GENETIC STRUCTURE OF THE BEE FROM CRETE ISLAND (GREECE)

P. HARIZANIS, Maria BOUGA

Laboratory of Sericiculture - Apiculture, Agricultural University of Athens, 75, Iera Odos, 118 55, Athens, GREECE
E-mail: mbouga@aua.gr

Abstract

The genetic structure of honey bee populations from different areas of Crete Island (Greece), corresponding to Apis mellifera adami*, (according to morphometric analysis Ruttner, 1988), were studied by means of RFLP's analysis of two mtDNA gene segments.*

Sixty samples were studied, taken from different queens. Total DNA was extracted, then 16s rDNA (965 bp) and CO I (1028 bp) gene segments were amplified using PCR. Seven and six restriction enzymes had at least one recognition site at 16s rDNA and CO I gene segments respectively.

Intrapopulation variation was revealed as regards the CO I gene segment digested with BstU I restricted enzyme.

It was found out that the genetic structure of these populations perhaps has been changed because of migratory beekeeping and commercial breeding, during last two decades. It seems that in Crete Island there are no pure populations of Apis m. adami*. Our data being compared with those from our previous research show that the honey bee from Crete seems to be similar to the honey bee populations from other areas of Greece.*

Keywords*:* Apis mellifera adami */ honey bee / mtDNA / genetic structure / Greece*

Introduction

Traditionally, intraspecific taxonomy of the honey bee *Apis mellifera* has been based on morphology. At present, 26 subspecies of *A. mellifera* are recognized on the basis of morphometric characters (RUTTNER 1988, 1992; SHEPPARD et al., 1997).

More recently, genetic tools, particularly DNA sequence analysis and allozyme electrophoresis, have been applied to the study of honey bee diversity. Mitochondrial DNA (mt DNA) has a number of properties that make it a favourite tool in systematics and population biology. It is generally maternally inherited without recombination. Thus, it enables precise detection of foreign haplotypes in populations. Only maternal inheritance of mtDNA has been demonstrated for honey bees (MEUSEL and MORITZ, 1993), thus all the workers and drones in a colony are sharing the same mtDNA as the queen. However, one has to take into account several technical improvements more or less recently introduced in hive management as the importation of foreign queens and migratory beekeeping.

On the basis of morphometric investigations, the honey bee populations from the Crete Island (South Aegean Sea, Greece) have been described by RUTTNER (1980) as *Apis mellifera adami*. According to RUTTNER (1988), *Apis m. adami* shows marked morphological similarities to Near East subspecies. Alloenzymic analysis (BADINO et al*.,* 1988) of honey bee populations in continent Greece (Thrace, Macedonia, Central Greece and Peloponesse) and in the island of Crete showed that there was a pure race in the island of Crete.

In our research honey bee populations from different areas of Crete Island, corresponding to *Apis m. adami*, according to morphometric analysis (RUTTNER 1988), were studied by means of RFLP's analysis of two mtDNA gene segments.

Our aim was to study the genetic structure of these populations, to find out if there is still a pure race of honey bee populations in Crete Island, and if there is coincidence with RUTTNER's (1988) morphometric analysis.

Materials and Methods

Bees from 60 colonies were collected from different areas from Crete Island (Chania, Rethymno, Heraklio and Lasithi). The sampling sites are shown in Figure 1. The honey bee populations from these areas correspond to the race *A.m. adami* according to morphometric analysis (RUTTNER, 1988).

The samples were transported to the laboratory alive, and stored at -80 ^{0}C until used. Total DNA was extracted from each individual, according to the protocol of HUNT and PAGE (1992), after minor modifications (BOUGA et al., 2003).

Mitochondrial DNA variation was analysed by RFLP's, performed on PCR amplified products. Two sets of primers were used for the amplification of 16s rDNA and CO I gene segments. The primers used for the 16s rDNA segment was the pair 5´-CAACATCGAGGTCGCAAACATC-3´ and 3´-AGTTGGGACTATGTTTTCCATG-5´ (Nielsen et al., 1994) and for the CO I segment was the pair 5´-GATTACTTCCTCCCTCATTA-3´ and 3´-AATAAGTCTGATAGGTCTAA-5´ (NIELSEN et al., 1999). The polymerase chain reaction (PCR) (SAIKI et al., 1988) was performed as described in BOUGA et al. (2003) as well as PCR conditions.

Figure 1 - Sampling sites in Crete Island: Chania, Rethymno, Heraklio, Lasithi.

Amplified mtDNA segments were digested with restriction enzymes. The informative restriction enzymes used for the16s rDNA gene segment were Ssp I, Dra I, Hinc II, EcoR I, Pst I and Alu I and for the CO I gene segment were Sau3A I, Fok I, Bcl I, Ssp I, BstU I, and Xho I.

The digested segments were then separated electrophoretically on 2% agarose gels in 0.5X TBE buffer, stained with ethidium bromide and visualized under UV light. The sizes of DNA fragments were compared to the PCR marker (Promega) run on the same gel and were calculated using DNAfrag 3.03 (NASH, 1991) program. A letter in order of appearance identified single restriction patterns. Composite genotypes for each individual were then defined from all the restriction patterns of the two mtDNA segments.

Results

The sizes of PCR-amplified mtDNA segments for all populations were found to be about 964bp and 1028bp for 16s rDNA and CO I gene segments respectively. Seven and six restriction enzymes had at least one recognition site on the amplified 16s rDNA and CO I segments respectively. Fragment patterns produced by each restriction enzyme for the two mtDNA segments are presented in Tables I and II.

Table I

Fragment size estimates (in base pairs) of all fragment patterns observed on mtDNA 16s rDNA gene segment among the populations studied

16s rDNA

Sau3A I		Ssp I		Dra I		Hinc II		EcoR I		Pst I		Alu I	
	A		A		A		A		A		A		A
548	—	628	—	557	—	598	—	492	—	621	—	572	—
416	—	336	—	407	—	366	—	472	—	343	—	392	—

Table II

Fragment size estimates (in base pairs) of all fragment patterns observed on mtDNA CO I gene segment among the populations studied

CO I

Sau3A I		FoK I		Bcl I		Ssp I		Bstu I			Xho I	
	A		A		A		A		A	B		A
371	—	476	—	465	—	487	—	1028		—	616	—
349	—	425	—	326	—	277	—	658	—		412	—
280	—	127	—	237	—	264	—	370	—			
28	—											

Intrapopulation variation was revealed as regards the CO I gene segment digested with BstU I restricted enzyme as it is shown in Figure 2.

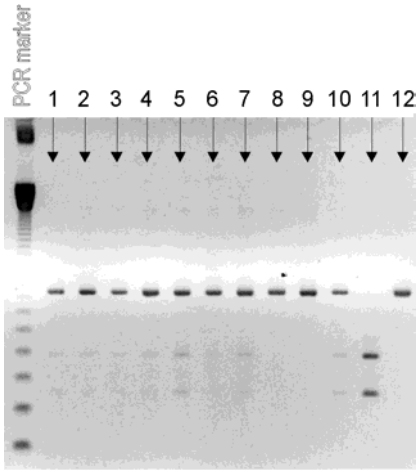

Figure 2 - Restriction fragment patterns after digestion with the BstU I enzyme in honey bee populations from: (1-3) Chania, (4-6) Rethymno, (7-9) Heraklio, (10-12) Lasithi.

The two haplotypes (composite genotypes), which were detected in the populations studied (letters in order correspond to the same order of fragment patterns shown in Tables I and II), the haplotype frequencies, and the sample size are presented in Table III.

Table III

Composite genotypes (haplotypes), haplotype frequencies and sample size (N) of all the populations studied

Haplotype	Composite genotype	Sample locality			
		CHANIA	RETHYMNO	HERAKLIO	LASITHI
Type 1	AAAAAAAAAABA	1.000	1.000	1.000	0.933
Type 2	AAAAAAAAAAAA				0.067
	N	15	15	15	15

Discussion

The study of mtDNA is of a particular interest in honey bees since it is the ideal marker of the colony – all individuals in the colony are sharing the same haplotype (excluding mutations), according to maternal inheritence.

The results of this study being compared with the results of our previous work (BOUGA et al., 2003) show that the honey bees from Crete Island look alike the honey bees from other areas of Greece, may be as a result of “importation” of queens from these areas. The results of Classical Morphometric Analysis

(HARIZANIS et al., 2001) on the same samples being compared with those of honey bees from Macedonia (North Greece) show that there is no statistically significant differentiation among these populations. The study based on Geometric Morhometric Analysis (HATJINA et al., 2002) shows that there is a little variability in honey bees from Crete Island.

Our results show that there is a unique haplotype in honey bees from Crete Island in frequency with low value. It seems that this haplotype is as a result of importation of foreign queens or it is of the pure race *Apis m. adami*.

In general, perhaps the genetic structure of honey bee populations has been changed because of migratory beekeeping and commercial breeding, during last two decades, and our data seem not to be in coincidence with RUTTNER's (1988) morphometric analysis, as concerning the existence of *Apis m. adami*.

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