A BRIEF REVIEW ON THE DETECTION OF LOCAL HONEY BEE POPULATIONS IN GREECE BASED ON GENETIC STRUCTURE STUDIES

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Subspecies of *A. mellifera* in Greece

The honey bee *Apis mellifera* L. is one of the most studied invertebrates. The species has a wide range distribution in the Old World and has been introduced by humans to many other countries worldwide. Its ecological and economic importance and, moreover, its social organization, have stimulated research in a wide variety of fields.

Traditionally, the intraspecific taxonomy of *A. mellifera* has been based on morphology. At present, 29 subspecies of *A. mellifera* are recognized on the basis of morphometric characters[1,2,3,4,5]. These subspecies are also described as "geographic races" because their distributions correspond to distinct geographic areas. Ruttner based on the application of numerical taxonomy using characters of "classical" morphometry concluded that the *A. m. adami*, *A.m. macedonica*, *A. m. cecropia* and *A. m. carnica* subspecies of *A. mellifera* exist in Greece[6] (**Fig. 1**).

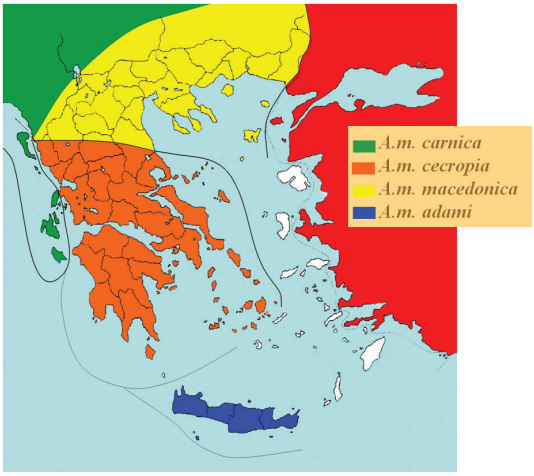

Fig. 1 *A.mellifera* subspecies in Greece according Ruttner 1988.

Specific studies on the genetic structure of honey bee populations in Greece

Classical morphometrics

There is only one comprehensive published study performed[7], (**Fig. 2**), in Greece, on the Greek subspecies of *A. mellifera*. According this study, that period there were no hybrid bees in Greece and different ecotypes existed in different geographical regions.

Geometric morphometrics

A new morphometry method, called geometric morphometrics, has been developed, based on the coordinates of landmarks located at vein intersections of the wings[8,9]. In a recent research honey bees collected from 32 different localities in Greece were studied based on the geometric morphometrics

1 Rutttner, 1988.
2 Rutttner, 1992.
3 Sheppard, Arias, Greech, Meixner, 1997.
4 M Engel**,** 1999.
5 Sheppard, Meixner, 2003.
6 Ruttner, 1988
7 Ifantidis, 1979.
8 Bookstein, 1991.
9 Smith, Crespi, Bookstein, 1997.

Fig. 2 Map of sampling regions (Ifantidis, 1979).

approach using, the coordinates of 19 landmarks located at wing vein intersections[10] (**Fig. 3**). The statistical analysis performed on the obtained data showed that honey bee populations from some Aegean islands (Chios, Astypalaia), from Kythira (an island close to Peloponnese) and from Crete island (Heraklion, Lasithi) can be discriminated based on this approach.

Alloenzymic approach

Alloenzymes (or also called allozymes) are variant forms of an enzyme that are coded by different alleles (number of alternative forms of the same gene) at the same locus. Many of the allozyme studies have contributed to understanding subspecies discrimination[11,12,13] revealing the existence of hybrid zones between them[14]. In addition, they have been used to analyze the phylogeny of *A. mellifera* on the basis of genetic distance matrices[15] and to detect significant genetic differences between commercial and feral honey bee populations[16].

Allozyme analysis of some Greek populations

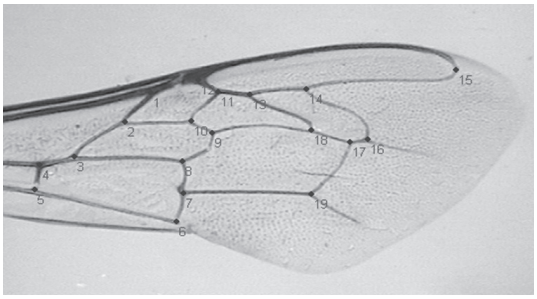

Fig. 3 19 landmarks located at wing vein intersection.

(Thrace, Macedonia, central Greece, Peloponesse) and populations on Crete showed the existence of a pure subspecies on Crete, as well as the possibility of distinguishing honey bee populations from northern and central Greece on the basis of allele frequencies[17].

On 2005 honey bee populations from different areas of Greece, Ikaria, Kasos, (Aegean islands), Kythira, Phthiotida (central Greece), Macedonia, were studied, using starch gel electrophoresis on ten different gene-enzyme systems[18] (**Fig. 4**).

Among the populations tested the highest percentage of polymorphic loci was found in the Phthiotida population. This observation could be due to gene flow, a hypothesis supported by the high frequency with which beekeepers in central Greece (including Phthiotida) are known to move their colonies because of changes in climate, flora, and other conditions[19]. The high level of polymorphism in central Greece contrasts with the situation in Kasos, which has the lowest percentage of polymorphic loci suggesting the maintenance of a rather pure honey bee population on that island.

Mitochondrial DNA (mtDNA) analyses

Mitochondrial DNA markers have been widely used to address population and evolutionary questions in *A. mellifera*, which was the first Hymenopteran for which the mitochondrial DNA sequence was published[20] (**Fig. 5**). The mitochondrial genome has been a very useful molecule for population genetic studies of *A. mellifera* and phylogenetic studies in the Genus *Apis*, as it contains regions with variable evolutionary rates.

10 Charistos, Hatjina, Bouga, Mladenovic, Maistros, 2014.
11 Sylvester, 1982.
12 Sylvester, 1986.
13 Daly, 1991.
14 Sheppard, McPheron, 1986.
15 Sheppard, Huettel, 1988.
16 Schiff, Sheppard, 1995.
17 Badino et al.,1988.
18 Bouga, et al, 2005.
19 Ifantidis, 1979.
20 Crozier, & Crozier, 1993.

Fig. 4 Sampling sites (Bouga et al., 2005).

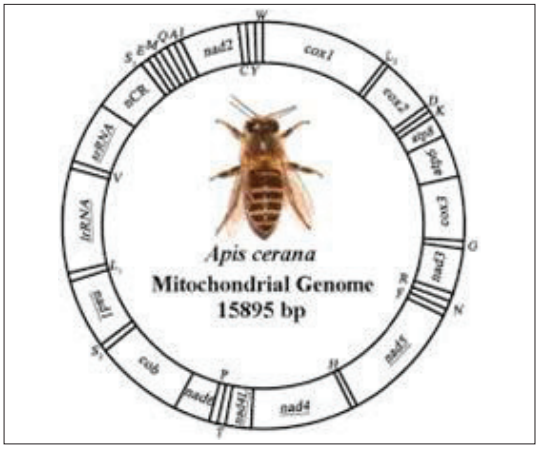

Fig. 5 Mitochondrial DNA.

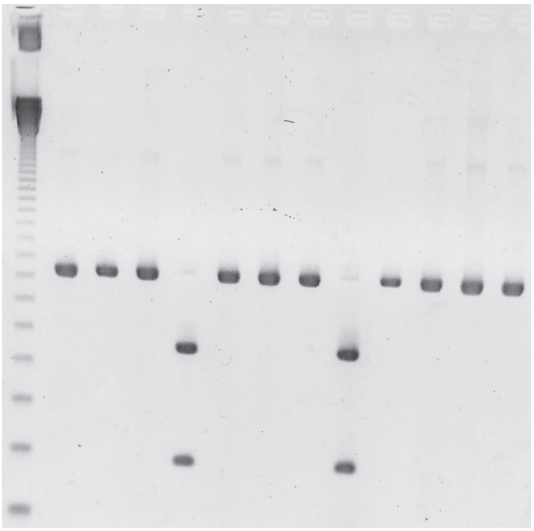

Fig. 6 *A. m. macedonica* diagnostic test.

The maternal inheritance of mtDNA, a property which has been demonstrated for honey bees denotes that all the workers and drones in a colony share the DNA of the queen[21]. Variation in the mtDNA of honey bees has been used to provide insight into their biogeography.

Diagnostic test for the discrimination of A. m. macedonica

Based on the results obtained using RFLP's molecular method (Restriction Fragment Length Polymorphisms) diagnostic patterns were revealed in the Macedonian honey bee population after the digestion of CO I (Cytochrome c oxidase subunit I, involving in the respiration) mtDNA gene segment, with the restriction enzymes (enzymes that cut DNA at or near specific recognition nucleotide sequences, known as restriction sites) NCO I and Sty I[22] (**Fig. 6**).

Sequencing analysis

On 2011 a study presents the first comprehensive sequencing analysis of *A. mellifera* subspecies occurring in Greece, and it is the first time that sequencing data from the ND5 mtDNA gene segment have been obtained at the population level[23]. Since honey bee mtDNA appears to be exclusively maternally inherited, the study of one worker per colony allows characterising the colony itself and the queen haplotype[24].

21 Meusel and Moritz, 1993.
22 Bouga, et al., 2005.
23 Martimianakis, et al., 2011.
24 Meusel and Moritz, 1993.

Haplotype 1	*A .m. cypria* (PYR,KOR, DAL1and 2), Aegean race near to *A. m. adami* (LMN 1,2,3,4, NIS 1,2), *A. m. adami* (CRE 4), *A .m. macedonica* (MAC 3,4,5,6 and 7, THR 1,2,3 and 4, JIJ), commercial strain (genetically improved *A. m. cypria* (SB), *A. m. cecropia* (SAR3, MES 1,3,4), *A. m. anatoliaca* (BAR), *A. m. meda* (OSM), *A. m. carnica* (LUB1,2,
Haplotype 2	*A. m. ligustica* (PER1,2, RAV1 and 2)
Haplotype 3	*A. m. cecropia* (SAR1), *A. m. carnica* (KEF2)
Haplotype 4	*A. m. cecropia* (SAR2)
Haplotype 5	*A. m. macedonica* (MAC1 and 2)
Haplotype 6	Aegean race near to *A. m. adami* (CHI1,2 and RHD1)
Haplotype 7	*A. m. adami* (CRE 2 and 3)
Haplotype 8	*A. m. cecropia* (MES 2), Aegean race near to *A. m. adami* (RHD 2,3,4), *A. m. ligustica* (FOR)
Haplotype 9	*A. m. carnica* (LIT)
Haplotype 10	*A. m. adami* (CRE 1)
Haplotype 11	*A. m. carnica* (GOR)
Haplotype 12	*A. m. cecropia* (LAR1 and 2)

Fig. 7 Haplotypes of honey bees studied (Martiminiakis et al., 2011)

Among the honey bees studied from Greece, one population from the island of Crete island was a unique haplotype (haplotype 10), as were populations from Larissa (Central Greece) (haplotype 12) (**Fig. 7**).

Conclusions

It is shown that based on the results of genetic studies on honey bees in Greece, using different genetic markers, there is a mixture of the populations due to the migratory beekeeping and the uncontrolled commercial practice.

Despite this, it also seems that there are still honey bee populations that there is the possibility to maintain local pure characteristics. There is the evidence that this happens especially to the bees from islands like Chios, Astypalaia, Kythira, Kasos and in a part of Crete island. It is also very interesting that something similar is for honey bee populations from Central Greece (Larissa).

The diagnostic test for *A.m.macedonica* is widely used for honey bees that it is supposed that belong to this subspecies that exists in this specific geographical area according Ruttner[25,26].

Perspectives

The research is ongoing using different approaches for the genetic study of honey bee populations in Greece.

The genetic markers can be applied in various honey bee populations of Greece, mainly on the bees from different islands, and there is the possibility to find out several local honey bees populations. It is well known that the local honey bee populations are better adapted to the local environmental conditions and they can survive more; so the detection of local bees can contribute to the sustainable development of apiculture.

25 Ruttner, 1988.

26 Stevanovic et al., 2010.

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