

MORPHOMETRIC, ALLOZYMIC AND MTDNA VARIATION IN HONEYBEE (*APIS MELLIFERA CYPRIA*, POLLMAN 1879) POPULATIONS IN NORTHERN CYPRUS

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Abstract

Morphometric, allozymic and mtDNA variability of honeybee populations *Apis mellifera cypria* from Northern Cyprus were investigated. Six populations (40 colonies) were analysed using 39 morphometrical characters. The Cytochrome B and Carboxyl Oxidase regions of the mtDNA were tested for *Bgl* II and *Hinf* I restriction enzyme profiles and the *Dra* I restriction fragment length polymorphism of the COI-COII intergenic region was also investigated. The mitochondrial ND2 region of was sequenced and compared with published sequences of other subspecies. Additional 55 colonies sampled later from the same localities were studied for allozyme variation using six enzyme systems. Very low levels of allozyme variation were detected, with an average heterozygosity of 0.006 ± 0.005 . The analysis of all mtDNA regions confirmed the European origin of Northern Cyprus honeybee populations. The digestion profiles found in Northern Cyprus were the same as reported for east European honeybees. The COI-COII intergenic region only possesses the Q sequence and the digestion profiles correspond to the C1 haplotype. Analysis of morphometric data, however, placed the honeybees from Northern Cyprus into the Oriental morphological lineage, together with *A. m. anatoliaca*, *A. m. meda*, *A. m. syriaca*, and *A. m. caucasica*. A phylogenetic tree constructed from the ND2 sequence data placed the Cyprus honeybees into the mitochondrial C- lineage, which includes both the morphological C- and O-lineages.

Keywords: Cyprus / genetic variation / *Apis mellifera cypria*

Introduction

Cyprus honeybee (*A. m. cypria*, Pollmann 1879) is another example of island subspecies like *A. m. ruttneri* in Malta, *A. m. adami* in Crete, *A. m. sicula* in Sicily. Similar to other island subspecies Cyprus honeybees are small in size, however their exotic appearance make them favorable among beekeepers in early days (RUTTNER, 1988).

In his morphometric studies, RUTTNER (1992) placed Cyprus honeybees in Oriental branch with *A. m. anatoliaca*, *A. m. syriaca*, *A. m. meda*, *A. m. caucasica*. As one can expect from its geographic position, *A. m. cypria* looks like their neighbours. They have almost equal distance to *A. m. anatoliaca* (74.6), *A. m. syriaca* (69.8), and *A. m. meda* (87.3). When they compared to *A. m. anatoliaca*, cyprus bees are smaller than Anatolian honeybee. Relative to body size their proboscis and legs are longer, but wings are shorter. Among oriental branch they have the longest and most slender abdomen (Index St6 84,6). Cubital index is very high in Cyprus bees (2.72). The most noticeable characteristic of Cyprus bees is the color of the abdomen. Cyprus and Syrian honeybees have the same type of color patterns which considered to be close relative to Egyptian bee (*A. m. lamarkii*, formerly, *A. m. fasciata*) (BR. ADAM, 1983). In terms of behavioural characteristics, Cyprus bees are very similar to *A. m. anatoliaca*, they are very aggressive and tend to swarm easily (BR. ADAM, 1983).

Despite being an island subspecies, it was observed that *A. m. cypria* has high morphometric variation (RUTTNER, 1988). Recent honeybee introductions from Turkey to Northern Cyprus could result in some changes in morphometry and one can ask how much these recent introductions affected honeybee colonies in Northern Cyprus. Besides there is no detailed study about allozyme, and genetic variation on DNA level.

To further address these questions related to the genetic variation in *A. m. cypria* and its relationship within O lineage, detailed sampling was undertaken. Here we report the results of complete analysis using mtDNA, morphometry and allozymes on Cyprus honeybees, *A. m. cypria*.

Materials and Methods

Total 40 colonies were sampled from 6 locations in Northern Cyprus. Samples were preserved in 90% ethanol for both morphometric and mtDNA analysis. An additional 55 colonies however were collected from mountainous area and frozen for biochemical variation in Northern Cyprus honeybee populations.

Morphometric analysis: A total 18 colonies was subjected to morphometric analysis. Between 11-15 worker bees per sample were dissected and measured for 39 morphometric characters according to RUTTNER et al. (1978). Most of the characters were measured with the help of a CCD camera with a morphometric measurement program (MEIXNER and MEIXNER, 1994). Pigmentation and pilosity were measured with a dissection microscope and an ocular micrometer. The statistical analysis was performed with the reference samples from the database of the Institut für Bienenkunde, Oberursel.

Allozyme analysis: After preliminary analysis, an additional 55 colonies were sampled from Mountainous area in detail for allozyme variation. Variability in six enzyme systems (*Pgm-1* and *Pgm-2*, *Hk*, *Mdh*, *Me*, *Est*, and *Pgi*) were utilized as described before (KANDEMIR and KENCE, 1995). Gene frequencies, summary of genetic variation parameters and deviations from H-W equilibrium were determined by using BIOSYS-1 program (SWOFFORD and SELANDER, 1981).

DNA Analysis: Total nucleic acids were extracted from 40 colonies followed methods described in SHEPPARD and MCPHERON (1991) and consisted of phenol-chloroform extraction protocol developed for high yield of intact circular mtDNA. DNA extractions were stored in -80°C . For mtDNA analysis, *COI*, *Cyt B* and *COI-COII* intergenic regions were amplified via polymerase chain reaction and digested with *Hinf-I*, *Bgl II*, and *Dra-I* respectively following the manufacturers recommendations (Table I). Gel fragments for *Hinf-I* and *Bgl II* digestions were separated on 2.5% agarose gel consisting of 1% standard BIO-RAD agarose and 1.5% Nu-Sieve agarose. *Dra-I* restriction digestion fragments were however separated on 10% and 7.5% polyacrylamide gels. Then gels were stained with ethidium bromide and photographed under UV light for documentation.

mtDNA regions, primer pairs and restriction enzymes used

Table I

Regions	Primer pairs	Rest. Enzyme	References
COI-F COI-R	5'-TTAAGATCCCCAGGATCATG-3' 5'-TGCAAATACTGCACCTATTG-3'	<i>Hinf-I</i>	Sheppard et al., 1994
Cyt B- F Cyt B- R	5'-TATGTACTACCATGAGGACAAATATC-3' 5'-ATTACACCTCCTAATTTATTAGGAAT-3'	<i>Bgl-II</i>	Sheppard et al., 1994
COI-COII- E2 COI-COII-H2	5'-GGCAGAATAAGTGCATTG-3' 5'-CAATATCATTGATGACC-3'	<i>Dra-I</i>	Garnery et al., 1993
ND2-ILE ND2-L1	5'-TGATAAAAGAAATATTTTGA-3' 5'-GAATCTAATTAATAAAAAA-3'		Arias and Sheppard, 1996

Results

Morphometry

Preliminary results showed that Cyprus bees belonged to Oriental branch. All samples from Cyprus were grouped with *anatoliaca*, *syriaca*, and *caucasica* subspecies. *A. m. lamarkii* was the closest subspecies to this cluster (Figure 1).

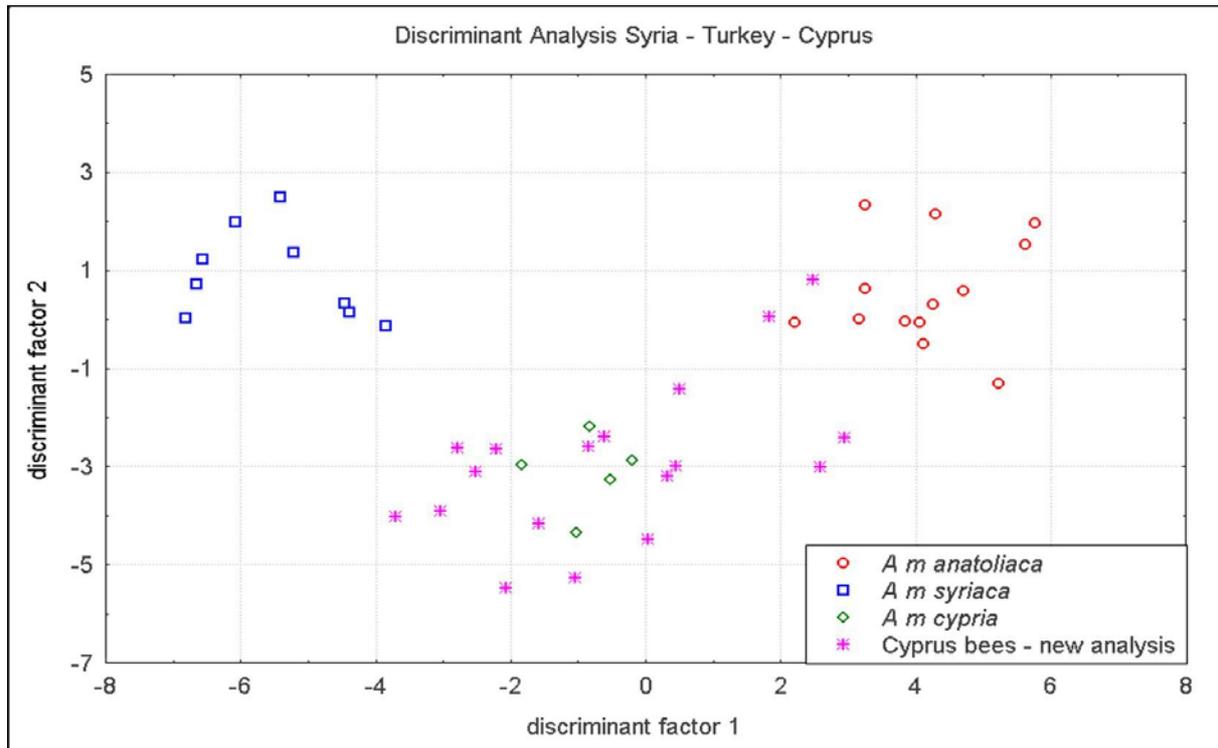


Figure 1 - Factor analysis of *A.m. anatoliaca*, *A.m. syriaca*, *A.m.cypria* (reference samples from Oberursel) and samples from Northern Cyprus.

Allozymes

Out of six enzymes assayed for biochemical variation, four were found to be polymorphic in Northern Cyprus honeybee populations. All enzyme systems were in Hardy-Weinberg equilibrium ($P > 0.05$). The overall heterozygosity for Northern Cyprus bees was calculated as 0.006 ± 0.005 .

mtDNA

CO-I region: Restriction of COI region with *Hinf I* restriction enzyme did not produce any restriction fragment. All colonies have the same type of undigested fragment on an agarose-gel resembling East Mediterranean C lineage.

Cyt B region: However digestion of this region resulted in two fragments which is a diagnostic site for European honeybees. All Cyprus colonies have this site indicating that they belong to C mtDNA lineage.

COI-COII region: There were no genetic variation in this region. Amplified PCR product showed that this region consists of only Q sequence as it is found in all C lineage. The digestion with *Dra-I* restriction enzyme did not yield any variation either. They all have the same type of restriction digestion profiles.

ND2 sequencing: ND2 gene from mitochondrial DNA was sequenced in both directions using ILE and L1 primers. A total of readable 650 bp was combined with other ND2 sequences deposited in the GenBank and were used in phylogenetic analysis. Three main clusters were visualized in phylogenetic tree constructed by Neighbor Joining. A, C and M lineages were clearly distinguished and O lineage was inserted into the A lineage. Among these lineages, Cyprus colonies were placed in the C lineage (Figure 2).

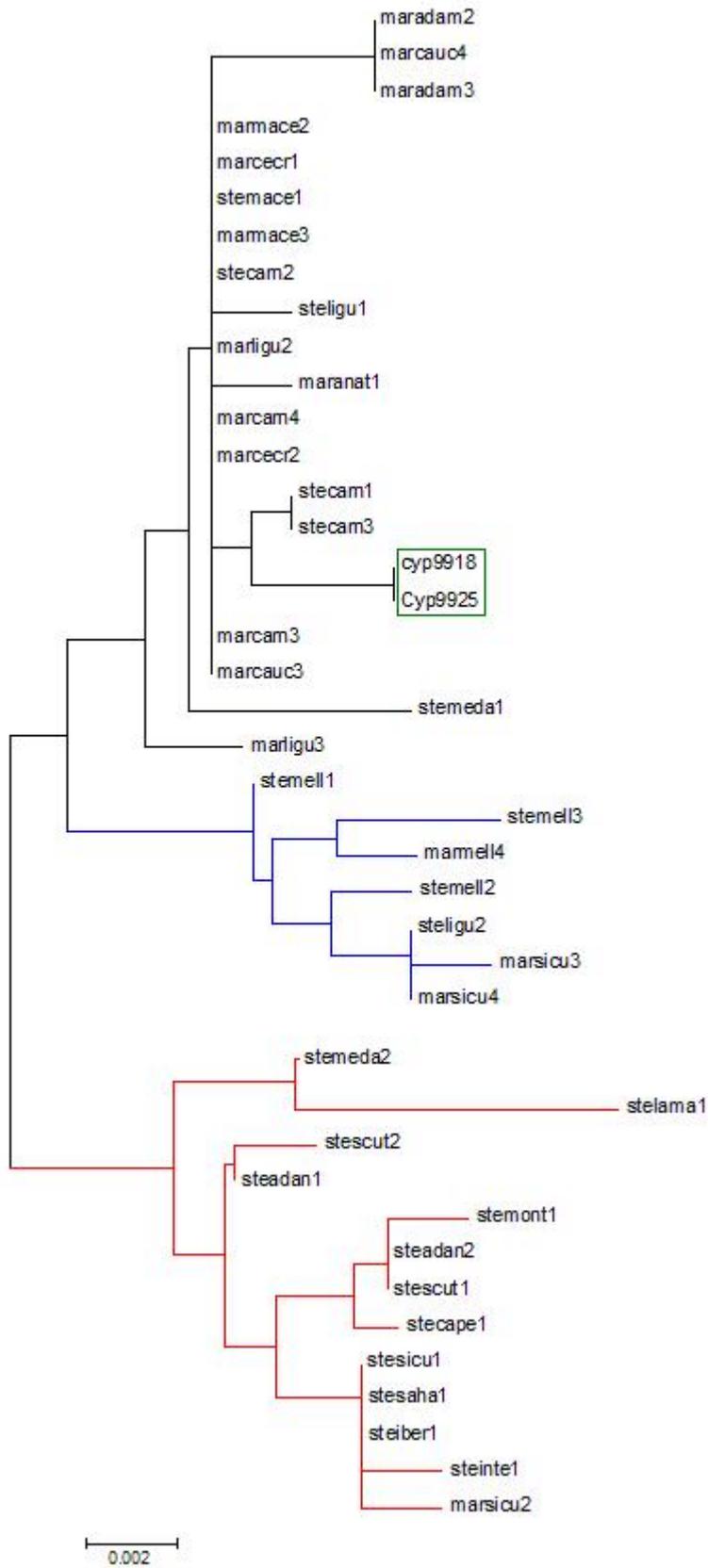


Figure 2 - A putative NJ tree showing the phylogenetic relationship of Northern Cyprus bees with the other honeybee subspecies based on 1000 replication (Black: C-lineage; Blue: M-Lineage and Red: A-lineage).

Discussion

Morphometry

In most cases, a clear allocation of the bees from Northern Cyprus to the samples classified as *A. m. cypria* from the reference collection resulted from the Morphometric analysis (Figure 1). Northern Cyprus bees were clustered with the O lineage subspecies as expected. New set of bees showed that Cyprus bees are still maintaining their morphometric structure except the effect of recent introduction as it was observed in the Factor analysis (Figure 1). Among the bees collected from the west of Northern Cyprus (Citrus orchards), there are some colonies which were more close to *A. m. anatoliaca* which is not surprising. Only three of samples - all from modern beekeepings - were addressed as *A. m. anatoliaca* in the analysis and are probably to be attributed to imported colonies from the mainland to Northern Cyprus. Therefore it can be concluded from these results that the bee population of the island to a large extent is still in the original form. From the analysis we also observed that *A. m. cypria* is more similar to *A. m. syriaca* and *A. m. anatoliaca* than *A. m. adami* and *A. m. meda*.

Allozymes

Morphometric analysis revealed that the western part of Northern Cyprus was influenced from the recent introductions. So that the new sampling was mostly done on the mountainous area to detect the biochemical variation in Northern Cyprus bees.

However there is not much variability in Northern Cyprus bees as might be expected from being an island subspecies. In addition to that this area is not polluted from an introduced genes, this could be the other reason of having low levels of heterozygosity in mountainous area. On the contrary there was a little genetic differentiation (F_{st} value is 0.038) among populations in this area. In terms of *Hk* and *Est* enzyme system populations are heterogenous. The presence of low levels of variability could be another support to morphometrics in terms of keeping an original status of Cyprus bees.

mtDNA

All samples from Cyprus showed the same mtDNA profile, which proves them clearly as a member of the mitochondrial C-Lineage. All different type of mitochondrial analysis (Cytochrome B region Bgl II digestion, Carboxyl Oxidase I region HinfI digestion, COI-COII region and Dral digestion) agreed upon this conclusion. In most of the analysis Cyprus bees are very similar to the neighbouring subspecies from Syria and Turkey, whereby populations from those countries exhibit much higher variability. Also the result of the sequence analysis of ND2 region showed that the samples of *A. m. cypria* were a member of the C-Lineage although they are morphometrically in O lineage.

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