

HONEY BEES IN SLOVAKIA: A REVIEW

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ABSTRACT

Beekeeping in the territory of Slovakia has a long tradition beginning in the late 18th century. Nowadays, six honey bee breeding stations, 58 reproduction farms and more than 15,000 registered beekeepers represent the apiculture sector in Slovakia. The population of indigenous Carniolan bee (*A. m. carnica*), that is the most common honey bee race in Slovakia is endangered due to crossbreeding with imported bees of unknown origin. The aim of our study was to characterize Carniolan honey bee population using molecular markers. A total of 79 Slovak Carniolan workers collected from breeding and reproduction farms in Slovakia were analysed and compared with 85 reference bees of *A. mellifera mellifera*, *A. mellifera macedonica*, *A. mellifera ligustica* and *Buckfast* using 10 microsatellite markers. Bayesian and frequency-based methods have been used to successfully assign 95 % of individuals to tested populations. Genetic structure analysis using the Bayesian clustering method incorporated in STRUCTURE package showed two distinct clusters in the Slovak population of *A. m. carnica* and its reasonable admixture with *A. m. macedonica* and *Buckfast* species. The tRNA^{leu}-cox2 intergenic region of mitochondrial DNA was sequenced in 66 Carniolan workers. We found that 71.2 % of samples correspond to 5 known haplotypes belonging to C lineage (C2c, C1a, C2d, C2e and C2z) and 28.8 % of samples have novel mutations in the sequenced region of mtDNA.

Key words: Carniolan honey bee; genetic diversity; individual-population assignment; molecular markers; mtDNA

INTRODUCTION

Beekeeping is an ancient tradition and honey bees have been kept in Europe for several millennia. Bees are critically important for the environment, sustaining biodiversity by providing essential pollination for a wide range of crops and wild plants. The majority of crops grown in the European Union depend on insect pollination, so the apiculture sector is an important part of the EU agriculture. Indeed, insect pollination, which is currently under threat in Europe, has an estimated economic value of € 22 billion per year in the EU alone (Nieto *et al.*, 2014). Bees contribute to human benefits also directly through the production of honey and other apiculture products such as pollen, wax for food processing, propolis in food technology, and royal jelly used as a dietary supplement and as an ingredient to food. There are around 630,000 beekeepers and 16 millions of hives

in the EU, producing 268,000 tons of honey in 2015 (https://ec.europa.eu/agriculture/honey_en). In recent years beekeepers have been reporting unusual weakening of bee numbers and colony losses, particularly in the USA and Western European countries (van Engelsdorp and Meixner, 2010). Since no single cause of declining bee colonies has been identified, several possible contributing factors have been suggested, acting in combination or separately. These include the effects of intensive agriculture and pesticide use, starvation and poor bee nutrition, viruses, environmental changes and attacks by pathogens or invasive species. The European Commission has taken important steps to diagnose and prevent bees' mortality, including actions on certain pesticides and a comprehensive study on honey bee colony mortality. There are many international research projects funded by the EU, like SMARTBEES, SWARMONITOR, SUPER-B, BEE DOC, COLOSS, EPILOBEE, etc.,

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as well as a strong support of beekeeping through national apiculture programs which aim at improving the general conditions for the production and marketing of honey and other apiculture products in the EU. In view of the important ecological and economic value of bees, there is a need to monitor and maintain healthy bee stocks, not just nationally but globally.

BEEKEEPING IN SLOVAKIA

In the territory of Slovakia, rational beekeeping started in the late 18th century, while new hive designs and movable frames were adopted in the second half of the 19th century. The first Union of Slovak beekeepers was founded in 1869, but regional organizations were active much earlier (Chlebo, 2010). The current structure of apiculture was formed 40 years ago by establishing 6 certified breeding stations and 58 reproduction stations (apiaries). Every breeding station keeps one of the main official lineages of the Slovak Carniolan honeybee (sometimes recognized as Carpathian Carnica sub-population). The main Slovakian lineages of the Carniolan bee include Košičanka, Carnica Sokol, Vojničanka, Tatranka, Sitnianska and Mošovčanka. In 2016 around 278,000 honey bee colonies and more than 15,500 beekeepers were registered in Central Registry of Beehives.

The Carniolan bee (*A. m. carnica*) is the most common and the only legal honey bee breed in Slovakia, originating from the mountainous north-western region of Slovenia (Kransko). Carnica's nearest relatives are the Italian bee (*A. mellifera ligustica*) and Macedonian bee (*A. mellifera macedonica*). These three breeds belong to C haplogroup (Ruttner, 1988). Carniolan bees are darker and larger than Italian bees. The characteristic yellow rings of *A. m. ligustica* being replaced by dark bands in *A. m. carnica*. Macedonian bee is also a dark one but with more yellow coloration at tergites and scutellum. The characteristic brood rhythm of *A. m. carnica* is a rapid build-up in spring, followed by a slow decline and an early cessation of brood rearing in the autumn (Ruttner, 1988; Milner, 2011). Carniolan bees are able to survive colder winters with a small winter cluster, they are very well adapted to the geographic and climatic conditions in the Central Europe and show high endurance, resistance to brood diseases and high honey yield (Chlebo and Kopernický, 2004).

The National Program of Stabilization and Development of Slovak Apiculture for 2010–2013 and other legislative documents justify the importance of beekeeping in Slovakia. The National Agricultural and Food Centre – Research Institute for Animal Production Nitra (NPPC – RIAP) is authorized as bee breeding

station and it manages the Central Registry of Hives in Slovakia. In addition, this institute has been approved as an artificial insemination service for all six breeding stations and offers morphometric control of breed purity and breed assignment. NPPC–RIAP has been involved in the COLOSS project aimed at monitoring of bee colony losses in the EU.

ANALYSIS OF BEE POPULATION USING MICROSATELLITES

Carniolan honeybee (*Apis mellifera carnica*) is considered an endangered subspecies of *Apis mellifera* in Slovakia due to its illegal crossbreeding with allochthonous subspecies. Morphometric studies have provided a large amount of information on the structure of *Apis mellifera* species, and morphometric parameters are still frequently used to determine the origin of bees. Measurements of morphometric characters are commonly taken according to Ruttner (1988), but this method cannot provide detailed information about the genetic structure of the population. Microsatellites are useful for testing of genetic variability on the level of subspecies and populations. During the last two decades, microsatellites have been used to analyze genetic diversity of honey bee populations in many countries (Garnery *et al.*, 1998; Dall'Olivo *et al.*, 2007; Uzunov *et al.*, 2014; Francis *et al.*, 2014; Nedic *et al.*, 2014). Despite more than a century-long tradition of beekeeping in Slovakia, genetic research of Slovak honeybees has not yet been done. Recently we started activities to adopt a molecular methods for characterization of Carniolan honeybee population in Slovakia using polymorphic microsatellite markers (Gasper *et al.*, 2013; Paál *et al.*, 2013; Šťastný *et al.*, 2014). A total of 79 samples of Slovak Carniolan honeybee from 19 regions of Slovakia, mostly collected at breeding and reproduction farms, were analyzed together with 85 reference samples of *Apis mellifera mellifera*, *Apis mellifera macedonica*, *Apis mellifera ligustica* and *Apis mellifera hybrid* (Buckfast). Ten microsatellite markers showing polymorphic information content higher than 0.5 were used for molecular analysis of bees. Bayesian and frequency-based methods implemented in a GeneClass software have been used to successfully assign 95 % of individuals to tested populations. After simulating 100,000 individuals using Bayesian Markov chain Monte-Carlo resampling, we assigned 87 % individuals to correct population. Population genetic structure was tested by the Bayesian clustering method incorporated in a Structure software (v 2.3.4). Overall, 92 % of the individuals were assigned to originally declared subspecies. According to Structure analysis, Slovak population of *A. m. carnica* seems to be

separated into two distinct clusters, showing the relatively high level of gene flow from *A. m. hybrid* (Buckfast) and *A. m. macedonica* subspecies. It might be caused by rebuilding the Slovak bee population after colony losses using queens of non-Carniolan origin.

ANALYSIS OF BEE POPULATION USING MITOCHONDRIAL DNA

Mitochondrial DNA is a small (about 16,000 bp), circular molecule that is transmitted intact by the queen to her offspring (workers and drones). The non-recombining maternal inheritance renders interpretation of mtDNA data straightforward, which in combination with relatively simple and inexpensive assays has made mtDNA one of the most popular markers in honey bee genetic studies. Based on morphological data 29 honeybee subspecies have been recognised (Engel, 1999; Sheppard and Meixner, 2003) and grouped in four morphological branches - C, M, A and O. The C lineage comprises seven subspecies: *A. m. carnica*, *A. m. ligustica*, *A. m. macedonica*, *A. m. siciliana*, *A. m. cecropia*, *A. m. cyprina* and *A. m. adami*. Upon the inclusion of mitochondrial molecular data, the O lineage has recently been identified as a sub-lineage of the African lineage A and been renamed to Z (Alburaki *et al.*, 2011). Finally, yet another mitochondrial lineage, named Y, has been identified in north-eastern Africa (Franck *et al.*, 2001), which also belongs to the context of the African lineages (Meixner *et al.*, 2013).

PCR-RFLP method based on amplification of tRNA^{leu}-cox2 region of mtDNA, followed by *Dra*I digestion, known as *Dra*I test (Garnery *et al.*, 1993) has been widely used in honey bee maternal identification, phylogeographical studies, understanding the complexities underlying natural hybrid zones, detecting introgression of foreign queens, among others (Meixner *et al.*, 2013). The combination of length and restriction site polymorphisms produced by the *Dra*I test has resolved over 100 haplotypes, nevertheless this method is unable to identify honey bees at the subspecies level. Therefore sequencing is the ultimate method for assessing mtDNA variation. In our study started in 2015, the tRNA^{leu}-cox2 intergenic region of about 570 bp was sequenced in 66 *A. m. carnica* workers, collected from 6 breeding farms and 21 reproduction farms in Slovakia. The sequence data were compared with complete mtDNA sequence of *A. m. ligustica* (L06178.1; Crozier and Crozier, 1993), as well as deposited sequences in GenBank using BLAST. We found that 71.2 % of samples correspond to 5 known haplotypes belonging to C lineage (C2c, C1a, C2d, C2e and C2z) and 28.8 % of samples have novel mutations in the sequenced region of mtDNA. The most frequent

haplotype C2c in Slovak bee population (36.4 %) is also typical and dominant for Slovenian *A. m. carnica* population (Sušnik *et al.*, 2004). Haplotype C2d, detected in 6.1 % of samples from Slovakia has been observed in Croatia, Canary Islands and in *A. m. macedonica* (Munoz *et al.*, 2009). Our group found this haplotype in several Buckfast bees from Slovakia and Denmark (unpublished data). Haplotypes C2e (4.5 %) and C2z (1.5 %) were detected in bees from Romania and deposited to GenBank (accession no. JQ977702.1, JQ754648.1). Interestingly, haplotype C1a, typical for *A. m. ligustica* was observed in 22.7 % bees from Slovakia. Čapek (2015) reported even higher occurrence of C1a haplotype (34.4 %) in 32 Slovak Carniolan honey bee samples. Six novel haplotypes are derived from C2c (11 bees, 16.6 %), C2v (4 bees, 6.1 %) and C2y (4 bees, 6.1 %).

CONCLUSION

To efficiently preserve Carniolan honey bee as a genetic resource in Slovakia it is necessary to know genetic characteristics of the population. Molecular markers like microsatellites and SNP in mitochondrial DNA could be useful for honey bee genetic studies. From our data we can conclude that honey bee population in Slovakia comprises predominantly Carniolan race (*A. m. carnica*). However, an apparent admixture of *A. m. ligustica*, *A. m. macedonica* and *Buckfast* bees has been observed. Therefore, simple and inexpensive genetic methods should be adopted and combined with standard morphometric tools to perform reliable bee race assignment, especially in case of breeding stations.

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