

VALERIA MALAGNINI, FEDERICO PEDRAZZOLI, PAOLO FONTANA,
GIOVANNI CILIA & CECILIA COSTA

HONEY BEES IN PANTELLERIA

Le api mellifere di Pantelleria

Apis mellifera L. has some peculiarities that make it a key organism for the conservation of biodiversity and, therefore, the global ecological balance (KEARNS *et al.*, 1998; AIZEN *et al.*, 2008). Unmanaged honey bee colonies have always been present in different areas and they have coexisted with managed colonies. In the last 35 years, with the transfer of the parasitic mite *Varroa destructor* Anderson and Trueman (NAZZI & LE CONTE, 2016) to Western honey bee colonies, unmanaged colonies have almost completely disappeared in most of Europe. However, in recent years, the reports of unmanaged colonies, which would sometimes be able to survive several years, have been increasing (CARTER *et al.*, 2016; HASSETT *et al.*, 2018; SEELEY, 2019). Unmanaged colonies are at the attention of the world of bee research and European beekeeping for the development, in such conditions, of resilience to a changing climate and to pathogens and parasites (MORITZ *et al.*, 2007; MIKHEYEV *et al.*, 2015), as well as for the protection of the indigenous subspecies of *Apis mellifera* (FONTANA *et al.*, 2018). On the Pantelleria island, a small island located between Sicily (Italy) and Tunisia, managed honey bee colonies belonging to local beekeepers coexist with unmanaged ones - 25 unmanaged colonies were recorded using the smartphone app BeeWild (FONTANA, 2020). In this study we describe the morphometry and haplotype distribution of the honey bee colonies living in this isolated and very peculiar environmental context, trying to point out the differences between managed and unmanaged colonies and whether there are genetic exchanges between them.

Bee individuals were collected from 32 colonies in the summer of 2021

and subjected to morphometric and molecular analyses. For morphometric analyses, 16 wing characters were used. A discriminant analysis including Pantelleria honeybees, *A. m. siciliana*, *A. m. ligustica*, *A. m. carnica*, *A. m. intermissa*, *A. m. caucasica* and *A. m. mellifera* was carried out. For molecular analyses, the non-coding region located between the tRNA^{Leu} and COII genes (originally named COI-COII intergenic region) was amplified (GARNERY *et al.*, 1993) and sequenced. The variability within colonies was assessed by comparing the sequences obtained from three individuals/colony and, as sequences were very homogeneous, one individual belonging to each colony was chosen. The software BioEdit and Mega X were used to check and correct the sequences and to build phylogenetic trees. As a reference, the sequences belonging to *Apis mellifera mellifera*, *A. m. ligustica*, *A. m. carnica*, *A. m. yemenitica*, *A. m. siciliana*, *A. m. iberiensis*, *A. m. intermissa*, and *A. m. ruttneri* and already present in GenBank were used. Evolutionary lineages and haplotypes for each individual were analysed by sequence blast on GenBank.

Based on the results of morphometric analysis 68% of the colonies were similar to *A. m. siciliana*, 4% to *A. m. ligustica* and 28% were hybrids (i. e. *A. m. siciliana* × *A. m. intermissa*). However Pantelleria honey bees cluster separately from *A. m. siciliana*.

Based on the mtDNA analyses we found that the A evolutionary lineage is dominant on the island (71% of the samples overall), and even more so in the unmanaged colonies (93%). The most frequent A haplotype was A1, which was found in *A. m. siciliana* during the reintroduction project APESLOW (DALL'OLIO *et al.*, 2014), followed by A4 and A2, also previously reported in *A. m. siciliana* (MUNOZ *et al.*, 2014). Results support the current existence in Pantelleria of an original population of honey bees close to *A. m. siciliana*, although beekeeping activities pose a risk of introgression. Furthermore, as the proportion of A lineage haplotypes was higher in the unmanaged colonies (93% compared with 50% in managed colonies), we can hypothesize that the local population has an adaptive advantage over imported genotypes. Further analysis must be carried out to better understand the identity of Pantelleria island honey bees.

Acknowledgements — We thank D. Almanza and A. Castello for their help during sample collection. This research was funded by Parco Nazionale di Pantelleria: “Direttiva agli enti parco nazionali 2019: Attività dirette alla conservazione degli impollinatori” - Direttiva MitE.

REFERENCES

- AIZEN M.A., GARIBALDI L.A., CUNNINGHAM S.A. & KLEIN A.M., 2008. ‘Long-Term Global Trends in Crop Yield and Production Reveal No Current Pollination Shortage but Increasing Pollinator Dependency’, *Current Biology*, 18.20: 1572–1575.

- CARTER L.J., SMITH M.L. & SEELEY T.D., 2016. 'How Honey Bee Colonies Survive in the Wild: Testing the Importance of Small Nests and Frequent Swarming', *PLOS ONE*, 11.3: e0150362.
- DALL'OLIO R., COSTA C., COSENZA M., BERGOMI P. & REALE S., 2014. Reintroduction of the endangered *A. m. siciliana* in Sicily: genetic and morphometric tools to support management of mating stations and conservation islands. P. 53 in: De la Rúa P. (ed.), Sixth European Conference of Apidology. *Universidad de Murcia – Servicio de Publicaciones*.
- FONTANA P., 2020. BeeWild, già più di 100 colonie censite. *Terrea di Mach*, 9 (Novembre): 3.
- FONTANA P., COSTA C., DI PRISCO G., RUZZIER E., ANNOSCIA D., BATTISTI A., CAUDURO G., CARPANA E., CONTESSA A., DAL LAGO A., DALL'OLIO R., DE CRISTOFAR A., FELICIOLO A., FLORIS I., FONTANESI L., GARDI T., LODESANI M., MALAGNINI V., MANIAS L., MANINO A., MARZI G., MASSA B., MUTINELLI F., NAZZI F., PENNACCHIO F., PORPORATO M., STOPPA G., TORMEN N., VALENTINI M. & SEGRÈ A., 2018. Appeal for biodiversity protection of native honey bee subspecies of *Apis mellifera* in Italy (San Michele all'Adige declaration). *Bull. Insectol.*, 71: 257–271.
- GARNERY L., SOLIGNAC M., CELEBRANO G. & CORNUET J.M., 1993. A simple test using restricted PCR-amplified mitochondrial DNA to study the genetic structure of *Apis mellifera* L. *Experientia*, 49(11): 1016–1021.
- HASSETT J., BROWNE K.A., MCCORMACK G.P., MOORE E., NATIVE IRISH HONEY BEE SOCIETY, SOLAND G. & GEARY M., 2018. 'A Significant Pure Population of the Dark European Honey Bee (*Apis mellifera mellifera*) Remains in Ireland', *J. Apicult. Res.*, 57.3: 337–350.
- KEARNS C.A., INOUE D.W. & WASER N.M., 1998. 'Endangered Mutualisms: The Conservation of Plant-Pollinator Interactions', *Annu. Rev. Ecol. Syst.*, 29: 83–112.
- MIKHEYEV A.S., TIN M.M.Y., ARORA J. & SEELEY T.M., 2015. 'Museum Samples Reveal Rapid Evolution by Wild Honey Bees Exposed to a Novel Parasite', *Nature Comm.*, 6.1: 1–8.
- MORITZ R.F.A., KRAUS F.B., KRYGER P. & CREWE R.M., 2007 'The Size of Wild Honeybee Populations (*Apis mellifera*) and Its Implications for the Conservation of Honeybees'. *J. Insect Conserv.*, 11.4: 391–397.
- MUNOZ I., DALL'OLIO R., LODESANI M. & DE LA RUA P., 2014. Estimating introgression in *Apis mellifera siciliana* populations: are the conservation islands really effective? *Insect Conserv. Divers.*, 7(6): 563–571.
- NAZZI F. & LE CONTE Y., 2016. Ecology of *Varroa destructor*, the Major Ectoparasite of the Western Honey Bee, *Apis mellifera*'. *Annu. Rev. Entom.*, 61: 417–432.
- NEUMANN P. & CARRECK N.L., 2010. Honey Bee Colony Losses. *J. Apicult. Res.*, 49.1: 1–6.
- SEELEY T.D., 2019. *The Lives of Bees: The Untold Story of the Honey Bee in the wild*. Princeton University Press, USA, 376 pp.

Authors' Address — V. MALAGNINI, F. PEDRAZZOLI, P. FONTANA, Fondazione Edmund Mach, Via E. Mach, 1 - 38067 San Michele all'Adige, Trento (I); C. COSTA, G. CILIA, CREA – Agriculture and Research Centre, Via di Saliceto, 80 - 40128 Bologna (I); email: valeria.malagnini@fmach.it

