

A HIGH-DENSITY AND TIME DYNAMIC DISTRIBUTION MAP OF HONEY BEE MITOCHONDRIAL DNA LINEAGES COVERING THE ENTIRE ITALY

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Abstract

Growing interest has emerged in the need to monitor the genetic integrity of the European *Apis mellifera* subspecies, which could be threatened by the human-mediated dispersion of non-native populations and lines. Honey contains environmental DNA (eDNA) traces from all organisms that directly or indirectly were involved in its production, including the DNA of the honey bees that produced it. Specific mitochondrial DNA (mtDNA) lineages (known as mitotypes) characterize several *A. mellifera* subspecies. Different mitotypes can be detected using honey as a source of honey bee DNA, providing approximate population genetic information useful for estimating the diffusion and frequency of honey bee mitotypes. In this study we present a snapshot of the diffusion of the main *A. mellifera* mtDNA lineages in Italy over five years (2018–2022). DNA was extracted from more than 3200 honey samples produced in all regions of the Italian peninsula, Sardinia and Sicily, during these five years. PCR products were analysed using a fragment size-based assay. Results confirmed that the C lineage was the most frequent mitotype throughout Italy except in Sicily where the A lineage was always highly represented. Other mtDNA haplotypes (A and M lineages) were present in almost all Italian regions. The updated distribution map of honey bee mtDNA lineages obtained in this study can be useful for designing and evaluating the potential effectiveness of conservation policies aimed at maintaining the integrity of honey bee genetic resources in Italy. Furthermore, we demonstrated that this approach based on the entomological footprint left in honey can provide a simplified yet extensive population genetic analysis, considering that each honey sample may contain the DNA of many different honey bees, from different colonies or even different apiaries.