.dlib.indiana.edu>). Data for
351 20-year averages of monthly temperatures were
352 available for all geographic areas. The regression of
353 the percentage of the non-melanic morphs (raw data
354 and arcsin transformed data) was based on mean
355 temperatures of particular months and mean temper-
356 atures of all combinations of two and three successive
357 month periods. The calculations were made using
358 SigmaStat 3.5 (Systat Software 2006).

359 Results

360 Micro-geographic variation

361 Micro-geographic variation in morph frequency
362 between closely positioned sampling sites of *Tilia*
363 ($N = 363$, $H = 10.998$, $df = 6$, $P = 0.088$) was not
364 significant (Table S2 in Supplementary Material).
365 There was no significant difference in the frequency of
366 morphs on particular hostplants, trees, herbs and crops
367 ($N = 860$, $H = 0.676$, $df = 2$, $P = 0.713$) (Table 1).
368 Also, no difference in the frequency of morphs was
369 found among stands of *Tilia*, *Acer* and *Betula*
370 ($N = 777$, $H = 1.404$, $df = 2$, $P = 0.496$) or when
371 host plants were ranked according to growth form, i.e.
372 low growing crop and herb vegetation vs. trees
373 ($N = 860$, $H = 0.665$, $df = 2$, $P = 0.415$) (data not
374 shown).

375 Temporal variation

376 Seasonal variation, i.e. in the percentage of the non-
 377 melanic morphs, in particular months from May to
 378 October in the Czech Republic (Table 2), significantly
 379 differed ($N = 761$, $H = 20.584$, $df = 5$, $P < 0.001$) and
 380 decreased from $90.9 \pm 1.45\%$ and $92.9 \pm 0.89\%$ in May
 381 and June to $88.8 \pm 0.75\%$ and $88.9 \pm 0.74\%$ in September
 382 and October. On trees, the frequency of light colour
 383 morph decreased significantly ($a = 2.831$, $b = -0.0347$,
 384 $R^2 = .0205$, $F_{1,760} = 15.961$, $P < 0.001$) (Fig. 2) over
 385 time.

386 Annual variation in the percentage of the non-melanic
 387 morphs increased significantly ($N = 784$, $H = 31.650$,
 388 $df = 8$, $P < 0.001$) from 2011 to 2018 ($a = -58.675$,
 389 $b = 0.0304$, $R^2 = 0.0348$, $F_{1,745} = 26.834$, $P < 0.001$)
 390 (Table 3). Calculated from these data, the frequency of
 391 the recessive *succinea* allele (mean = $95.8 \pm 0.36\%$)
 392 varied between 94.5% (in 2013) and 97.4% (2017)
 393 (Fig. 3). The mean absolute difference in frequency of
 394 the *succinea* allele between successive years was
 395 $0.6 \pm 0.22\%$ (c.i. 0.84%). The mean annual difference

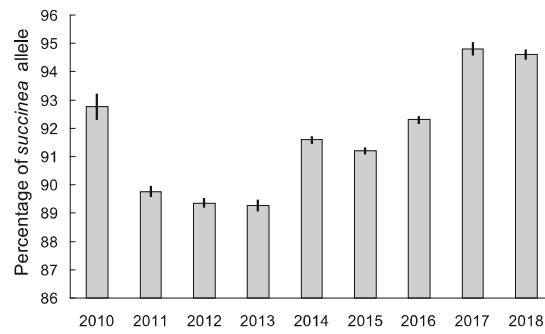


Fig. 3 Annual variation in frequency (%) of *succinea* allele in total annual samples of 2010–2018. Mean \pm 5% CI calculated using angular transformed data

calculated from the difference between minimum and maximum frequency of the *succinea* allele was 0.72%. 396 397

Macro-geographic variation 398

At a macro-geographical scale, the largest difference in colour polymorphism distribution of invasive, non-native *H. axyridis* populations is between America, 399 400 401

Table 2 The distribution of colour morphs of *H. axyridis* through the season, from 2010 to 2018

Month	Total	<i>succinea</i> N (%)	<i>conspicua</i> N (%)	<i>spectabilis</i> N (%)	<i>axyridis</i> N (%)	Σ melanic N (%)
May	774	695 (89.8)	15 (1.9)	62 (8.0)	2 (0.3)	79 (10.2)
June	2687	2450 (91.2)	58 (2.2)	175 (6.5)	3 (0.1)	236 (8.8)
July	7142	6347 (88.9)	152 (2.1)	637 (8.9)	6 (0.1)	795 (11.1)
August	6124	5475 (89.4)	138 (2.3)	507 (8.3)	4 (0.1)	649 (10.6)
September	5740	5050 (88.0)	138 (2.4)	543 (9.5)	10 (0.2)	691 (12.0)
October	2813	2498 (88.8)	57 (2.0)	255 (9.1)	3 (0.1)	315 (11.2)
Total	25,280	22,515 (89.3)	558 (2.2)	2179 (8.4)	28 (0.1)	2765 (10.7)

Table 3 Annual variation in distribution of colour morphs of *H. axyridis* in central Czech Republic, from 2010–2018

Year	Total N	<i>succinea</i> N (%)	<i>conspicua</i> N (%)	<i>spectabilis</i> N (%)	<i>axyridis</i> N (%)	Σ melanic N (%)
2010	897	796 (88.7)	15 (1.7)	86 (9.6)	0 (0.0)	101 (11.3)
2011	3930	3449 (87.8)	102 (2.6)	373 (9.5)	6 (0.2)	481 (12.2)
2012	2551	2241 (87.8)	75 (2.9)	233 (9.1)	2 (0.1)	310 (12.2)
2013	1744	1532 (87.8)	33 (1.9)	176 (10.1)	3 (0.2)	212 (12.2)
2014	3832	3391 (88.5)	99 (2.6)	339 (8.8)	3 (0.1)	441 (11.5)
2015	5008	4451 (88.9)	107 (2.1)	443 (8.8)	7 (0.1)	557 (11.1)
2016	4189	3767 (89.9)	89 (2.1)	327 (7.8)	5 (0.1)	422 (10.1)
2017	1301	1194 (91.8)	21 (1.6)	85 (6.5)	1 (0.1)	107 (8.2)
2018	2119	1948 (91.9)	25 (1.2)	145 (6.8)	1 (0.0)	171 (8.1)
Total	25,571	22,769 (89.2)	566 (2.1)	2207 (8.6)	28 (0.1)	2802 (10.8)

Table 4 Frequency of colour morphs in macro-geographic areas of America and Europe

	Total N	<i>succinea</i> N (%)	<i>conspicua</i> N (%)	<i>spectabilis</i> N (%)	<i>axyridis</i> N (%)
America					
Chile	780	780 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
USA	86	86 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Canada	1812	1812 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	2678	2678 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Europe					
Spain	1618	1256 (77.6)	125 (7.7)	237 (14.6)	0 (0.0)
Italy 1	4	2 (50.0)	1 (25.0)	1 (25.0)	0 (0.0)
Georgia	4	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Russia 1	544	497 (91.4)	0 (0.0)	47 (8.6)	0 (0.0)
Italy 2	1152	1139 (98.9)	4 (0.3)	9 (0.8)	0 (0.0)
Italy 3	327	256 (78.3)	13 (4.0)	58 (17.7)	0 (0.0)
Austria	51	48 (94.1)	1 (2.0)	2 (3.9)	0 (0.0)
Ukraine	45	39 (86.7)	0 (0.0)	6 (13.3)	0 (0.0)
Germany 1	362	347 (95.9)	4 (1.1)	11 (3.0)	0 (0.0)
Slovakia	19,197	17,451 (90.9)	365 (1.9)	1371 (7.1)	7 (0.0)
Czech Republic	22,105	19,585 (88.6)	518 (2.3)	1974 (8.9)	25 (0.1)
Germany 2	133	125 (94.0)	0 (0.0)	8 (6.0)	0 (0.0)
United Kingdom	3904	3101 (79.4)	238 (6.1)	565 (14.5)	0 (0.0)
Russia 2	3	2 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)
Germany 3	239	232 (97.1)	2 (0.8)	5 (2.1)	0 (0.0)
Denmark	100	91 (91.0)	0 (0.0)	8 (8.0)	1 (1.0)
Total	49,788	44,175 (88.7)	1271 (2.6)	4303 (8.6)	33 (0.1)

The areas are ranked according to mean geographic latitude, from south to north

402 which consists entirely of the non-melanic morphs,
403 and Europe, which consists of a mixture of several
404 colour morphs (Table 4). The morph composition of
405 populations in America was the same, despite the fact
406 that the species' distribution spans more than 30
407 degrees of latitude in the Northern (Nearctic region)
408 and Southern (Neotropical region) hemispheres. Con-
409 sequently, neither local or temporal trends were
410 identified, nor any macro-geographic variation in
411 morph distribution in American populations.

412 Throughout the area of Europe already invaded by
413 invasive, non-native *H. axyridis* populations, the non-
414 melanic morphs dominated in local populations. In
415 particular, in well-sampled areas (Table 4), the fre-
416 quency of the non-melanic morphs varied between
417 77.7% (Spain) and 98.7% (Italy 2). Melanic morphs
418 were present in all areas, including Georgia, where
419 they were collected after 2016 (data not shown). Of the
420 melanic morphs, *spectabilis* was the most frequent,
421 with proportions varying between 0.8% (Italy 2) and
422 17.7% (Italy 3). The *conspicua* morph was scarce: it

423 was absent from four areas (Russia 1, Ukraine,
424 Germany 2, Denmark), and in other areas represented
425 0.3% (Italy 2) to 7.7% (Spain) of totals. The *axyridis*
426 morph was found only in the Czech Republic,
427 Slovakia and Denmark and in all cases it repre-
428 sented $\leq 1\%$ of local populations. One individual of
429 the morph *aulica* was found in Czech Republic
430 populations.

431 In Europe, there were no monotonic latitudinal or
432 longitudinal trends in distribution of the non-melanic
433 morphs. The frequency of the non-melanic morphs
434 increased in general from north to south (Fig. 4a), with
435 a maximum at c. 44°N. The longitudinal trend was flat
436 hill-shaped (Fig. 4b), with maxima of the non-melanic
437 morphs between 10 and 30°E.

438 The distribution of morph frequencies in the
439 invaded area of Europe is concentric. In the central
440 parts of its recent (2018) distribution, the non-melanic
441 morphs were most frequent, representing more than
442 90% of the population in Slovakia, the Czech Repub-
443 lic, Germany, Denmark and Sweden (2018 data of

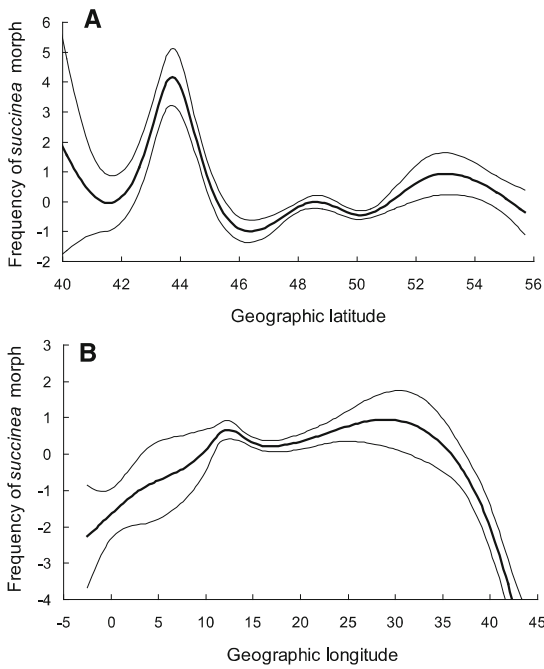


Fig. 4 Geographic trends in frequency of *succinea* morphs (logit(p) = log(p/1 - p) where p is proportion of *succinea* morphs in the populations modelled via GAM logistic regression)

444 Göteborg and Stockholm: N = 22 individuals, 95.5%
 445 non-melanic morphs, not included in the analysis).
 446 This morph was also frequent in the Po Valley of
 447 northern Italy. In contrast, in the margins of the current
 448 distribution (Spain and the United Kingdom in the
 449 west, and southern Russia (Russia 1) in the east) the
 450 percentage of the non-melanic morphs was under 80%
 451 (Fig. 4). Populations with a low proportion of the non-
 452 melanic morphs also occurred in Alpine regions of
 453 northern Italy (Italy 3). Melanic morphs contributed to
 454 the amount of melanic individuals in similar ratios
 455 (Table 4), with the rarer *conspicua* morph represent-
 456 ing about a quarter of melanic individuals (weighted
 457 average $3.0 \pm 1.47\%$), and the more common *spect-*
 458 *abilis* morph representing about three quarters of
 459 melanic individuals (weighted average $9.0 \pm 2.14\%$)
 460 (Table 4).

461 The frequency of the non-melanic morphs was not
 462 correlated with the meteorological data from the
 463 investigated areas. There was no significant correla-
 464 tion between the frequency of the non-melanic morphs
 465 and the average temperature of particular months of
 466 the growing season (April–October) or with periods

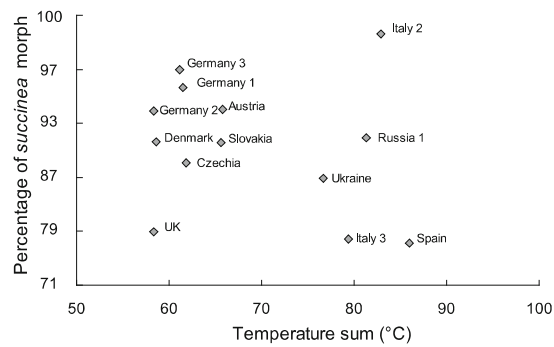


Fig. 5 The regression of the percentage of *succinea* morphs (angular transformation) in the geographic areas of Europe on sum of average May–August temperatures in these areas ($R^2 = 0.0457$, $a = 2.858$, $b = -0.00492$, $F_{1,12} = 0.527$, $P = 0.483$)

467 combining the average temperature over two or three
 468 months. The absence of a significant relationship is
 469 shown in Fig. 5 using a non-significant regression of
 470 arcsin percentage of the non-melanic morphs on sums
 471 of average temperatures over the May–August period
 472 in particular areas. The regressions calculated using
 473 the data for particular months and their combinations
 474 have nearly identical patterns of distribution of the
 475 data (not shown). This was because seasonal variation
 476 of temperatures at different individual localities were
 477 correlated, i.e. monthly temperatures were consis-
 478 tently high or low, with similar patterns of seasonal
 479 variation at multiple locations.

Discussion 480

Native and invasive populations 481

482 We found low variation in *H. axyridis* morph
 483 frequency in recently colonized areas compared to
 484 native areas (Dobzhansky 1933; Komai 1956;
 485 Zakharov and Blekman 2001; Noriyuki and Osawa
 486 2015). However, whilst the populations in America
 487 were monomorphic, this was not the case in Europe.
 488 There, 11.3% of melanic morphs (Table 3) was found,
 489 similar to that in east continental Asia, with a similar
 490 prevalence of the non-melanic morphs, but absence of
 491 a clear clinal trend in morph frequency (typical of
 492 populations from Japan (Komai 1956)). Macro-geo-
 493 graphic variation in the percentage of melanic morphs
 494 is likely to be greater than in native populations of

495	eastern continental Asia. Similar variation was also	In determining factors and mechanisms of origin of	541
496	observed at the micro-geographic scale: studies of	different morph composition in populations of partic-	542
497	populations in the Czech Republic revealed the	ular areas, we should distinguish two processes: (1)	543
498	absence of micro-geographic variation among sam-	maintenance of colour polymorphism within a popu-	544
499	pling sites spaced by tens of kilometres (Komai and	lation and (2) processes which lead to differentiation	545
500	Hosino 1951). Whilst we found no variation in morph	of geographic populations (Gray and McKinnon 2006;	546
501	frequency on particular host plants, our work on this	White and Kemp 2016).	547
502	question was only from one region, so may justify		
503	further study.		
504	Origin of polymorphism in recently colonised	Maintenance of morph frequency in local	548
505	areas	populations	549
506	The history of introduction of American <i>H. axyridis</i>	The mechanisms of the maintenance of colour poly-	550
507	populations from 1916 involved several intentional	morphism in <i>H. axyridis</i> are very likely similar in	551
508	attempts and unintentional introductions, but the	native (east Asia) and invaded (Europe) areas. Sea-	552
509	species failed to establish until the late 1980s (Lom-	sonal change in morph frequencies in populations of	553
510	baert et al. 2010; Roy et al. 2016), whilst the morphs of	<i>H. axyridis</i> in central Europe was smaller (fractions of	554
511	the introduced population(s) were not recorded. The	percent) and took place in the opposite direction.	555
512	probability of introducing a genetically recessive pure	Decreasing frequency of the non-melanic morphs	556
513	<i>succinea</i> population is greater than that of introducing	from late spring to early autumn appears to have been	557
514	a mixed population if the source population originated	reset by greater mortality of melanic morphs during	558
515	from east continental Asia (a large area including	the winter. Overwintering experiments (Honek et al.	559
516	eastern China, Korea, eastern Russia, north-eastern	2018) observed greater mortality in melanic than non-	560
517	Kazakhstan, Mongolia and northern Vietnam).	melanic morphs during the winter (Zdenka Marti-	561
518	Despite a restrictive political situation, transport in	kova and Alois Honek, unpubl.). However, this	562
519	the late 1980s was at least possible from some open	observation was made in an artificial hibernation site	563
520	countries, e.g. South Korea. In contrast, transport of	(Honek et al. 2018) and should be confirmed in	564
521	inoculum populations from Japan seems less probable	naturally assembled overwintering aggregations.	565
522	because of the prevalence of dominant melanic	Raak-van den Berg et al. (2012) did not observe an	566
523	morphs in this area (e.g. Noriyuki and Osawa 2015).	effect of colour morph on overwintering survival of <i>H.</i>	567
524	Furthermore, in the case of South America, the	<i>axyridis</i> in the Netherlands.	568
525	presence of only the non-melanic morphs are		
526	explained by the most probable source of populations	Differentiation in morph frequency	569
527	being eastern North America (Lombaert et al. 2010).	among geographic populations	570
528	Presence of melanic morphs in Europe points to co-	The origin of differences in morph frequency of <i>H.</i>	571
529	founders, inocula of populations containing dominant	<i>axyridis</i> populations inhabiting different geographic	572
530	melanic allelomorphs, likely escapees of commercial	areas implies a mechanism overcoming the stabilising	573
531	laboratory reared populations sold for biological	effect of recurrent seasonal variation in morph	574
532	control in glasshouses or, less likely, accidental	proportions (Wang et al. 2011). The area-specific	575
533	introductions of native populations from Japan. By	differences in morph frequencies may arise by (1)	576
534	admixture with populations from eastern North Amer-	long-term directed selection of particular morph	577
535	ica, this inoculum is likely to have contributed to the	composition, or (2) short-term processes, most prob-	578
536	establishment of invasive, non-native European pop-	ably random changes in small populations, e.g.	579
537	ulations. The far-reaching agreement between molecu-	founder effects or bottleneck effects.	580
538	lar and morphological data confirms the outcrossing	Consider first the selection of particular morph	581
539	event as a probable source of European populations	proportions in local populations which may imply	582
540	(Lombaert et al. 2010, 2011).	long term gradual changes in morph proportions.	583
		Indeed, long-term variation in <i>H. axyridis</i> morph	584

585 proportions was found in native populations (Komai
586 and Chino 1969; Bogdanov and Galalchij 1986). The
587 extent of these changes, e.g. more than 25% decrease
588 in the percentage of phenotypic non-melanic morphs
589 during a 50-year period in central Japan (Komai and
590 Chino 1969), is greater than the differences observed
591 among invasive non-native populations in Europe.
592 This may be because the data from native populations
593 are available for a longer period than the data of
594 invaded populations in Europe. However, only c. 7%
595 increase in the frequency of the phenotypic non-
596 melanic morphs was observed over a 55-year period in
597 Vladivostok, Russia (Bogdanov and Galalchij 1986),
598 which is similar to that observed in Europe.

599 A likely factor important for selection is climatic
600 difference between areas. Local climate, temperature
601 and humidity have proved the most important corre-
602 lates of morph frequencies in several species of
603 coccinellid (Dobzhansky 1933; Kryltzov 1956; Honek
604 1996; Sloggett and Honek 2012; Kawakami et al.
605 2015). Different thermoregulation capacities of colour
606 morphs have been advocated as a basis of the
607 maintenance of geographic differences in morph
608 frequency by climatic selection (Brakefield and Will-
609 mer 1985). In *H. axyridis*, the likely effect of climate is
610 different in native and invaded populations. Climate is
611 likely a driving factor of geographic variation in Japan
612 (Komai 1956). However, in Europe the effect of this
613 factor is unclear because of the limited variation in
614 morph proportions observed and the absence of a
615 correlation between climatic conditions and morph
616 frequency.

617 We may consider a possibility that geographic
618 differences originated by selection enforced through
619 affiliation to local complexes of Müllerian mimicry.
620 As with some other coccinellid species (Rothschild
621 1961; Frazer and Rothschild 1962; Pasteels et al.
622 1973), *H. axyridis* hemolymph contains distasteful and
623 poisonous alkaloids advertised by bright “warning”
624 coloration (aposematism) (Bezzerides et al. 2007;
625 Pruchova et al. 2014; Vesely et al. 2017). The species
626 may belong to a “Müllerian mimicry” complex of
627 species, with similar warning coloration and unpalata-
628 bility to predators. Here polymorphism presents a
629 problem if the species as a whole should be protected
630 by deceptive mimicry (Briolat et al. 2019). As in
631 *Adalia bipunctata* (L.) and *Adalia decempunctata* (L.),
632 polymorphic species composed of non-melanic and
633 melanic morphs may belong to different “circles” of

Müllerian mimicry (Brakefield 1985). Since warning
634 coloration does not confer full protection against
635 predation (Heikertinger 1932; Křištín 1988, 1991),
636 predators may exert different pressures on particular
637 mimicry complexes. The morph composition of a
638 species may thus be influenced by variation in local
639 pressures against particular circles of Müllerian mim-
640 ics. This scenario could be further investigated. 641

642 Due to the temporal stability of *H. axyridis* morph
643 frequency in local populations, it was assumed that the
644 populations are free of, or resistant to, selective
645 pressures and the differences may have originated by
646 founder effects, i.e. a random combination of morph
647 frequency in the initial populations. Spreading popu-
648 lations may have occasionally been reduced to small
649 migrant groups with randomly changed morph fre-
650 quencies which were subsequently maintained in local
651 populations. Alternatively, local populations may
652 have originated from several founder populations
653 whose morph compositions differed one from the
654 other. The eastern North America population, which is
655 likely one of the genetic sources of European popu-
656 lations (Lombaert et al. 2010), was certainly
657 monomorphic *succinea*. Neither origin or genetic
658 composition explaining colour morphs of these inocu-
659 lula populations are known. The explanation of macro-
660 geographic variation due to possible different origins
661 of source populations thus remains speculative. 661

662 Assembling data on world morph variation of
663 invasive, non-native *H. axyridis* populations con-
664 firmed a clear difference between the populations of
665 America and Europe. The results demonstrate insular
666 distribution of morph frequencies in local populations
667 of Europe, and pose an unsolved problem of the origin
668 of these differences. Further research is necessary to
669 elucidate (1) origin of this variation, (2) ecological
670 significance and (3) consequences of this variation for
671 applied problems of nature conservation and
672 agriculture. 672

673 **Acknowledgements** A.H., Z.M. and J.S. were supported by
674 the program VES19 INTER-COST No. MSMT-15739/2019-6
675 (MSMT ČR), the Project 17-06763S of the Grant Agency of
676 Czech Republic and the Ministry of Agriculture of the Czech
677 Republic, institutional support MZE-RO0418. M.B. by the long-
678 term strategic development financing of the Institute of
679 Computer Science (Czech Republic RVO 67985807), A.A.G.
680 and T.Z. by Grant FONDECYT 1180533, J.K. and P.Z. were
681 supported by the Scientific Grant Agency VEGA of the Ministry
682 of Education, Science, Research and Sport of the Slovak
683 Republic (Grants 2/0012/17 and 2/0032/19), and M.O.B. by 683

684 Russian Science Foundation 16-14-10031. We thank J.
685 Kohoutová and H. Uhlřířová for excellent technical assistance.

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