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Sustainable Beekeeping, from the south of the world

# **ABSTRACT BOOK**

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### PP-068

### mtDNA melting analysis for quick characterization of Africanized honeybee Apis mellifera populations using honey samples

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Honey contains mitochondrial DNA (mtDNA) from bees that produce it and can be used to identify their evolutionary origin, maternal lineage, or even subspecies. The mtDNA variation in Apis mellifera has been detected by different molecular methods (RFLPs, PCR-RFLPs, sequencing) and was previously used to characterize Africanized populations. High-resolution melting (HRM) analysis is a new technique that allows rapid identification of sequence polymorphisms in short amplicons generated by gPCR. The aim of this work is to identify the African mitotype in Apis mellifera populations by applying HRM. Honey samples were collected from 111 apiaries in 13 Argentine provinces between 2012 and 2018. Total DNA was extracted from sediment obtained by centrifugation of 10 g of honey and then subjected to qPCR in a final volume of 20 µl using a fluorescent intercalator and primers (APIS -F/Afr207R) that amplify a 207Pb product that allows discrimination between European and African origin. Positive controls (European/African) were used to corroborate qPCR product sizes and fusion curves. In addition, some products were purified and sequenced to confirm mitochondrial haplotypes. Only 26 honey samples from apiaries above 35° S in the provinces of Misiones, La Rioja, Catamarca, Formosa, Corrientes, Córdoba and Santa Fé showed an Africanized mitotype. The remaining apiaries below this border, mainly in the provinces of Buenos Aires, Rio Negro, Neuguén, and Entre Ríos, showed a European mitotype. The results are consistent with previous characterizations and with the distribution limit of Africanized populations in Argentina. Likewise, HRM analysis showed high sensitivity and effectiveness in mitotype characterization, as it requires very little DNA and requires only a single amplification reaction, reducing the cost and effort of sampling. In addition, it can be readily used in genetic selection programs in transition regions between European and Africanized populations, as it allows the identification of genetic resources of honey bee colonies based on the honey they produce.

#### PP-069

#### Informative STR markers for identifying genetic characteristics of Ukrainian steppe bees

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The preservation of the gene pool of Ukrainian steppe bees has recently become a significant problem due to their uncontrolled crossbreeding with gray mountainous Caucasians, Carniolans, and Buckfasts. This phenomenon leads to the loss of unique gene complexes of Ukrainian steppe bees, associated with increased adaptability to the specific conditions of the steppe and forest-steppe zones of Ukraine, with their unique floristic spectrum and continental climate.

One of the methodological approaches to preserving the gene pool of the autochthonous subspecies of bees is a set of selection-genetic measures aimed at developing identification and selectively significant molecular-genetic markers, creating genetic reserves of Ukrainian steppe bees, and combining methods of classical and marker-associated selection for their main productivity traits.

The purpose of our own research was to create highly informative STR markers for determining the population and individual ethological characteristics of Ukrainian steppe bees.

We analyzed the primary DNA sequences (Apis mellifera) from the GenBank database: DNA AJ509537, AJ509586, and AF140070 and constructed our own designed primers using the FastPCR ver 6.1 program. We selected the optimal conditions for amplification to combine the primers in a multiplex system. When analyzing the population of Ukrainian steppe bees (village of Machukhy, Poltava region), a total of 5 alleles were detected in each locus with levels of actual heterozygosity ranging from 0.667 to 0.917 for AJ509537 and AF140070, respectively. After carrying out an ethological experiment to determine the level of aggressive response to an external stimulus using a classical method modified by us, working bees were selected for further determination of their affiliation with genetic patrilineages using the developed STR markers. The scientific hypothesis of the predominant influence of the parental genotype (drone contribution) on the degree of defensive behaviour expressionr is confirmed, which can be used in breeding programs aimed at reducing the aggressiveness of the Ukrainian steppe bee.

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