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MORPHOMETRIC AND MtDNA ANALYSIS IN HONEYBEE POPULATIONS (*Apis mellifera* L.) OF NORTH AND NORTHWEST IRAN

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ABSTRACT. Morphometric and mitochondrial DNA analysis were used to find out the extent of variation in honeybee populations in Iran. Honeybee samples were collected from Five location in north and north west of Iran. 23 characters were measured for each sample in morphometric studies. *Dra*I restriction enzyme was used for restriction of amplified mtDNAand restricted fragments were separated in polyacrylamide gel. In morphometric analysis, five Iranian populations clustered into four distinct groups by PCA. The *Dra*I restriction exhibited 4 bands with sizes of 422, 64, 47, and 41 base pair, which corresponds to C1 pattern.

KEY WORDS. *Apis mellifera* L, morphometric, mitochondrial DNA, Iran

INTRODUCTION

Two hypotheses have been stated about evolution of honeybees. According to the one hypothesis, honeybees evolved in near Ethiopia in Africa and extend over Africa and then Middle East and Europe (Wilson 1971). However the second hypothesis explains that, although representatives of most of bee species were original to all the continents, bees belonging to the genus Apis were originally to be found only in the Old World, namely southern part of Caspian Sea and they were distributed to Africa and Europe (Ruttner 1988). The aim of this study is to determine amount of genetic diversity in 5 Iranian honeybee populations. Therefore morphometric studies and mitochondrial DNA analysis used to find out the extent of variation in honeybee populations in Iran. Migratory bee keeping and importing of queens are two main factors in increasing of homozigosity and loosing of diversity between local honeybee populations. Genetic diversity between populations can be used in studies for improving of honeybee

races which are economically valuable and more resistant to diseases. Our study focuses on honeybee populations of north-western Iran.

MATERIAL AND METHODS

Honeybee workers collected from 5 different locations belonging to 5 provinces Urmia, Sarein, Viladereg, Amol and Alborz regions which are located in north and North West of Iran.

Morphometric Measurements:

For each sample 23 characters were measured. 11 angular, 8 metric characters of forewing, and 4 metric of hindlegs (Figure 1). In this study NTSYS (Rohlf, 1992) computer program was used for analyzing morphometric data. Principle Component Analysis (PCA) was performed by SYNTAX software.

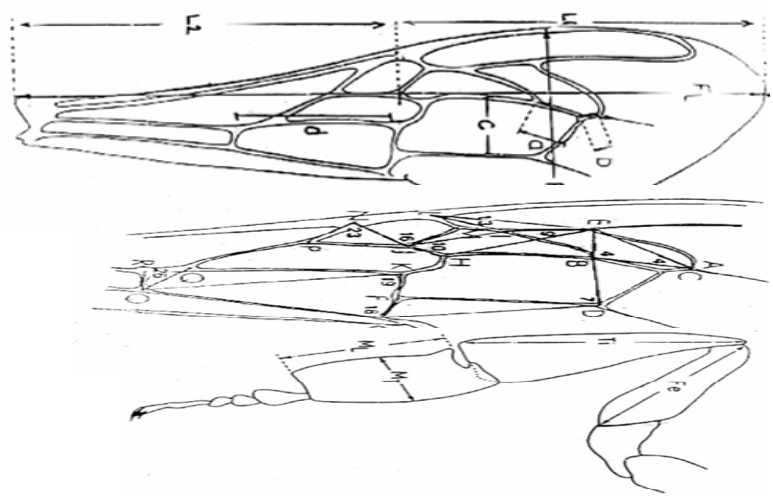

Figure 1. *23 parameter measured in forewings and hindlegs*

Mitochondrial DNA Amplification by PCR:

DNA extraction was done from homogenized thorax using phenol-chloroform extraction and ethanol precipitaion method. The tRNAleu-COII intergenic region of the mtDNA was amplified with the corresponding 1x buffer, 2 mM Mgcl2, 0.3mM of each dNTP, 0.25µM of primers E2 (5'-GGCAAGAATAAGTGCATTG-3') and H2 (5'-CAATATCATTGATGACC-3') (Garnery *et al.*1992). 3 units of Taq DNA polymerase in a total volume of 100 µl. Polymerase chain reaction were submitted to an initial denaturation of 5 minutes at 97 ºC, followed by 30 cycles of 95ºC for 30 seconds, 50ºC for 90 seconds and 72ºC for 90 seconds. 5µl of PCR product was electrophorased in a 2% agarose gel in order to check the result of the reaction. In a sterile tube 13.3µl sterile deionised water, 2µl restriction enzyme buffer, 0.2µl acetylated BSA (10µg/µl) and 3µl DNA, were mixed by pipetting. Then 0.5µl of *Dra*I restriction enzyme (TTTAAA) was added to the mixture.

After mixing and adding 1 drop of mineral oil on each tube, the mixture was incubated at 37ºC for 3-4 hours. Restricted fragments were separated in 10% polyacrylamide gel for separating long fragments and in 3.6% gel for separating shorter fragments.

RESULTS AND DISCUSSION

In morphometic analysis the first three components explain 94.82% of the total variation. Five Iranian populations clustered into four distinct groups in PCA. Although Urmia, Amol and Alborz were clustered into three separate clusters, Sarein and Viladereg were grouped closer and distinctly separated from other Iranian populations (Figure 2).

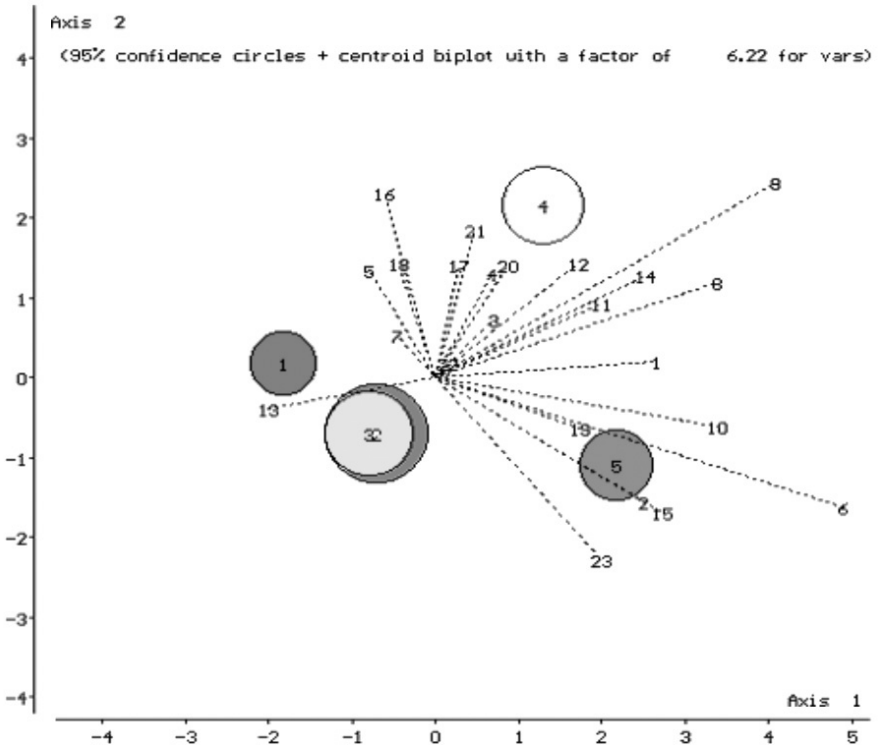

Figure 2. *Clustering of five Iranian honeybee populations on the bases of morphometric analysis, axis 1 vs. 2 (1:Urmia, 2: Sarein, 3: Viladereg, 4:Amol, 5: Alborz).*

Among the 23 variables, in the first component although FL, BDG and d variables have the highest loading, MW and FB show negative loading. FGD and ML gave the highest positive loading and BDG gave negative loading in second component. The third component is positively loaded by 'a' and ML and negatively loaded by EAB variables. The highest negative correlation is observed between HEI and OKF variables. Restriction fragment data obtained from these studies, showed the existence of only one haplotype in populations. The *Dra*I restriction exhibited 4 bands with sizes of 422, 64, 47, and 41 base pair, which corresponds to C1 pattern (Figure 3).

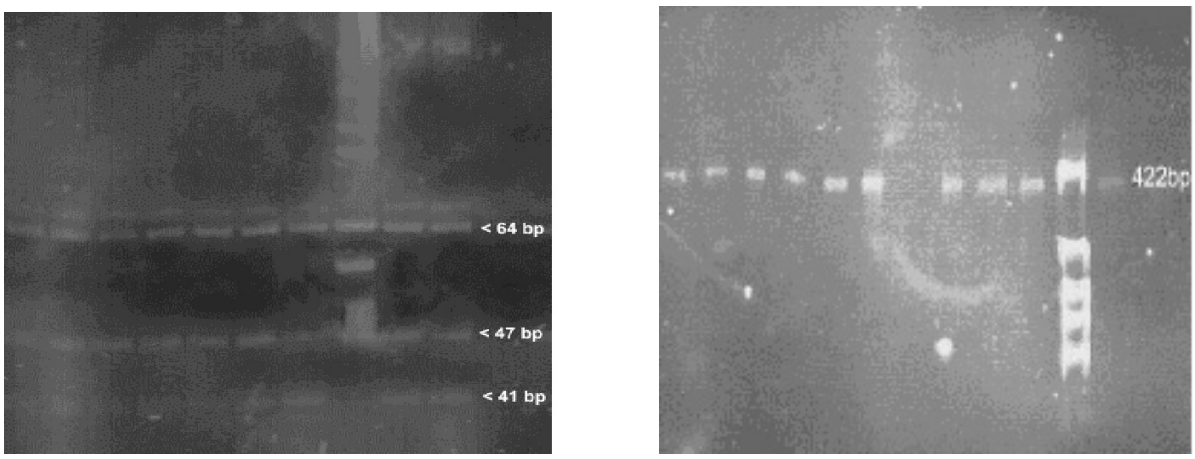

Figure 3. *Fragments of mtDNA in 3,6% and10% acrylamide gel.*

This pattern is similar to the results reported by Garnery 1993. C1 pattern also was observed in Turkish honeybees especially in populations of eastern and northeastern Turkey. The data obtained from mtDNA analysis did not suggest apparent distinction between *A. m. meda* and other Anatolian populations (Palmer *et al*. 2000). All the samples studied presumably belonged to *A.m.meda* subspecies.

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