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Morphometric identification of honey bee subspecies reveals a high proportion of hybrids within a Mediterranean protected area

Lise Ropars*, Laurence Affre and Benoît Geslin
IMBE, Aix Marseille University, Avignon Université, CNRS, IRD, Marseille, France

The importance of natural protected areas for the preservation of locally adapted subspecies (or local genotype) of honey bees has been recently emphasized in the literature. In western Europe, initiatives have emerged to protect the native subspecies i.e., the Western European dark bee, *Apis mellifera mellifera* (Hymenoptera: Apidae). Here, we investigated the honey bee subspecies diversity in a Mediterranean protected area, Calanques National Park, near Marseille, France. We found that the population of honey bees is mainly composed of hybrids between *Apis mellifera mellifera* and *Apis mellifera intermissa*. These hybrids might have a better resistance to the Varroa mite and a better adaptation to arid Mediterranean climate. Before promoting the native honey bee subspecies, *Apis mellifera mellifera*, it appears essential to identify honey bee subspecies used by beekeepers in protected areas.

Keywords: honey bee subspecies; *Apis mellifera*; beekeeping; Mediterranean protected area; conservation programs

While Geldmann and González-Varo (2018), suggested to ban the installation of honey bee colonies in protected areas to neutralise the indirect competition for floral resources between honey bees and wild pollinators (see also Geslin et al., 2017; Mallinger et al., 2017), Alaux et al. (2019) argued that native subspecies of honey bees must be promoted within protected areas to develop local and adapted genotypes. In France, the Western European dark bee, *Apis mellifera mellifera* is the native subspecies (De la Rúa et al., 2009), but it has been exposed to hybridization with other non-native subspecies coming from African, North Mediterranean, or Oriental lineages. Commercial honey bees used by beekeepers, are nowadays derived from outcrosses between several *Apis mellifera* subspecies in order to improve honey productivity, or behavioral traits such as calmness, foraging efficiency and resistance to a wide range of temperature or to pathogens (Neumann & Blacqui re, 2017). To promote locally adapted honey bees, conservation programs have emerged mostly targeting *A. m. mellifera* (Alburaki et al., 2011; De la R  a et al., 2009; Parc Nationaux de France, 2016). Those programs suggest favouring this subspecies for beekeeping activities within natural areas (Alaux et al., 2019). But to date, few information exists on honey bee subspecies installed in natural protected areas and it remains difficult to know what honey bee subspecies are used by beekeepers on these territories.

Through a two-year field campaign from February to June 2017 and 2018, conducted in the western part of Calanques National Park (near Marseille, France — 43  13'N, 5  28'E) (Ropars, Affre, Aubert, et al., 2020),

we analyzed the subspecies diversity of honey bees (Figure 1). This protected area, composed of a terrestrial core of 85 km   and an adhesion area of 26 km  , is characterized by Mediterranean low vegetation habitats (phrygana and shrubland), Mediterranean forests and rocky habitats. In the spring period, when the scrubland was flowering (composed of rosemary, *Rosmarinus officinalis*, rockrose, *Cistus albidus*, and thyme, *Thymus vulgaris*), nearly 450 honey bee colonies were installed in the park mostly by transhumant beekeepers to produce scrubland honey (Calanques National Park authorizations). When weather conditions were favorable for pollinators activity (> 15  C, no windy or cloudy days), we captured honey bees foraging on wild flowers. These captures were performed on 17 sites within the protected area and were part of a larger sampling protocol (see Ropars, Affre, Aubert, et al., 2020; Ropars, Affre, Schurr, et al., 2020).

We used the Apiclass website (<http://apiclass.mnhn.fr>) to identify honey bee subspecies based on wings morphometrical characteristics (Baylac et al., 2008). Apiclass references a subspecies-curated database of more than 5000 samples to analyze wings based on 19 points corresponding to the intersections of the main veins of bee forewings, returning an affiliation percentage for each subspecies and evolutionary lineage (Nawrocka et al., 2018). We submitted to Apiclass photographs of the right wings of 172 individuals of *A. mellifera*, taken with a binocular magnifier (  10) equipped with a camera (TOUPCAM TM 5.IMP). We considered an individual pure for a given subspecies if its affiliation was 90% or higher, otherwise it was considered a hybrid of several

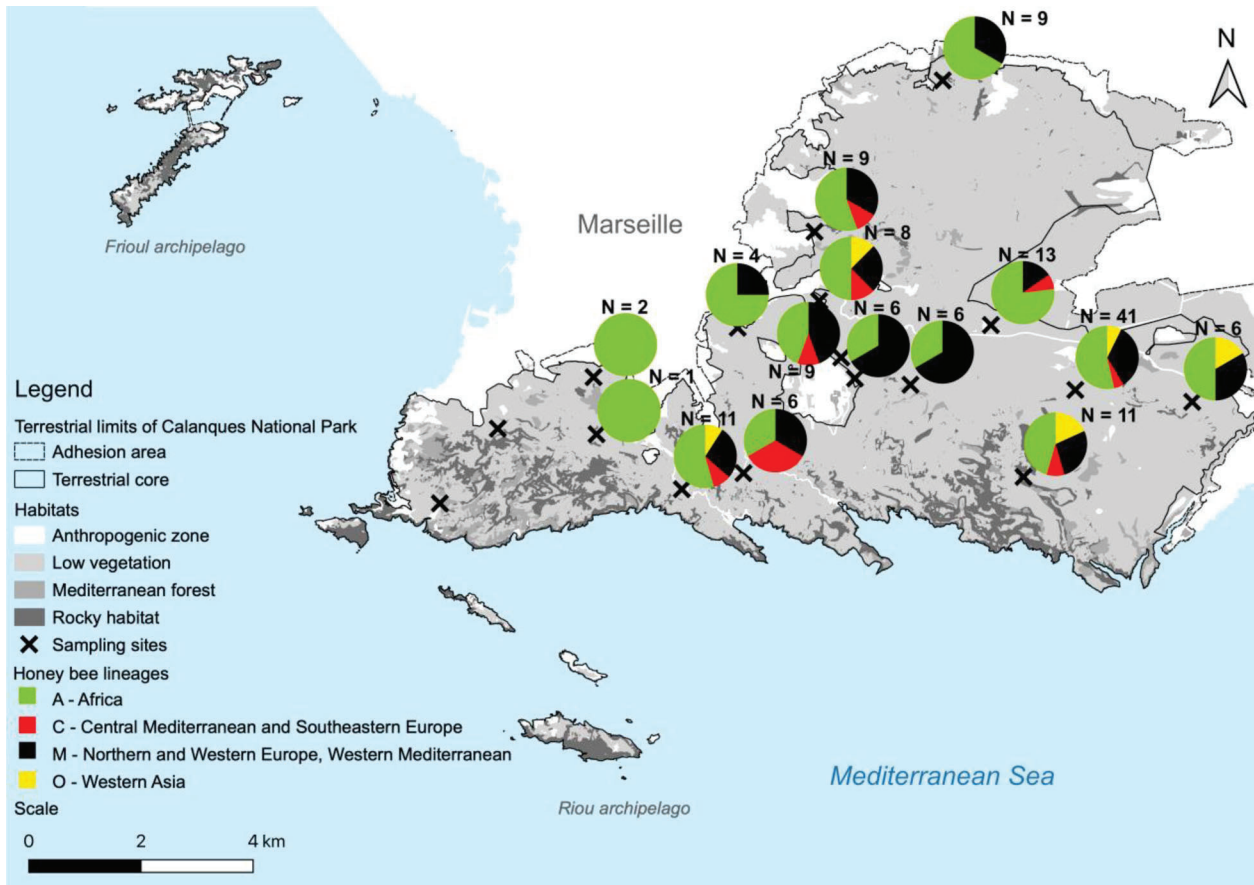


Figure 1. Distribution of honey bee lineages within the western part of Calanques National Park. Terrestrial limits and habitat data were obtained from Calanques National Park authorities.

Table 1. List of honey bee subspecies and lineages with the number of individuals and the corresponding percentage.

Honey bee subspecies	N	Percentage
<i>Apis mellifera anatoliaca</i>	2	1.4%
<i>Apis mellifera carnica</i>	1	0.7%
<i>Apis mellifera caucasica</i>	2	1.4%
<i>Apis mellifera intermissa</i>	1	0.7%
<i>Apis mellifera mellifera</i>	11	7.7%
Hybrids	125	88.0%
Honey bee lineages	N	Percentage
Africa	77	54.2%
Western Asia	8	5.6%
Northern and Western Europe	47	33.1%
Central Mediterranean and Southeastern Europe	10	7.0%

The total corresponded to 142 individuals as 30 on the 172 individuals did not give any result using the *Apiclass* software ($\rho \geq 0.05$).

subspecies (Lehébel-Péron, 2017). We also noted each sample's affiliation to the different evolutionary lineages (M - Northern and Western Mediterranean, A - African, C - Central and Southeastern Europe, and O - Oriental, Western Asia).

Within Calanques National Park, *Apiclass* successfully processed 142 of the 172 individuals (Table 1), revealing that 17 (12%) could be assigned to a single subspecies, while the remaining 125 (88%) were of hybrid origin - mostly involving *A. m. mellifera* and *A. m. intermissa*. In

France, two other National Parks (protected areas, Category II, UICN) have conducted inventories of honey bee subspecies. Within the Vanoise National Park, a genetic study conducted in 2016 and 2017 on 359 honey bee revealed that the number of hybrids reached 36–38% (Grosset et al., 2019). Likewise, Cévennes National Park hosts a low percentage of hybrids (31%) which could be explained by the retaining of traditional beekeeping practices and especially trunk apiaries (Lehébel-Péron, 2017). Given these previous studies, the percentage of hybrids (88%) can be considered high in Calanques National Park and few beekeepers appeared to use the native subspecies of honey bee, *A. m. mellifera*.

The common use by beekeepers of hybrids between *A. m. mellifera* and *A. m. intermissa* could be first related to the tolerance of *A. m. intermissa* against the Varroa mite, *Varroa destructor* (Kefuss et al., 2004). Also, as *A. m. intermissa* might be better adapted to arid Mediterranean climates (De La Rúa et al., 2004), the introgressions of *A. m. intermissa* with *A. m. mellifera* could therefore be beneficial for beekeepers who install their colonies in the South of France.

It is worth noting that the high use of hybrids is not consistent with the beekeepers' perception about the origins of their colonies (from an informal questionnaire

distributed in fall 2017), and most of them believed that they managed the Western dark honey bee, *A. m. mellifera*. At this stage, it remains difficult to understand if the high abundance of *A. m. intermissa* is due to a lack of knowledge, a willingness of beekeepers to use this subspecies or to natural hybridizations with feral colonies. Yet, it questions the relevance of conservation actions promoted by land managers for *A. m. mellifera* in Calanques National Park. Indeed, even if actions for the conservation of *A. m. mellifera* are established in some protected areas, it remains difficult, based on our results, for beekeepers to prevent their colonies from hybridizations. Also, there are very few information about the potential of protected areas to harbor feral populations of honey bees (Requier et al., 2019). Moreover, honey bees can swarm, sometimes over quite long distances and there are no ways to control the natural installation of swarms coming from managed colonies into protected areas (Schmidt, 1995). Males in search of virgin queens could also fly very long distances (up to 15 km, Jensen et al., 2006) and consequently breed with feral or locally adapted populations.

Before promoting the Western European dark bee, *Apis m. mellifera* subspecies, it appears essential to identify honey bee subspecies used by beekeepers within protected areas. As stated by Henriques et al. (2020), the use of wing geometric morphometrics offers a reliable and accessible tool for exploring the population structure of honey bees. Finally, we only used the *Apiclass* methods on honey bees captured on flowers and future studies should investigate the origin of honey bees and their local adaptation through genetic studies at colony level.

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