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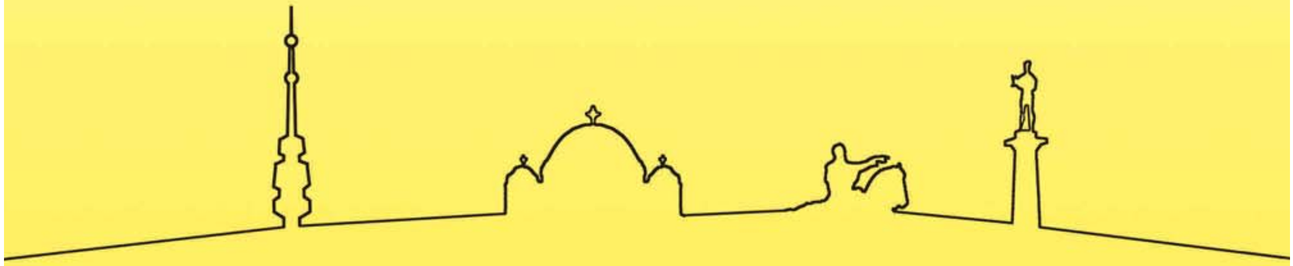

A MOLECULAR TOOL TO DETECT GENETIC INTROGRESSION FROM SPERMATHECA CONTENT

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The genetic integrity of *Apis mellifera mellifera* is threatened by introgression in many places of its native distribution, after recurrent importations of commercial queens typically belonging to the divergent C-lineage. A growing interest in keeping and protecting *A. m. mellifera* has motivated the development of conservation programs in many places of Europe. As part of the conservation efforts, isolated mating stations are set to avoid unwanted crosses, but these are not always effective as matings with unwanted drones are frequently reported. An interesting method to monitor the degree of isolation of mating stations could be through genetic analysis of the queen spermatheca contents. While this method implies that queens selected for monitoring are sacrificed, it can be a powerful way of assessing the effectiveness of mating stations because it would allow easy detection of unwanted alleles. Here, we developed an SNP-based tool suited to the analysis of DNA extracted from spermatheca or from pooled DNA of varying sources. To that end, we first designed an SNP panel from whole-genome sequence data generated from 228 drones, of which 148 belonged to the M-lineage (117 *A. m. iberiensis* and 31 *A. m. mellifera*) and 80 to the C-lineage (46 *A. m. carnica* and 34 *A. m. ligustica*). A total of 5,007 highly differentiated SNPs was found. Based on different criteria, 130 SNPs were selected to be included in the genotyping tool. This tool is based on the NEBNext Direct Genotyping Solution that allows high-throughput, sequence-based target genotyping of single-individual or pooled DNA. To assess the tool's sensitivity and accuracy, 142 samples (DNA extracted from spermatheca and tissue, as well as known DNA mixtures) were genotyped. After removing the problematic SNPs, 81 were retained and these were able to provide an estimate of the pool introgression level with great accuracy. This tool represents a significant advance in the genetic analysis of honey bee colonies with a variety of applications, including breeding and conservation of *A. m. mellifera*.

Keywords: SNPs, Pools, Spermatheca content