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BIOCHEMICAL GENETIC STUDIES IN HONEYBEES *Apis mellifera L.*

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ABSTRACT

Biochemical genetic polymorphisms were investigated in the Egyptian and Carniolan races and their Hybrid genotype. Esterase isozyme bands showed that, whereas Est-C_2 band appears in common among all populations studied and cannot be considered as a genotypic marker, Est-A_1 band was restricted to the Egyptian race. However, Est-A_2 band appeared in common in both the Carniolan and the Hybrid indicating a closer genetic relationship between them.

The distribution of Malate dehydrogenase electrophoretic pattern indicated that two third of Mdh isozymes were common in the three populations studied, while the remaining third was common in both the Carniolan and Hybrid indicating a closer genetic relationship between them. Restricted bands were represented by the extremely high molecular weight bands denoting the Egyptian race (Bands 1 and 2) and the ~ 53 ~ 54 Kda band which strictly appeared only in Carniolan and Hybrid slots.

These results interprets the presence of obvious genetic variations between Egyptian and Carniolan races and that the Hybrid is closely related to the Carniolan rather than to the Egyptian race.

Comparisons between protein fractions of the Hybrid with both in Egyptian and Carniolan races proved that the great majority of the Hybrid fractions have been transmitted from the Carniolan race. However the remaining Hybrid fractions were transmitted from the Egyptian race. These results interpreted the higher similarity between Hybrid and the Carniolan race and may indicate that this Hybrid had been obtained from Carniolan queens mated by Egyptian drones.

INTRODUCTION

Extensive studies have been carried out concerning the biochemical polymorphism in bee populations in Egypt as in other parts of the world. Most of these studies focused on the analysis of Esterase (Est) and Malate dehydrogenase (Mdh) isozymes. In addition, the distribution of SDS polyacrylamide gel electrophoretic protein fractions was also investigated.

Mestriner (1969) was the first to study biochemical polymorphism in bee *Apis mellifera*. Using starch-gel electrophoresis and a discontinuous buffer system, he detected three main protein zones in the pupae of drones and workers derived from queens that were artificially inseminated from a single drone. The data for esterase allozymes activity were consistent with the hypothesis of a single codominant locus. He then suggested a mono factorial type of inheritance.

Bitondi and Mestriner (1985) using an electrophoretic technique studied esterase isoenzymes in homogenized extracts of Africanized worker honeybee pupae. The electrophoretic mobility of these isoenzymes was modified by prior incubation of the extract with one or other of 6 thiol

reagents. The results suggested that of the 6 esterases identified, 1,2 and 5 contained reactive sulphydryl group, whereas 3,4 and 6 did not. They suggested that variations in the esterases of honeybees are probably determined by 6 distinct gene loci.

Moritz (1988) .determined the activity of malate dehydrogenase (Mdh). The total protein content also determined by isoelectric focusing (Mdh) showed very low activity in both drones and workers just after emergence, but it rose to its maximum after 4 days in drones and 9-10 days in workers, and remained at this level throughout the investigation (16 days).

Lee *et al.* (1989) studied (Mdh) and on specific esterase polymorphism in *Apis mellifera* and *Apis cerana* in South Korea. In the former species, the (Mdh) locus showed 3 alleles, with frequencies of 0.67 (S), 0.21 (F) and 0.21 (M). The esterase in *Apis mellifera* showed frequencies of 0.86 (S) and 0.14 (F), but in *A. cerana* the esterase was monomorphic.

Yim *et al.* (1990) carried out an epigenetic study for 13 enzymes including esterase in *Apis mellifera* and *Apis cerana*. They showed that the distributions of the enzyme varied in different parts. They also considered esterase allozymes to be diagnostic for the two species.

El-Barbary and Youssef (1994) concluded that esterase-6 isozyme is present through all preadult stages and during againg of workers. It is controlled by one locus with two alleles "S" and "F" giving rise to three different electrophoretic patterns according to genotype. "S" was the common allele while "F" was mainly present in heterozygotes. (FF) genotypic structures were expressed only during early larval stage.

Del Lama *et al.* (2001) detected four aminopeptidases in *Apis mellifera* by starch gel electrophoresis. These enzymes were characterized on the basis of their substrate preference, effect of inhibitors, tissue and ontogenetic developmental distribution. Lap-A activity was present in all tissues and developmental stages. They observed four electrophoretic variants of Lap-D with an uncommonly high intralocus heterozygosity level. Segregational analyses demonstrated the absence of close linkage between Lap-D and Est-la, Est-2, Est-5, Est-6, Mdh-1, HkK-1 and Pgm-1 loci of *Apis mellifera*

MATERIAL AND METHODS

The material used in this study was two races of *Apis ,mellifera* known as the Egyptian race *A ,mellifera lamarckii* and the Carniolan race *A. mellifera carnica* as well as the Hybrid. The colonies of each race were headed by newly mated queens of almost equal age.

For the experimental work three Egyptian colonies each headed by a newly mated queen, were brought from The Bee Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation El-Dokky, Cairo, as well as three Carniolan colonies each also headed by a newly mated queen. Three Hybrid colonies randomly mated (Carniolan queens X unknown drones) were provided by the Apiary of the Faculty of Agriculture in Alexandria.

Biochemical genetic polymorphism:

Studies on biochemical polymorphism has provided many genetic markers for the analysis of problems of development and evolution. In the present study, genetic differences among and between Egyptian, Carniolan and Hybrid populations of *Apis mellifera* were revealed by examining isozyme polymorphism and analyzing their total protein fractions by SDS polyacrylamide gel electrophoresis.

1- Detection of esterase isozymes:

Ten samples of the newly emerged workers were collected from each colony of the Egyptian, Carniolan and Hybrid bees. Each sample was kept in a vial in a deep freezer till until uses.

Starch gel electrophoresis was carried out according to El-Metainy *et al.* (1977). Electrode buffer and staining solution were prepared according to Shaw (1965) and Shaw and Keen (1967). Each sample was homogenized in separate couples in 0.1 m1 of distilled water, according to Bitondi and Mestriner (1983). Each homogenate was absorbed on a 2 x 10 mm strip of filter paper, placed on the middle surface of a gel plate and kept at 4°C for about one hour, and the filter papers were then removed. Electrophoresis was carried out with a constant current of 13-14 v/cm for 2 hours at 4°C. Then the gel plates were stained at room temperature with esterase staining solution for at least 30 minutes. The best plates were selected, photographed and their diagrams were drawn.

2-Detection of Mdh (Malate dehydrogenase) isozymes:

Nine adult workers were taken alive from each tested colonies in fine net boxes to the laboratory where they were immediately frozen at- 18°C until they were used.

The thoraces and heads of adult bees were crushed at room temperature in approximately equal volumes of 300 µ 0.05 M HCl pH 8.0 girding buffer and the homogenate was centrifuged at 10000 rpm for 10 minutes (Cornuet, 1979). The pellets were then removed and the supernatant containing enzyme was transferred to a new tube and frozen at-18°C until they were used.

Electrophoresis was performed in 7 percent acrylamide gel vertical slabs (19.8 x 26.8 x 1.5 cm) gel electrophoresis apparatus. All glass plates were washed with tap and distilled water, then surface was sterilized with ethanol. The gel solution was swirled and poured using 15 well combs (1.5 mm) were used. Gels were left to polymerize for at least 30 minutes. The bridge or electrode and gel buffers were carried out according to Hubby and Lewontin (1966).

3- Protein electrophoretic studies:

Sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE) was performed for water and non-water soluble protein fractions according to the method of Laemmli (1970) which was modified by studier (1973).

The individual worker bee samples were homogenized in a cool mortar with 2 ml of extraction buffer, then vortexed for 2 min. Samples were shaked for two hours, then centrifuged for 15 min at 14000 rpm at 4°C. The

supernatant containing water soluble proteins was transferred to a new tube and the pellet was removed and kept at 0°C until utilization.

The gels were gently agitated for 1 hr. After removing the solution, a new destining solution was added to the box. Agitation was resumed until gel background became clear.

The molecular weight estimates were calculated using a Simple Basic computer program analysis (GEL works ID). Documentation system using SDS molecular weight marker (Sigma) 70 L Kit 66.000 K da to 14.200 Kda.

RESULTS

1- Esterase isozymes:

Esterase in honeybee like in other Hymenoptera occurs in six different isozymes. Their expression in a population depends, among other factors, on the genotype, developmental stage, intensity of inbreeding and the relative stability of the environment. Each isozyme, synthesized by structural genes at a single locus, may include one, two or multiple forms of allozymes according to the number of alleles persisting at this locus. These allozymes, together with isozymes, are considered as a source of genetic variability of biological significance.

The photograph of esterase isozymes electrophoretic pattern and its descriptive zymogram, for the Egyptian race of *Apis mellifera Lamarckii* is shown in Figure (1). In this figure it can be seen that the pattern reveals two bands. The first is cathodal and located, according to its relative mobility at location 2. This band will be consequently designated as (C2). Meanwhile second band is anodal, located at position 1, and will be referred to as (AI). It can also be observed in Figure 1. The C2 band is relatively more dense than AI. This observation reflects a higher activity of Est-C2 rather than Est-AI. This result applies to all samples examined (10 slots).

Furthermore, examination of Est-C2 and Est-A_1 bands for variability reveals that both loci are expressed in all samples studied and are monomorphic, where no allozyme polymorphism can be detected.

Considering the esterase electrophoretic pattern in the Carniolan race, the photograph and descriptive zymogram of this pattern are shown in Figure (2). Here, Est-C_2 band is still present but a new lighter cathodal band appears (Est-C_3) in all samples examined. On the other hand, the anodal pole for Carniolan race shows a rather different picture with that observed for the Egyptian race. Here, two newly arisen anodal bands appear. They are Est-A_2 and Est-A4. They are both consistent with all samples but Est-4 was slightly more dense than Est-A_2, It can be noted that Est-A_1 band which was found in the Egyptian race has completely disappeared in the Carniolan esterase pattern.

Variability examination of the cathodal as the anodal isozymes (Fig.3) shows that all Est-C_2, Est-C_3, Est-A_2 and Est-A_4 were monomorphic.

The electrophoretic pattern of esterase isozyrnes in the Hybrid is shown in Figure 3. It can be seen in this figure that Est-C_2 band in the

cathodal pole is still there, but in 5 samples of ten it was polymorphic demonstrating the presence of at least two alleles in this locus. Slots 1'2'3 . 4 and 10 represent heterozygotes carrying the genotype F/S (the allele F for fast and S for slow), Consequently they express a Hybrid band consisting of Est-C_2 F and Est-C_2/S at the Est-C_2 locus. The remaining cathodal bands (slots from 5 to 9) were homozygous carrying FF genotype. Examining the esterase activity in the hybrid bands at may be observed that the allele "F" is more dense than ."S" allele, giving reason to conclude that the allele "F" expresses higher esterase activity than "S".

As the anodal pole of the Hybrid esterase pattern is concerned (Fig. 3), only one band, of Est-A2 appears in all samples. The activity of this band is far less from that of the cathodal band and it's consistently monomorphic. From the results of esterase electrophoretic patterns shown in Figures 1, 2, and 3 for. Egyptian, Carniolan and Hybrid populations, it may be concluded that the esterase pattern exhibited by each of the two races and the Hybrid was fairly different. Although the cathodal Est-C_2 band was present in the three patterns and had relatively higher activity, this band was exclusive in the Egyptian race. It was accompanied by another cathodal band (Est-C_3) in Carniolan, then it turned to be polymorphic in the Hybrid. Regarding the anodal pattern, Est-A_1 appeared exclusively in the Egyptian race. It was replaced by two bands, Est-A_2 and Est-A_4 in the Carniolan then one band, Est-A_2 remained in the Hybrid; All the anodal bands for all of the two races and the Hybrid type were monophorphic.

(2) Ma1ate dehydrogenase isozymes:

Genetic differences among and between workers in the two races and the Hybrid of honeybee under investigation also appeared using an additional biochemical marker; the Malate dehydrogenase isozymes system. The photograph and descriptive zymogram of the "Mdh" electrophoretic pattern in Egyptian adults is shown in Figure (4). From the data presented in this figure, it can be demonstrated that the banding pattern of "Mdh" was totally cathodal. In all the nine samples examined from the Egyptian race, the pattern consisted of four cathodal bands: Mdh-C_3, Mdh-C_4, Mdh-C_5, and Mdh-C_6. From the point of view of their activity, Mdh-C_6 isozyme showed the highest activity as expressed by band density, followed by Mdh-C_5 isozyme. Mdh-C_4 band, on the other hand, showed the lowest activity in the Egyptian race. As the level of polymorphism is considered, it can be clearly observed that all bands representing Mdh pattern in the Egyptian race were monomorphic.

With regard to the Carniolan race, the Mdh isozyme pattern is presented in the photograph and its descriptive zymogrann shown in Figure (5). Examining this pattern, it can be seen the occurence of the four isozymes previously detected (Mdh-C_3-Mdh-C_6), in addition to the two newly arising isozyme: Mdh-C_1 and Mdh-C_2). The latter, as the other four bands, was common in the Carniolan samples examined, but the former (Mdh-C_1) isozyme appeared only in 4 samples out of nine. Mdh isozyme activity, on the other hand, was largely distributed over bands instead of being concentrated in one or two. The pattern in the Carniolan race showed a wide distribution of activity over bands. Furthermore, examination of this pattern with regard to

genetic variation clearly demonstrate that all isozymes expressed were, again, monomorphic.

With respect to the Hybrid Mdh isozyme pattern shown in Figure (6), it can be seen that the pattern consists of six bands namely from Mdh-C_1 to Mdh-C_6. Moreover, isozyme activity is widely distributed over the whole array of bands. This pattern again shows that all isozymes appearing in the Mdh system are monomorphic.

By examining the Malate dehydrogenase isozyme system for the Egyptian, Carniolan and the Hybrid populations shown in Figures (4 , 5 and 6) respectively, it may be concluded that the Egyptian race, *Apis mellifera* shows clear and distinct differences from both the Carniolan race and the Hybrid. While four isozyme Mdh cathodal bands Mdh-C_3, Mdh-C_4, Mdh-C_5 and Mdh-C_6 were common all over the three populations under investigation and appeared in all samples, two additional extra-bands (Mdh-C_1 and Mdh-C_2) occurred in both the Carniolan race as in the Hybrid. Another discriminative feature for Egyptian race is that the malate dehydrogenase activity was expressed mainly in the Mdh-C_6 followed by Mdh-C_5. On the other hand, this activity which appeared mostly over the whole range of bands accused in both the Carniolan race and the Hybrid. It is also interesting to note that the totality of malate dehydrogenase bands appearing in all slots representing the Egyptian, Carniolan and Hybrid samples were all monomorphic.

(3) SDS polyacrylamide gel electrophoresis:

The protein fraction of the three populations of honeybee under investigation was detected and identified using a molecular genetic powerful discriminative technique with relatively high resolution; the SDS polyacrylamide gel electrophoresis (SDS-PAGE). The samples examined by this method were haemolymph extracted form honeybee workers. Four fingerprint replicates were assayed for each population and were denoted on the pattern as E, C and H standing for Egyptian, Carniolan and Hybrid populations, respectively. In addition, a standard reference protein marker was simultaneously assayed in order to detect the molecular weight (MW) and relative mobility (Rf) of each band appearing on the pattern.

The data presented by applying this approach become rather clear and informative emphasizing the discriminative fingerprint of each population (Table 1). By examining this table, it can be clearly observed that several bands appear in common all over the pattern (colored in yellow). Such protein fractions cannot play the role of "marker proteins" since they can be detected and identified in all populations and samples. In addition to these fractions, (Table 1) shows another type of bands which appear scattered as seen in separate patterns denoting different populations, but they prove to be common bands as shown in (Table 1). The example for this type of bands resides in fractions of molecular weight from 21.19 (colored in pink) plotted with a range of relative mobility extending from 0.470 to 0.479.
Protein fractions colored in light green and dark green also represent good examples of this type of common bands. It is important to note that common bands are much more frequent than uncommon ones and represent the great majority of protein fractions in the pattern.

Table (1): Relative mobility and protein fraction distribution in Egyptian, Carniolan and Hybrid populations.

Band #	Rf	E1	E2	E3	E4	C1	C2	C3	C4	H1	H2	H3	H4
1	0.079		139.772	137.375									
2	0.138		115.138	105.642	111.08			86.466	90.911	85.443			
3	0.174		85.619										
4	0.176				84.347								
5	0.178		80.889										
6	0.199	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]			
7	0.203												[illegible]
8	0.216				62.136								
9	0.217							61.962					
10	0.22		59.963	60.161		60.631							
11	0.224	58.509							58.899				59.09
12	0.225						57.637			58.168	57.812		
13	0.227											57.044	
14	0.234					54.185	53.502	54.444	54.524	53.903	53.72	53.641	54.812
15	0.254	48.502											
16	0.256												46.419
17	0.279	42.063	41.685	41.55	41.644	39.52	40.699	40.267	39.783	40.336	39.337	39.604	39.445
18	0.324	34.636	34.749	35.148	34.111	35.116		34.646	34.88	34.66	34.682	35.192	35.047
19	0.372	28.414		26.822	29.806	29.546	30.188	29.628	29.744	29.865	30.213	29.842	29.728
20	0.376		28.738										
21	0.421	25.409	25.331	25.422	25.575	26.294	26.407	26.171	25.528	25.461	26.458	26.345	26.087
22	0.47	21.76	22.163	22.217	21.98	23.192	23.146	22.171	22.876			23.118	
23	0.473												22.716
24	0.476										22.96		
25	0.479									22.605			
26	0.516	20.182	20.019	20.218	20.149	20.873	21.011	20.967	21.165	20.963	20.969	21.075	21.059
27	0.596	18.801	18.765	18.671	18.729	18.636	18.722	18.843	18.72	18.686	18.643	18.707	18.624
28	0.673	18.203	18.017	17.967	17.974	17.682	17.572	17.711	17.619	173842	17.602	17.627	17.66
29	0.749	17.92	17.779	17.683	17.742	17.145	17.031	17.224	173105	17.059	16.977	16.966	17.098
30	0.832	17.786	17.613	17.501	17.581	17.706	16.75	16.943	16.858	16.775	16.592	16.622	16.749
31	0.908	[illegible]			[illegible]	[illegible]			[illegible]	[illegible]	[illegible]	[illegible]	

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On the other hand, further examination of the data presented in (Table 1) reveals that appearance is exclusively restricted to the pattern of a certain population. Examples of such fractions are those of high molecular weight ranging between ~139~ and ~111 Kda, figuring exclusively in the pattern of Egyptian race and disappear completely in both the Carniolan and the Hybrid patterns. Another example for band specificity in relation to strain is clearly shown by the fraction having molecular weight 54 Kda which appear at Rf value 0.234 and prove to be restricted to all samples derived from the Carniolan and Hybrid populations but show complete absence from all samples of the Egyptian race.

It can be generally noted that most of the differences between populations were observed in the upper part of the pattern which has high molecular weight protein fractions rather than in the lower part of the pattern where common bands with low molecular weight fractions predominate.

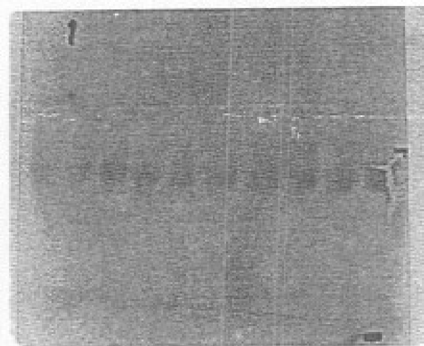
Considering the coefficiency of similarity values presented in Table 2, together with the pheno gram showing phylogenetic relationship between different genotypes illustrated in Figure (7), it can be seen that C_1, H_2 and H_3 samples as well as C_4 and H_1 were completely identical giving a similarity coefficient of "I'. H_1 However, comparison between Carniolan and Hybrid patterns revealed a general mean of 0.927.

On the other hand, the Egyptian race showed rather lower similarity coefficients with both Carniolan and Hybrid bees with average estimates of 0.861 and 0.863, respectively.

It can be obviously noted that similarity between Carniolan and Hybrid samples give higher values rather than similarity between each of them and the Egyptian race.

Table (2): Coefficient of similarity between different genotypes representing the Egyptian, Carniolan and the Hybrid as revealed by SDS-PAGE

		E1	E2	E3	E4	C1	C2	C3	C4	H1	H2	H3	H4
Lane		1	2	3	4	5	6	7	8	9	10	11	12
1	E1	1											
2	E2	0.87	1										
3	E3	0.87	1	1									
4	E4	0.9	.097	0.97	1								
5	C1	0.89	0.83	0.83	0.86	1							
6	C2	0.85	0.79	0.79	0.81	0.88	1						
7	C3	0.86	0.87	0.87	0.9	0.89	0.92	1					
8	C4	0.9	0.9	0.9	0.93	0.93	0.89	0.97	1				
9	H1	0.9	0.9	0.9	0.93	0.93	0.89	0.97	1	1			
10	H2	0.89	0.83	0.83	0.86	1	0.88	0.89	0.93	0.93	1		
11	H3	0.89	0.83	0.83	0.86	1	0.88	0.89	0.93	0.93	1	1	
12	H4	0.93	0.8	0.8	0.83	0.89	0.92	0.93	0.9	0.9	0.89	0.89	1



Bands
+

Est-A_1

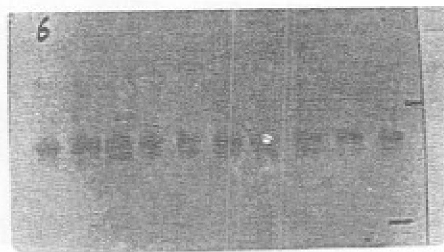
O.L

Est-C_2

−

Fig. (1): Photograph and discriptive zymogram showing the Esterase isozymes pattern in adult stages newly emergent in Egyptian race.

Key of band densities v. high ▇ > ▨ > ▨ > ▨ > ▭ v. low



Bands
+

Est-A_1

Est-A_2

O.L.

−

Est-C_2

Est-C_3

Fig. (2): Photograph and discriptive zymogram showing the Esterase isozymes pattern in adult stages newly emergent in Carniolan race.

Key of band densities v. high ▇ > ▨ > ▨ > ▨ > ▭ v. low

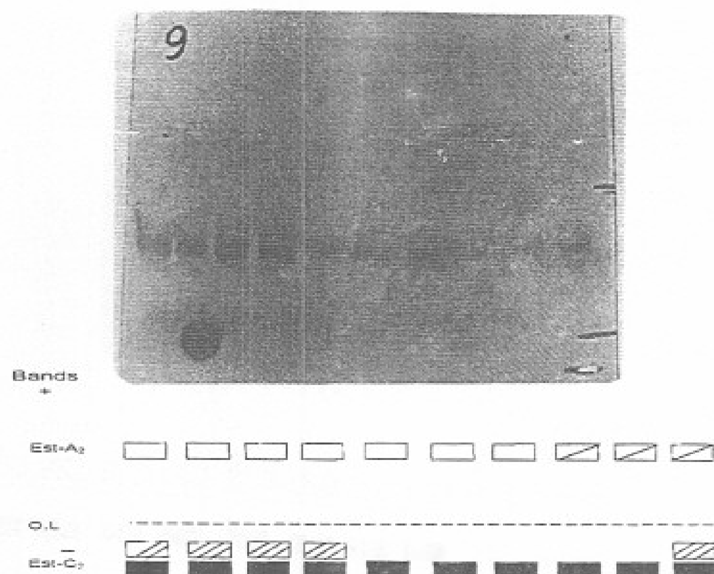


Fig. (3): Photograph and discriptive zymogram showing the Esterase isozymes pattern in adult stages newly emergent in Hybrid.

Key of band densities v. high > > > > v. low

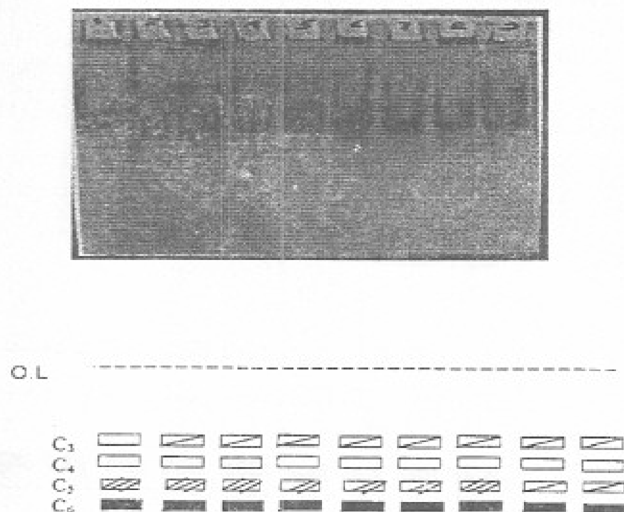
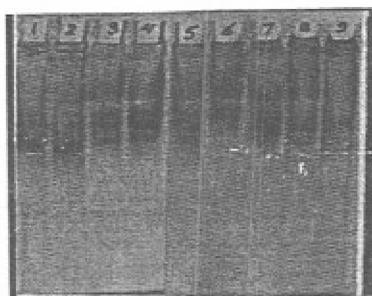


Fig. (4): Photograph and discriptive zymogram showing the Malate dehydrogenase (Mdh) isozymes pattern in adult worker stage of Egyptian race

Key of band densities v. high > > > > v. low



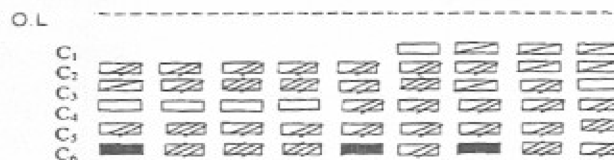
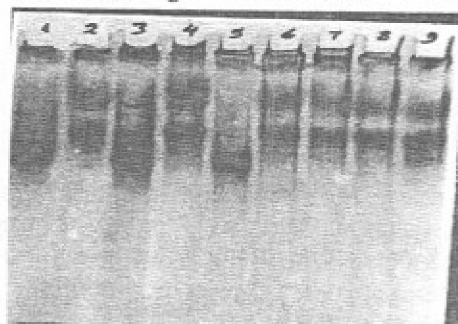


Fig. (5): Photograph and discriptive zymogram showing the Malate dehydrogenase (Mdh) isozymes pattern in adult worker stage of Carniolan race.

Key of band densities v. high > > > > v. low



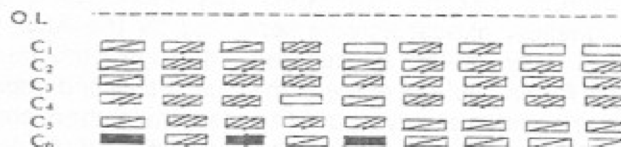


Fig. (6): Photograph and discriptive zymogram showing the Malate dehydrogenase (Mdh) isozymes pattern in adult worker stage of Hybrid.

Key of band densities v. high > > > > v. low

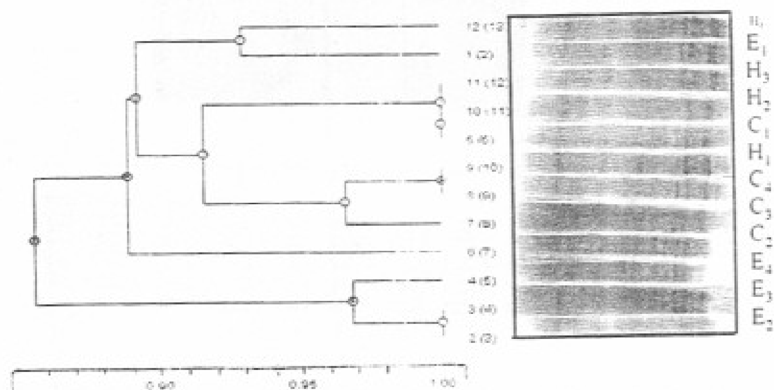


Fig. (7): Phenogram showing phylogenetic relationship between different geneotypes reprenting the Egyptian, Carniolan and the Hybrid population.

DISCUSSION

The data on esterase electrophoretic activity revealed the presence of a constant common cathodal band with considerable activity (Est- C2) in the three tasted populations. Whereas this band was monomorphic in both the Egyptian and Carniolan races, it was polymorphic in the Hybrid. This finding agrees with those of Badino *et al.* (1983) who studied population structures of *Apis mellifera* from North Italy and South France. The two honeybee varieties, almost entirely separated by the Alps, hybridize with each other in very limited Alpine area. Although both populations were monomorphic for an allele M at the Mdh locus, the Hybrid population showed isozyme polymorphism at this locus. Therefore the authors considered the M allele as a dignostic marker, which allows the distinction, with fairly good/ accuracy, between both populations and the Hybrid.

Furthemore, the anodal esterase bands appearing in different patterns were Est-A_1, Est-A_2 and Est-A_4, and Est A_2 in the Egyptian, Carniolan and Hybrid populations, respectively. All these anodal bands were consistent and monomorphic. Examining these results, a number of points may be noted : (a), Est- A_1 anodal band was restricted to the Egyptian race and might be considered as genetic marker to this race (b) Est- A_2 and Est-A_4 have replaced Est-A_1 in the Carniolan race, the former maintained its activity

in the Hybrid forming a common band in both populations: (c) all esterases appearing in the Hybrid population get their origin from the Egyptian or Carniolan races or both.

Examination of the Malate dehydrogenase isozyme patterns in the three populations supports and confirms the previous points about 67 % of the bands are common to all of the three populations, and more than 90% are strictly common to only the Carniolan and Hybrid ones. Such findings may account for the results obtained in many instances during this investigation regarding to physiological characteristics of the three populations of honeybee with the conclusion that the Hybrid bee population is closely related to the Carniolan rather than to the Egyptian race.

In addition, the conclusions drawn from the SDS-polyacrylamide gel electrophoresis analysis were informative and conclusive. The pattern of protein fractions electrophoregram showed only two distinct fingerprints . The - first denoting the Egyptian race and was characterized by the presence of extremely high molecular weight protein fractions (~139- ~ 111 Kda). The second, denoting both the Carniolan and the Hybrid populations, was characterized by the presence of a distinct and consistent protein fraction (53~ 54 Kda). Therefore, each of these two fingerprints included its own genetic marker. Such findings are in accordance with those of Akey and Hung (1990) who found two proteins with molecular weight 42 and 52 Kda to be specific to *A. mellifera scutellata* and Africanized honeybees. It can also be concluded that most of the differences between populations were observed in the upper part of the pattern. The lower part consisted mostly of common bands.

Taking into account the results of the SDS-PAGE technique, together with those of isozyme genetic variability, it can be concluded that the Hybrid population is closely phylogenetically related to the Carniolan rather than to the Egyptian race.

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دراسات وراثية بيوكيميائية على نحل العسل

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تناول هذا البحث دراسة المشابهات الانزيمية لـ Malate Dehydrogenase, Esterase وكذلك تحليل البروتين فى سلالتين من نحل العسل هى سلالة النحل المصرى وسلالة النحل الكرنيولى وكذلك الهجين وأوضحت نتائج الدراسة ما يلى :

١- أظهر التفريد الكهربى لنشاط مشابهات أنزيم الاستريز حزمه سالبة ($Est\text{-}C_2$) حيث ظهر شكل مشترك ومنسق بين الثلاث عشائر تحت الدراسة. وقد أظهرت تلك الحزمة تعددا فى الأليلات فقط لدى العشيرة الهجين.

٢- الحزم الموجبة التى ظهرت فى العشائر الثلاث كانت ($Est\text{-}A_1$) و – ($Est\text{-}A_4$, $Est\text{-}A_2$ – $Est\text{-}_2$) فى النحل المصرى والكرنيولى والهجين على الترتيب وكانت جميعها ثابتة فى جميع العينات وغير متعددة الأليلات.

٣- يتضح من توزيع الحزم لمشابهات إنزيم الاستريز أنه فى حين ان الحزمة $Est\text{-}C_2$ تظهر مشتركة بين العشائر الثلاث ولا تمثل علاقة وراثية إلا ان الحزمة $Est\text{-}A_1$ تعتبر مميزة للسلالة المصرى حيث تقتصر عليها. كذلك فإن الحزمة $Est\text{-}A_2$ تظهر مشتركة يبين كل من السلالة الكرنيولى والنحل الهجين مما يشير إلى وجود تقارب وراثى بينهما.

٤- تبين من التفريد الكهربى لمشابهات انزيم Malate Dehydrogenase أن جميع الحزم سالبة ولم تظهر أى حزم مقتصرة على سلالة أو مميزة لها. وقد ظهرت الحزم من $Mdh\text{-}C_3$ إلى $Mdh\text{-}C_6$ فى السلالة المصرى كما ظهرت الحزم من $Mdh\text{-}C_1$ إلى $Mdh\text{-}C_6$ فى كل من الكرنيولى والهجين مع ظهور اختلاف فى توزيع نشاط الأنزيم عند الهجين.

٥- يتضح من توزيع الحزم لمشابهات انزيم Malate Dehydrogenase أن ثلثى الحزم تظهر مشتركة فى الثلاث عشائر فى حين ان الثلث الباقى يظهر فى السلالة الكرنيولى والنحل الهجين فقط مشيرا إلى التقارب الوراثى بينهما.

٦- اوضح تحليل البروتين ان الحزم ذات الوزن الجزئى المرتفع (~139~ 111 KDa) يقتصر وجودها على السلالة المصرى فقط ، كذلك فإن الحزمة (~53~54 Kda) فقد ظهرت فى جميع عينات السلالة الكرنيولى والنحل المصرى فقط وهذه النتيجة تشير إلى وجود اختلافات وراثية واضحة بين سلالتى المصرى والكرنيولى وان النحل الهجين تربطه علاقة وراثية أكبر من الكرنيولى عنه مع السلالة المصرى.

٧- بفحص الشكل التخطيطى للعلاقة الوراثية وكذلك قيم معامل التماثل بين الثلاث عشائر تبين وجود تطابق كامل بين بعض عينات السلالة الكرنيولى والهجين بمتوسط عام للتماثل ٠,٩٧٢ فى حين ان السلالة المصرى أظهرت تماثل مع كل من الكرنيولى والهجين بمتوسط عام للتماثل ٠,٨٦١ و ٠,٨٦٣ على الترتيب.

٨- أشارت المقارنات بين الحزم البروتينية فى الهجين وبين كل من السلالة المصرى والكرنيولى أن أغلب الحزم فى الهجين قد نقلت إليه من الكرنيولى وأن البقية من تلك الحزم نقلت اليه من المصرى وهذا يفسر درجة التماثل العالية بين الهجين والكرنيولى وكذلك يمكن أن تشير إلى ان النحل الهجين المستخدم فى تلك الدراسة قد نتج عن تزاوج ملكات كرنيولى مع ذكور مصرى.