GENETIC ANALYSIS FOR SOME HONEY BEES FROM MIDDLE EAST WITH RAPD-PCR MARKERS

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INTRODUCTION

In spite of morphometrical analysis is very powerful method in discriminating populations and morphological characters but have several drawbacks such as their polygenic determinism, which hinders their use in population genetic studies (Estoup *et al.* 1995). To understand the dynamics of selection in natural population of honeybees one needs suitable inheritable markers. Allozymes have proved to be very useful in many other species but have brought little additional information to our understanding of honey bees. The main reason is the low level of allozyme polymorphism (Packer and Owen, 1992). In recent years, Various DNA markers have been developed which avoid such problems and several authors have shown the power of nuclear DNA to reveal genetic variability in honey bees (Hall, 1990). Mitochondrial DNA (mtDNA) has been shown to be very useful for classification of honey bees. Subspecies specific mtDNA variability has been found repeatedly used to analyze the biogeography of honeybees (Garnery *et al.*, 1992). Nucleotide sequence data of mtDNA have been used to produce phylogenetic trees of the 4 *Apis* species: *cerana, dorsata, florea* and *mellifera* (Garnery *et al.* 1991).

Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) is another category of DNA markers evidenced in honey bees. These markers have been successfully used to build a genetic map of 300 loci (Hunt and Page, 1994). Herein the present studies used RAPD-PCR tools for analysis zing the genetic relationship among honeybees collected from different region of Middle East. Two subspecies collected from Egypt, (*Apis mellifera lamarckii* and *A. m. carnica*); two species from Sudan (*A. m. sudanensis* and *A. florea*) and only one species fromYemen (*A. m. yemenitica*).

MATERIAL AND METHODS

100 mg tissues of each sample of honeybee workers of *Apis mellifera lamarckii* (E); *A. m. carnica* (C); *A. m. sudanensis* (S); *A. florea* (F); and *A. m. yemenitica* (Y) were ground in a small mortar under liquid nitrogen with a pestle. 1.5 ml of homogenate buffer (Flook *et al.*, 1992) and incubated at 65 °C for 80 minutes with occasional inverting. Phenol was buffered and used according to the method discussed by Ausubel *et al.* (1989). 5 ug RNAse was added and incubated at 37 °C for 1 hour. Samples were diluted by adding TE buffer to final concentration of 25 ng / ul. Genomic DNA was analyzed with RAPD- PCR technique according to the method described by Williams *et al* (1990) with slight modification. Denaturation, annealing, extension were repeated 35 cycles at 94 °C, 40 °C, 72 °C for 1 min., 1 min. and 2 min. respectively. The post extension was for 5 minutes at 72 °C. DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. The patterns were photographed by using Polaroid Camera.

Statistically, similarity index (Nei and Li, 1979) and Unweighed pair-group arithmetic average (UPGMA) cluster analysis (Lynch and Milligan, 1994) were used for data obtained.

RESULTS AND DISCUSSION

RAPD-PCR products were generated by using 10-base long oligonucleotides of a five rbitrary sequence on genomic DNA isolated from honeybees. In order to investigate the differences of genetic materials, Patterns of interest were those that produced clearly distinguishable major products. By using the arbitrary sequences, the number of produced fragments were 38 distributed as 8, 5, 9, 8 and 8 fragments with primer OPA-1, OPA-3, OPA-5, OPA-7 and OPA-9 respectively (Figure 1). The molecular sizes of these fragments ranged from 202 bp to 1015 bp. The highest molecular size was detected by using primer OPA-3 with *A. m. lamarckii* and *A. m. sudanensis* where the lowest molecular size was detected in all honeybees by using primer OPA-1. The highest number of monomorphic fragments (6) were produced by using primer OPA-9, in contrast only one monomorphic fragment was generated by using primer OPA-3. DNA generated by using RAPD-PCR was analyzed statistically by the similarity index and the obtained data showed that the similarity among different samples were high and ranged from 0.74 to 0.92. Where the highest value was present among bees collected from *A. florea* and *A. m. yemenitica* (0.92), while the lowest value was between *A. m. lamarckii* and *A. florea* 0.74 (Table 1). By using random primer OPA-1, eight

fragments were produced in all investigated honeybees. The molecular sizes of these fragments ranged from 792 to 202 bp. Three monomorphic fragments of molecular sizes 429, 353, 295 and 202 bp were detected in the five samples of honeybees except fragment (353 bp) was absent in *A. m. carnica*. There were five DNA fragments were generated by using OPA-3. This primer showed complete similarity between honey bees *A. m. lamarckii* and *A. m. sudanensis*. One DNA fragment of molecular size 355 bp only was present as common (monomorphic) fragment among honeybee samples. The total number of fragments of RAPD-PCR patterns produced by using primer OPA-5 were nine fragments. Out of these fragments, four fragments were monomorphic for different samples of honey bees and their molecular sizes were 540, 472, 383 and 354 bp. While six fragments were present as common fragment between honeybees from Egypt, *Apis mellifera lamarckii* and *A. m. carnica* and also six fragments were monomorphic between honeybees collected from Sudan *A. m. sudanensis* and Yemen *A. m. yemenitica*. Primers OPA-7 and OPA-9 produced 16 fragments (eight fragments for each), ten fragments detected as common fragments by using these primers. So the two primers (7 & 9) showed the highest value of similarity among honeybees.

UPGMA cluster analysis revealed the presence of four nodes for different samples of honeybees (Figure 2 and Table 2). The first node includeded two species of honeybee *A. florea* and *A. m. yemenitica*, they were very close (0.1112) and the same result present in the second node which included also two subspecies *A. m. lamarckii* and *A. m. sudanensis* (0.1719) and they are closest subspecies, while the third node included honeybees, *A. m. lamarckii, A. m. carnica* and *A. m. sudanensis* (0.3480), and the last one was all honeybees studied (0.4198).

TABLE (I)

Estimation of similarity coefficient among different collection of honey bees from middle east

	C	S	F	Y
E	**0.86**	**0.90**	**0.74**	**0.75**
C		**0.75**	**0.76**	**0.75**
S			**0.80**	**0.78**
F				**0.92**

TABLE (II)

UPGMA Cluster analysis using Nei's (1972) original distance

Nodes	Distance	Samples
1	0.1112	F Y
2	0.1719	E S
3	0.3480	E C S
4	0.4198	E C S F Y

A comprehensive review , based on morphology and behaviour, has been discussed by Alexander (1991). Molecular biology has added powerful tool for analysis of genetic variation, so PCR-amplified mtDNA have been used in conjunction with morphometric analysis to identify Africanized bees (Nielsen *et al.* 2000). RAPD markers are a highly resolving and helpful tool for investigation of variability and provide a simple technology which can be used to rapidly distinguuish species, strains and sexes in laboratory conditions (Vidigal *et al.* 1994; Roberts and Crawford, 2000). RAPD-PCR markers provide reliable data for mapping in haploids, if the primers are properly screened and selected (Hunt and Page, 1995). 10 % of the western honey bee (*Apis mellifera* L.) genome is comprised of sequences repeated 100 or more times (Crain *et al.*, 1976). In this investigation the bees genome were isolated from the whole body tissue of the workers and the DNA analysis was executed by a battery of five arbitrary primers. Our results show that they produced 38 fragments and the highest number of monomorphic fragments were generated by using primer OPA-9.

Samples of honey bees were consistently distinguished from each other by the presence or absence of RAPD fragments. By using five random primers there were 23 monomorphic bands were found between honey bees *A. florea* and *A. m. yemenitica*, where the similarity index between them was 0.92. So our study by UPGMA analysis showed that *A. m. yemenitica* is more closer to *A. florea* (0.1112) . The total number of monomorphic fragments between Egyptian honeybees *A. m. lamarckii* and Sudanese *A. m. sudanensis* were 26 fragments, these subspecies showed high value of similarity (0.90). We proved that they were the nearest subspecies by UPGMA analysis which was 0.1719. While the total number of monomorphic bands of RAPD-PCR patterns for subspecies *A. m. lamarckii*, *A. m. carnica* and *A. m. sudanensis* were 21 fragments, and

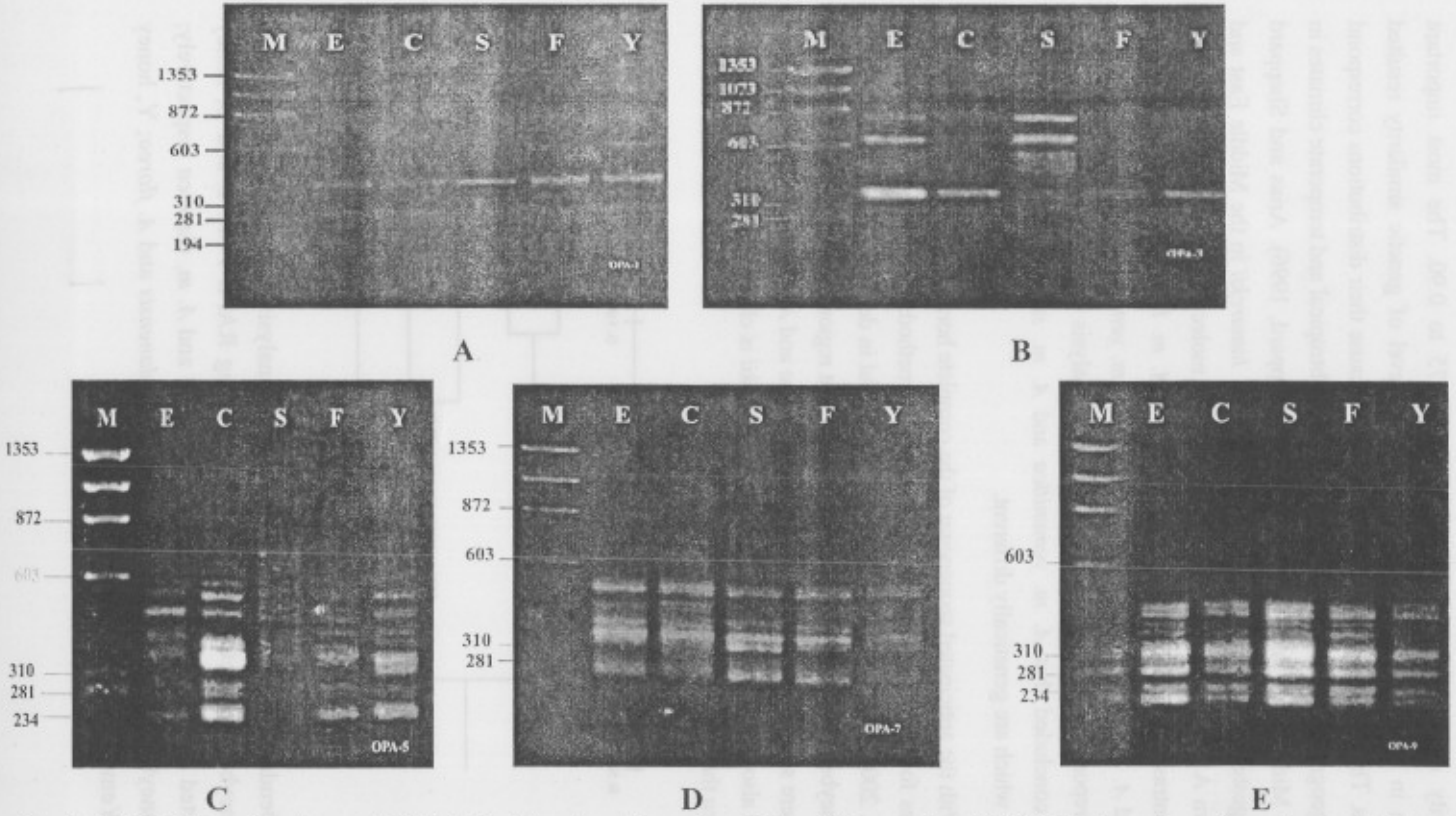

Fig. (1) RAPD-PCR profile of genomic DNA isolated from honeybees collected from different region of Middle East by using random primer (A) OPA-1; (B) OPA-3; (C) OPA-5; (D) OPA-7; (E) OPA-(9). Lane M, Standard marker; lane E and C, honey bees collected from Egypt *Apis mellifera lamarckii* and *A. m. carnica* respectively; lane S and F honeybees collected from Sudan, *A. m. sudanensis* and *A. florea* respectively; lane Y, honey bees from Yemen.

the similarity index ranged among them from 0.75 to 0.90. The most important observation in the present data show that a high level of genetic similarity resulted among bees. The subspecies are geographic races because their distributions correspond to distinct geographic areas ranging across tropical, subtropical and temperate climates in Africa, the Middle East and Europe (Arias and Sheppard, 1996). Arias and Sheppard (1996) suggested that the nearest subspecies to *A. m. lamarckii* in the Middle East and Northeastern Africa is *A. m. yemenitica* although no molecular data available for these taxa. In contrast, Our molecular data showed that *A. m. lamarckii* is closer to *A. m. carnica* and *A. m. sudanensis* than *A. florea* and *A. m. yemenitica*, similar obsevations have been reported in a previous study on protein analysis by Abou Zeid *et al.* (2002), Who also concluded that *A. m. yemenitica* and *A. m. sudanensis* are two separate subspecies, which are genetically different.

With the anticipated sequencing of the complete honey bee genome, there will be opportunities for further refinement of identification methods to include nuclear markers (Pinto *et al.* 2003). RAPD-PCR amplification are useful in detecting the genetic similarity among honeybees which were collected from different regions of middle east. The results presented here suggest that the two species of *A. florea* and *A. m. yemenitica* were closest species and also the Egyptian honey bee *A. m. lamarckii* is closer to *A. m. sudanensis* and *A. m. carnica* than *A. florea* and *A. m. yemenitica*.

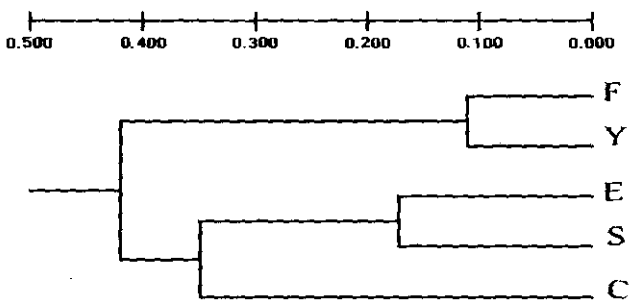

Fig. (2): Dendrogram based on UPGMA cluster analysis showing the relationship among honeybees collected from middle east using RAPD-PCR. E and C, honey bees collected from Egypt *Apis mellifera lamarckii* and *A. m. Carnica* respectively; S and F honeybees collected from Sudan, *A. m. sudanensis* and *A. florea*; Y, honey bee from Yemen, *A. m. yemenitica*.

SUMMARY

In the present study, RAPD-PCR technique was used to compare genetic material of the honey bees collection from Egypt (*Apis mellifera lamarckii* and *A. m. carnica*), Sudan (*A. m. sudanensis* and *A. florea*) and Yemen (*A. m. yemenitica*). similarity index and Unweighed pair-group arithmetic average (UPGMA) cluster analysis were used for data obtained. Screening of genomic DNA by using five arbitrary primers showed high genetic similarity among different collection of honeybees and *A. m. lamarckii* is closer to *A. m. carnica* and *A. m. sudanensis* than *A. florea* and *A. m. yemenitica*.

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