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Developing reduced SNP assays from whole-genome sequence data to estimate C-lineage introgression in the Iberian honeybee (*Apis mellifera iberiensis*)

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The honeybee has been subject to a growing number of threats. In Western Europe one such threat is large-scale introductions of commercial strains (C-lineage), which is leading to introgressive hybridization and even the local extinction of native populations (M-lineage). Here, we developed reduced assays of highly informative SNPs from 176 whole genomes to estimate C-lineage introgression in M-lineage subspecies *Apis mellifera iberiensis*. We started by evaluating the effects of sample size and sampling a geographically restricted area on the number of highly informative SNPs. We demonstrated that a bias in the number of fixed SNPs ($F_{ST}=1$) is introduced when the sample size is small ($N \leq 10$) and when sampling only captures a small fraction of a population's genetic diversity. These results underscore the importance of having a representative sample when developing reliable reduced SNP assays for organisms with complex genetic patterns. We used a training dataset to design four independent SNP assays selected from pairwise F_{ST} between the Iberian and C-lineage honeybees. The designed assays, which were validated in holdout and simulated hybrid datasets, proved to be highly accurate and can be readily used for monitoring populations not only in the native range of *A. m. iberiensis* in Iberia but also in the introduced range in the Balearic islands, Macaronesia, and South America, in a time- and cost-effective manner. While our approach used the Iberian honeybee as model system, it has a high value in a wide range of scenarios for the monitoring and conservation of potentially hybridized domestic and wildlife populations.

Polymorphisms in cytochrome P450 versus cline distribution of evolutionary lineages in *Apis mellifera iberiensis*

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Honey bees (*Apis mellifera*) are the most prominent and economically important pollinator species worldwide. However, the reported decline of its populations in several regions of the world over the last decades is of concern. The causes are manifold, including the spread of pathogens and parasites, malnutrition and habitat loss, climate change and xenobiotics, especially pesticides. Among the main mechanisms used by insects to cope with the adverse effects of xenobiotics is the metabolic resistance mediated mainly by three superfamilies of enzymes: the cytochrome P450 monooxygenases, the glutathione transferases and the carboxylesterases.

We hypothesize that the genetic background influences the sensitivity to pesticides or detoxification capacity of different honey bee populations, ecotypes and subspecies. The Iberian Peninsula provides an interesting scenario to study the genetic variability of the cytochrome P450 genes given the co-occurrence of two clinally distributed evolutionary lineages, as a result of secondary contact.

In this study, the genetic variability of six genes of the cytochrome P450 superfamily (CYP6AS3, CYP6AS4, CYP6AS5, CYP6AS7, CYP6AS12 and CYP6AS17) was analyzed in the Iberian honey bee (*Apis mellifera iberiensis*) to provide more information on the mechanisms of resistance to xenobiotics and to identify the genetic variation involved in local adaptation. Genomic signal of selective sweeps was detected in three genes, of which CYP6AS5 presents the highest number of point mutations under selection, being proposed as a candidate gene to perform gene expression studies. We discuss the correlation between the variability of P450 genes and the distribution of the evolutionary lineages in the Iberian Peninsula. The identification of polymorphisms in these genes promises to shed light on the relationship between diversity and xenobiotic tolerance of *A. m. iberiensis*.