

BIOCHEMICAL STUDIES AND MITOCHONDRIAL DNA FINGERPRINTING OF HONEY BEE IN SAUDI ARABIA

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ABSTRACT

Honey bee is an important agricultural insect as they pollinate more than 90 crops and providing honey and other commercial products. The study of genetic relationships (diversity) is important for the selection of suitable diverse parents to obtain races with disease resistance and desirable traits. Traditionally, honey bee genetic diversity was based entirely on morphological markers. However, polymerase chain reaction (PCR) -based markers are suitable for an estimation of genetic relationships or diversity in honey bee. Also, protein markers still used as a molecular marker for genetic diversity in honey bee and many other organisms. In addition, mitochondrial DNA (mtDNA) fingerprinting method was efficiently used for studying the genetic diversity but it was not applied with honey bee before.

Eleven stocks of *Apis mellifera* were used to study the genetic fingerprinting at the molecular and biochemical levels. The results of molecular analysis in mitochondrial DNA (mtDNA) showed that the total number of fragments were 79 with an average number of 7.9 fragments /primer. The polymorphism ranged from 25% to 100% with 11 unique bands. On the other hand, the biochemical analysis revealed that the gene or genes which are responsible for protein synthesis are the same in the eleven stocks. These data would lead to a conclusion that the differences between tolerant and sensitive stocks to mites did not affect the protein concentration in the different stocks. Some colonies showed significant excess of amino acid polymorphism so the concentration of amino acid was affected by tolerant and sensitivity to mites in these colonies.

These results led to the possibility of using mtDNA as a reliable and quick method for studying the genetic fingerprints in these stocks, as well as the biochemical levels.

Key words: amino acid, *apis mellifera*, mtdna, protein, RAPD.

1. INTRODUCTION

Honey bee is an important agricultural insect pollinating more than 90 crops and providing honey and other commercial products. Although the taxonomic description of honey bees from the African continent dates back to 1804 when the French entomologist Latreille named a bee from Senegal *Apis adansonii* (Latreille, 1804), the study of honey bees from this continent remained superficial and incomplete until far into this century. For more than 150 years, *Apis mellifera adansonii* was used as the only name for all the bees of sub-Saharan Africa (Buttel-Reepen, 1906 and Kerr, 1958), although it became known later that substantial geographic variability exists among honey bee populations of this vast region (Ruttner, 1976 and 1988). Analysis of mtDNA variation, proved that samples which were morphometrically identified as *A. m. monticola* had a unique haplotype, in addition to sharing other haplotypes with *A. m. scutellata*, thus

lending additional support to the 'refugial hypothesis' (Ruttner, 1988). Morphometric analysis of *A. m. litorea* suggests that it is a distinct subspecies (Lou *et al.* 1998), but no molecular data are currently available to better describe its relationship to the neighboring *A. m. scutellata*.

Genomic fingerprints are increasingly used to study the relationships at the intra- or even interspecific level. The fingerprints are obtained by visualizing many parts of the genome. Differences in these fingerprints between individuals are interpreted as genetic distances. Obviously, the differences should reflect variations in DNA rather than artifacts due to a non-robust method. Furthermore, the method should provide the appropriate level of discriminatory power and it should be relatively rapid and cheap, especially in large-scale population genetics studies (Williams *et al.*, 1990 and Chapuisat *et al.*, 1997).

Up till now the most widely used PCR-based multilocus DNA fingerprint techniques are random amplified polymorphic DNA (RAPD) (Vos *et al.*, 1995) and the more recently proposed amplified fragment length polymorphism (AFLP) (Pejic *et al.*, 1998). RAPD is generally regarded to be less time consuming, while the AFLP method is more robust (Williams *et al.*, 1990 and Mueller and Wolfenbarger 1999).

The mite *Varroa jacobsoni*, initially an ectoparasite of the bee *A. cerana*, was first found on *A. mellifera* in the 1950s. When this mite parasitized *A. cerana*, it remained limited to the local infested area. However, a rapid man-aided dispersal was observed soon after contact with *A. mellifera*, since the contact between *Varroa* and *A. mellifera*, caused serious problems worldwide. The effects of the parasite on honey bees range from weakening to death of parasitized colonies, with the intensity of the problem being directly related to the level of infestation. Climate and *A. mellifera* race were found to be important factors that affect the development of this parasite. However some types of *A. mellifera*, show resistance to *Varroa comparedto* bee races. One of the factors that is thought to be involved in this resistance is the fact that the reproductive ability of *Varroa* females is known to be lower in resistant bees. Some scientist observed that *A. cerana* has a strong defense mechanism against *Varroa jacobsoni*. When resistance *A. cerana* workers were artificially infested with varroa females, 99% succeeded in ridding themselves of the parasite by performing body movements that expelled or removed it from their body. It was also observed that many of the mites that left the host workers were killed by the infested worker itself or its fellow workers. Lowery and Randall (1977) collected mites from the bottom of resistance bee colonies, and they noticed that the occurrence of mutilations in several parts of their body, indicating possible attacks by the workers. The objectives of the present study were to determine the genetic variability in mDNA, proteins and amino acids among eleven stocks of *Apis mellifera* tolerant and sensitive to mites.

2. MATERIALS AND METHODS

2.1 *Apis mellifera* stocks

Eleven colonies representing three honeybee races namely; Balady (*Apis mellifera yementica*) tolerant to mites, Mostord (*Apis mellifera carnica*) sensitive to mites and Balady hybrids (hybrids between Balady and Mostord) moderately tolerant. These colonies were collected from the gene pool of the Bee Research Unit of King Saud University. The eleven

colonies were four Balady, three Mostord and four Balady hybrids. Mostord bees were imported from Egypt whereas the Balady bees were brought from the south of the kingdom (Al Ghamdi, 2002). This research has been conducted in the Biotechnology department, Taif university, Faculty of Science, Kingdom of Saudi Arabia during the years of 2006-2007. The flies were incubated at 25°C in the optimal medium (10 gm cornmeal, 10 gm sucrose, 10 gm yeast, 1.5 gm agar-agar and 2 ml propionic acid) / 100 ml water.

2.2 Determination of total protein extracts

The protein content of the different genotypes of *A. mellifera* was determined according to the method of Cohen *et al.* (1989).

2.3 Determination of total amino acid extracts

The amino acid composition of the experimental samples was determined using HPLC-Pico-Tag method according to Millipore Cooperative procedure (Latorre *et al.*, 1986).

2.4 Molecular analysis of mtDNA experiments

2.4.1 Extraction of mtDNA

mtDNA was extracted from the different genotypes of *A. mellifera* using mini preparation (mini prep) method that is performed in much shorter periods according to Latorre *et al.* (1988) and Nei and Tajima (1981).

2.4.2 Polymerase chain reaction with random primers for mtDNA

RAPD-PCR was carried out according to the procedure given by Vos *et al.* (1995). Ten random primers (10-mer) were purchased from Operon kit A (Operon technologies, Inc., Alamada, CA, USA) and screened for its ability to polymorphisms. The primer sequences were as follows:

Primer	5' Sequence 3'
OPA04	5'-AACGGGCTG -3'
OPA05	5'-AGGGGTCTTG-3'
OPA09	5'-GGGTAACGCC-3'
OPA11	5'-CAATCGCCGT-3'
OPA14	5'-TCTGTGCTGG-3'
OPA15	5'-TTCCGAACCC-3'
OPA16	5'-AGCCAGCGAA-3'
OPA17	5'-GACCGCTTGT-3'
OPA19	5'-CAAACGTCGG-3'
OPA20	5'-GTTGCGATCC-3'

PCR reaction was carried out in a volume of 50 µl containing 1 µl of 50 ng/µl template DNA, 4 µl of 10 µM decamer oligonucleotide primer from, 0.5 µl of 5 u / µl of *Taq*. DNA polymerase (Finpoll), 5 µl of 10x buffer (500 mM Kcl, 100 mM Tris-Hcl pH 9.0 and 1% Triton x-100), 2 µl of 50 mM

MgCl₂, 5 µl of 2 mM dNTPs (dATP, dCTP, dTTP, dGTP) and deionized dd H₂O.

PCR amplification was performed in a thermal cycle programme for one cycle of 3 min. at 94°C, 40 cycles of 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72°C, and a final cycle of 5 min at 72°C.

2.4.3 Electrophoresis of PCR products

The amplification products were resolved by electrophoresis in a 1.5% agarose gel (Sigma) containing 0.5 µg /ml ethidium bromide in 1x TAE buffer. After electrophoresis, the RAPD patterns of the PCR products were visualized under UV light.

2.5 Statistical analysis

The test of (χ^2) and (2x2) analysis were used aiming to clarify the significant and non-significant patterns of RAPD analysis and total protein in different stocks of *A. mellifera* used in the study according to Moretto and Joao de Mello (1999).

3. RESULTS AND DISCUSSION

To shed some light on the different genotypes of *A. mellifera* which have been used in this study, the present data may reveal some aspects for comparing eleven stocks. The criteria of these comparisons include differences in tolerance and sensitivity to mites, the variability in mtDNA, also in the total protein content and total amino acids in all of these stocks.

3.1 Determination of total proteins

Table (1) shows no variability in the total protein content between them, however the total protein concentrations for all eleven stocks of *A. mellifera* was measured and the data presented in Table (1) ranging between 16% to 22.76% with an average of 19.19%. The statistical analysis (χ^2) and comparing each strain with the other on the

Table (1) : Total protein concentrations in the eleven stocks of *Apis mellifera* as determined according to the method of Lowery and Randall (1977).

Stocks	Protein conc. %	Calculated (χ^2) values
Balady 3	19.21	
Balady 5	20.14	0.0219
Balady 6	20.96	0.076
Balady 7	16.94	0.1425
Balady 8 hybrid	22.76	0.0023
Balady 9 hybrid	19.87	0.1892
Balady 10 hybrid	19.11	0.3181
Balady 11 hybrid	16.00	1.1789
Mostord 12	18.37	0.0962
Mostord 13	20.23	0.0010
Mostord 14	17.45	0.1294

protein level encourage us to suggest that the total protein concentrations among the stocks could not detect variations among these eleven stocks. In other words there are no significant variations in the concentration of these proteins. These results demonstrated that the gene or genes which are responsible for protein synthesis are the same in the experimental strains. These data would lead to a conclusion that the protein concentration had no influence in tolerant and sensitive stocks in response to mites.

3.2 Determination of total amino acids

The data obtained from the present work show some characteristics for total amino acid and protein concentrations of the eleven stocks of *A. mellifera* in case of using HPLC-Pico-Tag method according to Millipore. The concentrations of the total amino acids of the eleven stocks of *A. mellifera* are shown in (Table 2). From the presented values, the highest amino acid ratio in the first strain is for arginine and estimated with 4.739%, while the lowest amino acid ratio is for four amino acids and estimated with 0.00%. By a comparison, it was also found that the highest amino acid ratio is for aspartic and ranged this was tree between 0.556% and 7.334%. with serine and ranged from 1.543 and 6.891. Also, it was observed that the amino acid therionine in all the stocks was absent and estimated with 0.00%. Moreover, the lowest amino acid ratio in all the stocks was, for methionine ranging between 0.004% and 0.119%.

On the other hand, the essential amino acid aspartic increased in the fifth strain and estimated with 7.33%, while it was decreased in all experimental strains and ranging between 0.557% and 5.428%. Also, serine was increased in sixth strain and estimated with 6.891%, while it was decreased in all the experimental strains and ranging between 4.898% and 1.543%. The concentrations of the total amino acids in the tolerant stocks ranged from 0.00 % and 4.739 %, in case of sensitive stocks total amino acids ranged from 0.00% and 5.428 % .While in the hybrid stocks, the total amino acids ranged from 0.00% and 7.334%. From these data, some stocks showed significant excess of amino acid polymorphism within and between them. Rand *et al.* (1994) found that the significant excess of amino acid polymorphism within both *Drosophila melanogaster* and *Drosophila simulans* species is localized in one region of the protein. They added that mtDNA evolution has been faster in *D. melanogaster* than in *D. simulans* and the population structure of mtDNA is distinct in these species. A comparison within and between *D. melanogaster*, *D. simulans*, and *D. yakuba* species

concerning the ratio of amino acid replacement change to synonymous change that reveals a relative excess of amino acids replacement polymorphism compared to the neutral prediction of William *et al.* (1994). Rand and Kann (1996) stated that both *Drosophila* and human mtDNA show very significant excess of amino acid polymorphism. A pattern of excess amino acid polymorphism holds across the entire mitochondrial genome when *D. melanogaster* and *D. simulans* were compared (Rand, 2001). Also the representation of cysteine appears to correlate positively with the complexity of the organism. Miseta and Csutora (2000) investigated the occurrence of cysteine in known proteins of various species. Cysteine is a relatively rare amino acid within the proteins of investigated organisms and protein of the fruit fly contained somewhat less cysteine (1.90%).

3.3. Isolation of mtDNA

The insect mitochondrial genomes are closed circular and have the universal genetic code. The genome size varies from 14,422 bp (in *M. bicolor*) to 19,517 bp (*D. melanogaster*). All insect mtDNA are similar in size except for *D. melanogaster* mtDNA which is larger compared to other species of *Drosophila* due to the presence of a noncoding 4553-bp region at the end of genome and it has 14 coding genes compared to 13 in most insects (Chandra *et al.*, 2006). Mitochondrial DNA (mtDNA) size among the *D. melanogaster* species subgroup varies from 16.0 to 19.0kb. However, the mtDNA size among the *D. melanogaster* species subgroup varies from 16.0 to 19.0 kb. However, the mtDNA size variation within the *D. melanogaster* species complex ranges from 18.0 to 19.0 kb. Shah and Langley (1979) estimated the size of mtDNA in *D. melanogaster* to be 18.6 kbp. In comprehensive studies, Hale and Singh (1986 and 1991) recorded many cases of mtDNA size variations in *D. melanogaster*. They classified the mtDNA size to 14 different size classes began with 18.2 kbp until 19.9 kbp, with predominant of 18.6 kbp class size. Choi and Choo (1993) estimated mtDNA size to be 18.9 kbp in *D. melanogaster*. This size variation may be due to cases of insertion or deletion and to the presence of transposable genetic elements.

3.3.1 RAPD-PCR in mtDNA of eleven stocks of *A. mellifera*

mtDNA has been the most widely studied region of eukaryotic genomes and has played a critical role in the development of population and evolutionary genetics (Rand *et al.*, 1994). Rand (2001) demonstrated that mtDNA was a neutral genetic marker. Also the analyses of *Drosophila*

mitochondrial genomes, especially those on *D. melanogaster* were among the first ones devoted to animal organisms (Polan *et al.*, 1973). While in honey bee mtDNA variations were detected in many subspecies (Eixner *et al.*, 2000; Sheppard *et al.* 96, 1999).

Mitochondrial DNA (mtDNA) variation is an important tool in the analysis of phylogenies of honey bee (Canovas *et al.*, 2008).

MtDNA is an important tool in the analysis of phylogenies of closely related species in population structures of individual species (Latorre *et al.*, 1988). mtDNA is appropriate because of its uniparental inheritance, non recombogenicity, small size, and elevated rate of mutation (reviewed in Hale and Singh, 1991).

The use of RAPD-PCR is aiming to show fast and reliable discrimination of any variations. The purified mtDNA of the eleven different stocks was produced when they are used as templates for RAPD-PCR, characteristic reproducible multiple-band profiles of amplified fragments. Out of 22 tested primers, only ten succeeded in this test. The results of RAPD-PCR analysis in mtDNA using primer (OPA04) are illustrated in Fig. (1) and Table (3). The molecular size of the 9 PCR products ranged from 250 bp to 1500 bp. Two common bands of 450 bp, and 400 bp were observed in the eleven stocks of *A. mellifera* species and exhibited 22.2% monomorphism, while the seven remaining bands were polymorphic with percentage 77.8%.

Four bands in the eleven strain of *A. mellifera* species with primer (OPA05) are illustrated in Fig. (1) and Table (4) at a molecular size ranging between 190 bp to 400 bp. There were three common bands with molecular size of 190 bp, 200 bp and 300 bp present in all species which exhibited 75% monomorphism. The remainder one band was polymorphic with percentage 25%.

The results of RAPD-PCR with primer (OPA09) are shown in Fig. (1) and Table (5). The molecular size of these products ranged from 250 bp to 900 bp. This primer gave 6 polymorphic bands with percentage 100%.

The RAPD-PCR products of primer (OPA11) are illustrated in Fig. (1) and Table (6). It is obvious that the total number of bands is 8 with molecular size ranging from 250 bp to 1100 bp. The results also indicated that there was one common band with molecular sizes of 450 bp, while all the remaining bands were polymorphic. The polymorphic bands exhibited 87.5% with one positive unique band with molecular sizes of 450 bp in stock number 8.

The results of RAPD-PCR analysis using primer (OPA 14) are depicted in Fig. (1) and

Table (2): Total amino acid concentrations (%).

Amino acids	Stocks										
	1	2	3	4	5	6	7	8	9	10	11
Aspartic	3.039	2.031	1.731	0.557	7.334	4.608	2.530	2.064	5.428	1.305	0.599
Glutamic	2.462	3.960	3.413	5.055	2.717	3.716	2.377	4.557	2.731	3.599	2.008
Serine	2.938	2.759	3.389	1.816	4.898	6.891	2.985	1.798	1.543	1.891	1.830
Glycine	1.927	0.771	0.210	0.374	0.000	0.000	1.061	0.235	0.000	0.187	0.842
Histedine	1.830	1.057	2.586	0.999	1.797	0.000	0.000	0.967	0.860	1.332	1.975
Arginine	4.739	0.369	0.685	0.000	0.000	0.287	2.901	0.000	0.860	1.781	0.000
Therionine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Alanine	0.000	3.084	2.043	2.157	2.140	1.481	4.882	2.423	1.525	2.583	1.970
Proline	0.000	0.326	0.328	0.307	0.373	0.147	0.423	0.252	0.168	0.141	0.163
Tyrosine	0.257	2.060	0.672	1.355	1.427	1.269	0.252	1.455	0.887	0.851	0.655
Valine	0.345	0.006	0.875	1.326	0.481	0.525	0.219	0.544	1.046	1.685	1.769
Methionine	0.000	0.011	0.004	0.201	0.000	0.000	0.089	0.004	0.102	0.119	0.102
Cystine	0.007	1.519	0.202	0.000	0.225	0.212	0.162	0.646	0.000	0.000	0.000
Isoleucine	1.568	0.476	3.356	0.000	0.000	0.000	0.595	0.521	3.215	4.392	5.223
Leucine	0.006	0.491	0.685	2.787	1.242	0.727	0.368	0.488	0.000	0.000	0.000
Phenylalanine	0.049	1.189	0.733	0.000	0.000	0.000	0.115	0.025	0.000	0.000	0.000
Lysine	0.032	0.020	0.040	0.000	0.118	0.000	0.144	0.013	0.000	0.357	0.308

Table (3): Scoring of RAPD-PCR markers for the eleven stocks of mtDNA generated by RAPD-PCR in case of using primer OPA 04.

Scoring of multiple-band profiles	Different genotypes of <i>Apis mellifera</i>											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
	+	-	-	-	+	+	-	-	-	-	-	1500
	+	+	+	-	+	+	+	+	-	-	-	900
	+	+	-	-	-	-	+	+	-	+	+	800
	-	-	+	-	+	+	+	+	-	+	+	700
	+	+	-	-	-	-	+	+	-	-	-	600
	+	+	+	-	+	+	+	+	-	+	+	500
	+	+	+	+	+	+	+	+	+	+	+	450
	+	+	+	+	+	+	+	+	+	+	+	400
	+	-	-	-	-	-	-	+	-	-	-	250

Table (4): Scoring of RAPD-PCR markers for the eleven stocks of mtDNA using primer OPA 05.

Scoring of multiple-band profiles	Different genotypes of <i>Apis mellifera</i>											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
	-	-	-	-	+	+	+	-	-	-	-	400
	+	+	+	+	+	+	+	+	+	+	+	300
	+	+	+	+	+	+	+	+	+	+	+	200
	+	+	+	+	+	+	+	+	+	+	+	190

Table (5): Summary of RAPD-PCR markers used to identify eleven stocks of mtDNA in case of using primer OPA 09.

Scoring of multiple-band profiles	Different genotypes of <i>Apis mellifera</i>											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
	+	-	-	-	-	+	-	-	-	-	-	900
	+	-	-	-	-	-	-	-	-	-	-	700
	-	+	+	+	+	+	-	+	-	+	+	600
	-	+	+	+	+	-	+	+	-	+	+	500
	+	+	+	+	+	+	-	-	-	+	+	300
	-	+	+	+	+	-	-	+	+	+	+	250

Table (6): Scoring of RAPD-PCR markers for the eleven stocks of mtDNA using primer OPA 11.

Scoring of multiple-band profiles	Different genotypes of <i>Apis mellifera</i>											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
	+	+	+	-	+	+	+	+	-	-	-	1100
	+	+	+	-	+	+	+	+	-	-	+	1000
	-	-	-	-	+	+	-	-	-	-	+	900
	+	-	-	-	-	+	+	+	-	+	+	700
	+	-	-	-	-	-	-	+	-	-	-	600
	+	+	+	+	+	+	+	+	+	+	+	450
	-	-	-	-	-	-	-	+	-	-	-	300
	+	-	-	-	-	-	-	-	-	+	+	250

Table (7): Diagnostic bands of eleven stocks of mtDNA generated by RAPD-PCR in the case of using primer OPA 14

Scoring of multiple-band profiles	Different genotypes of <i>Apis mellifera</i>											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
-	+	-	-	-	-	-	+	-	-	-	-	800
-	-	-	+	+	+	+	-	+	+	+	+	700
+	-	-	-	-	-	-	-	+	-	-	-	500
+	+	+	+	+	+	+	+	+	-	-	-	450
-	-	-	-	+	+	+	+	-	-	-	-	300
-	-	+	+	+	+	+	-	-	+	+	+	250

Table (7). The results reveal that this primer produced 6 bands with molecular sizes ranging from 250 bp to 800 bp. In the eleven stocks, the six bands were polymorphic with percentage 100%.

there was one band with a molecular size of 500 bp present in stocks number 1. This band might be considered as specific positive marker.

Ten bands with primer (OPA16) are illustrated in Fig.(1) and Table (9) with molecular

Table (8): Scoring of RAPD-PCR markers for the eleven stocks of mtDNA using primer OPA 15

Scoring of multiple-band profiles	Different genotypes of <i>Apis mellifera</i>											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
-	-	-	-	-	-	+	-	+	+	-	+	900
-	-	-	-	-	-	-	-	-	+	-	+	600
+	-	-	-	-	-	-	-	-	-	-	-	500
-	+	-	-	-	-	+	+	-	-	+	-	400
-	+	-	+	+	+	+	-	-	+	-	+	450
+	+	+	+	+	+	+	+	+	+	+	+	300
+	+	+	+	+	+	+	+	+	+	+	+	270
-	-	-	-	-	-	+	-	+	-	-	-	250
+	+	+	+	+	+	+	+	+	+	+	+	200
+	+	+	+	+	+	+	+	+	+	+	+	100

Table (9): Summary of RAPD markers used to identify eleven stocks of mtDNA in the case of using primer OPA 16.

Scoring of multiple-band profiles	Different genotypes of <i>Apis mellifera</i>											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
-	-	-	-	-	+	+	-	-	-	-	-	1500
-	-	-	-	-	+	+	-	-	-	-	-	1400
-	-	+	-	+	+	-	-	-	-	-	-	900
+	-	+	-	-	-	-	+	-	-	-	-	800
-	-	+	+	+	+	-	+	+	+	+	+	700
+	+	+	+	+	-	-	+	+	-	-	+	600
+	+	-	+	+	+	+	+	+	-	-	-	500
+	-	+	+	+	+	+	+	-	+	+	+	400
-	-	+	+	+	+	+	+	+	-	-	-	300
-	-	+	+	+	+	+	+	-	+	+	+	270

Fig. (1) and Table (8) show the results of RAPD-PCR with primer (OPA15) and illustrated that the molecular size of the PCR products ranged from 100 bp to 900 bp. It gave 6 polymorphic bands with different molecular sizes which exhibited 60% and four monomorphic bands which exhibited 40%. On the other hand,

size ranging between 270 bp to 1500 bp. The remainder 10 bands were polymorphic with percentage 100%.

RAPD-PCR products with primer (OPA17) are illustrated in Fig. (1) and Table (10). This primer gave 9 polymorphic bands with different molecular sizes ranging between 200 bp to 1000

Table (10): Scoring of RAPD-PCR markers for the eleven stocks of mtDNA using primer A 17.

Scoring of multiple-band profiles	Different genotypes of <i>Apis mellifera</i>											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
	+	+	-	+	+	+	+	-	-	+	-	1000
	+	+	-	+	+	+	+	-	-	+	-	900
	+	+	+	+	+	+	+	+	+	+	+	700
	-	-	-	-	-	-	+	-	-	-	-	500
	+	+	+	+	+	+	+	+	+	+	+	450
	-	-	-	-	-	-	+	-	-	-	-	400
	-	-	-	-	-	-	+	-	-	-	-	300
	+	+	+	+	+	+	+	+	+	+	+	250
	-	-	-	-	-	-	-	+	-	-	-	200

bp and four unique bands at molecular sizes of 500 bp, 400 bp, 300 bp and 200 bp which could be used as positive molecular marker for stock number 7 and 8. These polymorphic bands exhibited 66.7%. Three common bands of 700 bp, 450 bp, and 250 bp were observed in the eleven stocks.

present in the eleven stocks, while the other bands were polymorphic with percentage 75%. However, three bands with molecular size of 700 bp and, 150 bp were detected only in stock number 6 and 9 and could be used as a positive marker for these stocks; while one band sized 250 bp could be used as a negative marker.

Table (11): Summary of RAPD markers used to identify eleven stocks of mtDNA in the case of using primer OPA 19.

Scoring of multiple-band profiles	Different genotypes of <i>Apis mellifera</i>											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
	-	-	-	-	-	+	-	-	-	-	-	700
	+	+	+	-	-	-	-	-	-	-	-	600
	+	-	-	-	-	+	-	-	-	-	-	550
	+	+	+	+	+	+	+	+	+	+	+	500
	-	-	-	+	+	+	-	-	+	-	-	400
	+	+	+	+	+	+	+	+	+	+	+	300
	-	+	+	+	+	+	+	+	+	+	+	250
	-	-	-	-	-	-	-	-	+	-	-	150

Table (12): Scoring of RAPD-PCR markers for the eleven stocks of mDNA using primer OPA 20.

Scoring of multiple-band profiles	Different genotypes of <i>Apis mellifera</i>											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
	-	-	-	+	+	-	-	-	-	-	-	2000
	+	+	+	+	+	+	+	-	-	-	-	1500
	-	+	+	+	+	+	+	-	-	-	-	1400
	-	+	-	-	-	+	+	-	-	+	-	900
	+	+	+	+	+	+	+	+	+	+	+	800
	-	+	-	+	-	+	+	+	+	+	+	500
	+	+	+	+	+	+	+	+	+	+	+	400
	-	-	-	-	-	-	-	+	-	-	-	350
	-	-	-	-	-	-	-	+	-	-	-	300

The results of RAPD-PCR analysis using primer (OPA19) are depicted in Fig. (1) and Table (11). The results revealed that this primer produced 8 bands with molecular sizes ranging between 150 bp and 700 bp. Two common bands with molecular size of 300 bp and 500 bp were

The results of primer (OPA20) are shown in Fig. (1) and Table (12). This primer produced 9 bands with molecular size ranging between 100 bp to 1875 bp. There were three polymorphic bands which exhibited 33.33% and six common bands with molecular sizes of 930 bp, 910 bp, 360 bp,

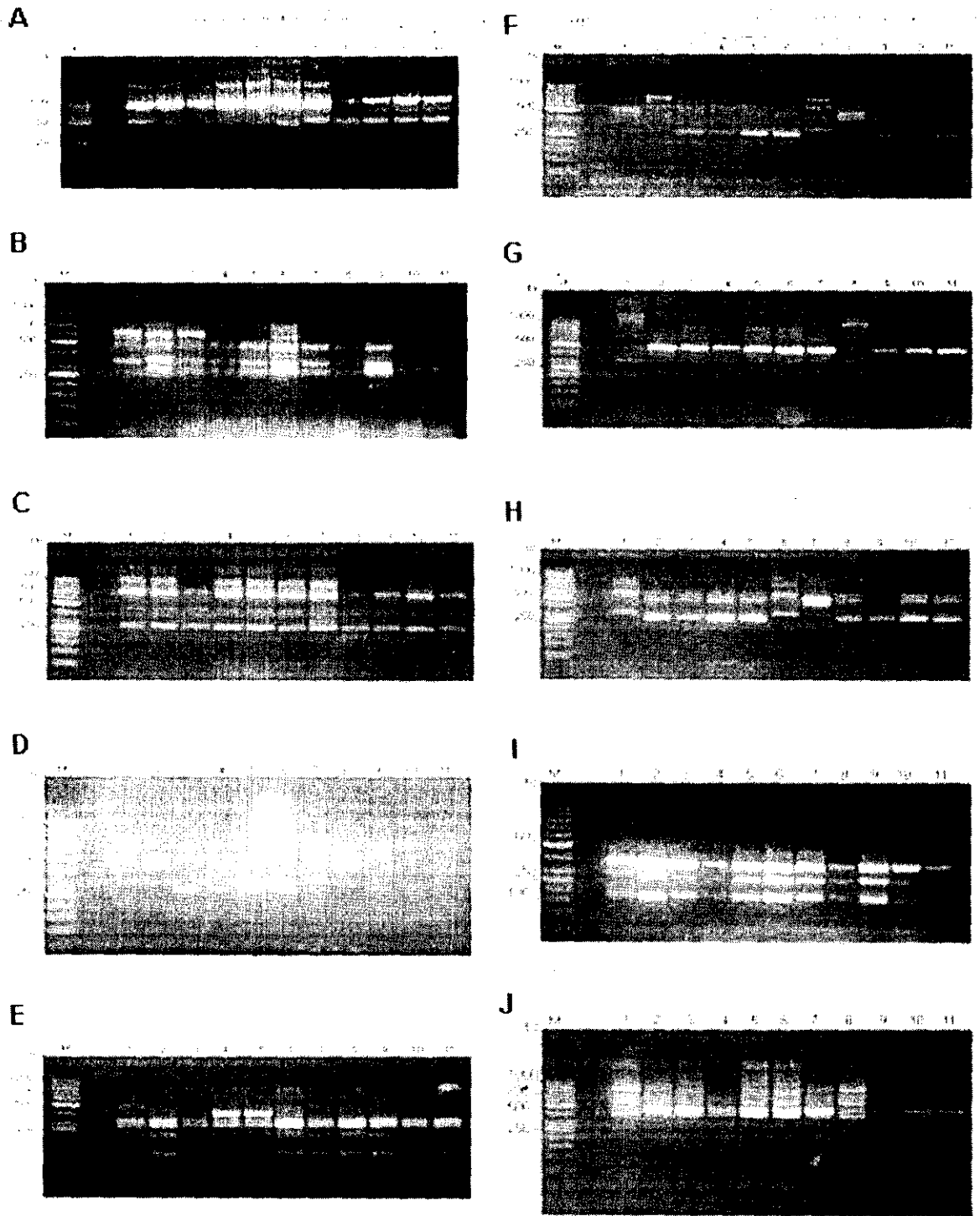


Fig.(1): RAPDs amplification of mtDNA of *Apis mellifera*. M, molecular marker -50 bp DNA ladder, (1) Baldy 3, (2) Baldy 5, (3) Baldy 6, (4) Baldy 7, (5) Baldy 8 hybrid, (6) Baldy 9 hybrid, (7) Baldy 10 hybrid, (8) Baldy 11 hybrid, (9) Mostord 12, (10) Mostord 13, (11) Mostord 14. Primers: (A) OPA 20, (B) OPA 19, (C) OPA 17, (D) OPA 16, (E) OPA 15, (F) OPA 14, (G) OPA 11, (H) OPA 09, (I) OPA 05, (J) OPA 04.

Table (13): Summary of electrophoretic separation pattern of RAPD-PCR products using the ten primers.

Primer	Polymorphic bands		Monomorphic bands		Unique bands	Total bands
	No.	%	No.	%		
OPA04	7	77.8	2	22.2	No	9
OPA05	1	25	3	75	No	4
OPA09	6	100	No	0	+	6
OPA11	7	87.5	1	12.5	+	8
OPA14	6	100	No	0	No	6
OPA15	6	60	4	40	+	10
OPA16	10	100	No	0	No	10
OPA17	6	66.7	3	33.3	+,+,+,+	9
OPA19	6	75	2	25	+,+,-	8
OPA20	7	77.8	2	22.2	+,+	9
Total	62	78.5	17	21.5	11	79
Average	6.2		1.7			7.9

300 bp, 260 bp, and 210 bp.

Conclusions

Ten primers were screened with the mtDNA of the 11 *A. mellifera* genotypes. These primers generated reproducible and easily scorable RAPD profiles. These produced multiple band profiles with a number of amplified mtDNA fragments ranging from 6 to 10 (Table 13). The total number of fragments produced by the ten primers was 79 with an average number of 7.9 fragments / primer. While the number of polymorphic fragments ranged from 1 to 10. A maximum number of 10 amplicons were amplified with primer OPA15 and OPA16, while the minimum number of 4 fragments was amplified with primer OPA05. The highest number of polymorphic bands (10) was obtained with primer OPA16 that exhibited 100% polymorphism. The average number of polymorphic fragments / primer among the 11 *A. mellifera* genotypes was 6.2.

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دراسات كيمو حيويه والبصمه الوراثيه ل د ن ا الميتوكوندريا في نحل العسل في المملكة العربية السعودية

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ملخص

يعتبر نحل العسل من الحشرات الزراعية المهمة اقتصاديا حيث يقوم بتلقيح ما يزيد عن ٩٠ محصولا و هي خشرة منتجة للعسل بالإضافة إلى منتجات تجارية أخرى ، و تعتبر دراسة التنوع الوراثي مهمة لانتخاب الأبناء التي تتميز بقدرتها على المقاومة للعديد من الأمراض و الصفات الاقتصادية الأخرى ، و قديما اعتمدت دراسات التنوع الوراثي على الصفات المظهرية للحشرة في التمييز بين الأصول الوراثية لنحل العسل و التي تميزت ببساطتها . و توجد الآن تقنيات وراثية أكثر دقة لتقدير التنوع الوراثي في نحل العسل تعتمد على واسمات جزيئية باستخدام تفاعل البلمرة التسلسلي البسر (PCR) و منها واسمات الباندات العشوائية (RAPD) ، كما توجد طريقة جزيئية أخرى لم تقدم من قبل و هي البصمة الوراثية لسلاسل النحل للانتخاب ضد الأمراض من خلال الدنا الميتوكوندري (mtDNA) . تعتبر التقنيات السابقة مجتمعة بالإضافة إلى الواسمات البروتينية من أقوى النظم المستخدمة في مجال الواسمات الجزيئية . استخدمت إحدى عشرة سلالة من نحل العسل لدراسة البصمة الوراثية لها على المستوى الجزيئي والكيموحيوي . وأوضحت الدراسة وجود حوالي 79 حزمة بمعدل ٧,٩ حزمة لكل بادئ و تراوحت نسبة تعدد المظاهر من ٢٥% إلى ١٠٠% مع وجود ١١ حزمة فريدة . ومن ناحية أخرى على المستوى الكيمو حيوي تدل النتائج على أن الطفرات لا تؤثر على تركيز البروتين في السلالات المختلفه بينما أظهرت بعض السلالات زيادة معنوية في الأحماض الامينية متعددة المظاهر . تؤدي هذه النتائج إلى إمكانية استخدام دنا الميتوكوندريا كطريقة سريعة ومفيدة في دراسة البصمة الوراثية لهذه السلالات .

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