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GENETIC DISTANCE BASED ON ELECTROPHORETIC ISOESTERASES OF TWO SOLE SPECIES FROM MEDITERRANEAN SEA AND LAKE QARUN, EGYPT.

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ABSTRACT

*Specimens of **Solea aegyptiaca** and **Solea vulgaris** were obtained from two different localities, Abu-Kir Bay off Alexandria and lake Qarun, during 1993-1994. Esterase isozymes were compared electrophoretically, using three organ tissues; heart, liver and kidney, to determine the phylogenetic relationship between **Solea aegyptiaca** and **Solea vulgaris**, and also to know if transplanted sole in Lake Qarun has become a distinct population. From results of esterase patterns in various organ tissues, it may be concluded that Est-3A as well as, Est-2A were the only loci invariably expressed in all organ tissues of both species analyzed. The gene frequencies estimated for allelic variants in different organ tissues indicated that Est-3A locus had a tendency of high polymorphism regarding to all studied organ tissues.*

*The Nei's genetic distance values, between the two species, **Solea vulgaris** and **Solea aegyptiaca**, are varied between 0.025 & 0.101, while the genetic distance between **Solea aegyptiaca** populations from Abu-Kir Bay and Lake Qarun was 0.024. The phylogenetic*

*relationships based on genetic distance revealed that **Solea vulgaris** and **Solea aegyptiaca** are genetically diverged, as well as, the transplanted **Solea aegyptiaca** in Lake Qarun had become a distinct population differing from that in Abu-Kir Bay off Alexandria.*

INTRODUCTION

The sole fishes were transplanted into Lake Qarun from the Egyptian Mediterranean off Alexandria, the successful acclimatization of sole fish has been established since it became a major part of the lake fisheries (El-Zarka, 1963).

The electrophoretic methods have proved to be useful in species identification (Allendorf and Utter, 1979). Furthermore, according to Tave and Smitherman (1980); Menezes ***et al.*** (1993), the electrophoresis can give an independent estimate of the level of variation within a population without an extensive survey of morphological and other quantitative traits.

The esterases are investigated in several organisms as useful genetic markers in tissue differentiation and population variation (Masters and Holmes, 1975). Ligny (1968) employed esterases as genetic markers in flounder (***Pleuronectes flesus***) and plaice (***Pleuronectes platessa***); Ridgway ***et al.*** (1970) in Atlantic herring; Ahuja ***et al.*** (1977) in Xiphophorine fish (***Platypoecilus maculatus*** and ***Xiphophorus helleri***); Hindar (1986) and Dempson ***et al.*** (1988) in Arctic Charr (***Salvelinus alpinus***) and El-Deeb and Essa (1992) in Tilapia species.

The literature pertaining to the genetics for sole fishes has been cited by some workers in different localities. Quignard ***et al.*** (1984) made a biosystematic studies on ***Solea vulgaris*** complex in the Gulf of Lion by using biochemical genetics to differentiate between ***Solea vulgaris*** and ***Solea aegyptiaca***. Pasteur ***et al.*** (1985) gave a survey about biochemical genetic polymorphisms of ***Solea vulgaris*** with details of the techniques employed. Basaglia ***et al.*** (1988) estimated the evolution of enzymatic patterns of some isozymes during the development of ***Solea vulgaris*** using the biochemical electrophoretic method.

The present study was carried out by analyzing esterase isozyme electrophoretically in order to reveal biochemical genetic markers and to find out the phylogenetic relationships between ***Solea vulgaris*** and ***Solea aegyptiaca*** from Abu-Kir Bay and ***Solea aegyptiaca*** from Lake Qarun, as well as, to know if the transplanted sole in Lake Qarun has become a distinct population by estimating the genetic distances between ***Solea aegyptiaca*** from Lake Qarun, ***Solea aegyptiaca*** and ***Solea vulgaris*** from Abu-Kir Bay.

MATERIAL AND METHODS

Random samples of sole fishes were taken from two different localities Lake Qarun and Abu-Kir Bay waters during 1993-1994.

For electrophoretic esterase analysis, three organs; heart, liver and kidney were taken from 37 alive specimens of ***Solea vulgaris*** ranged from 170 to 210 mm in total length, 64 alive specimens of ***Solea aegyptiaca*** varied from 170 to 280 mm in total length, from Abu-Kir Bay and 72 alive specimens of ***Solea aegyptiaca*** ranged between 150 and 260 mm in total length from Lake Qarun.

Discontinuous polyacrylamide gel electrophoresis (Disc-PAGE) was carried out by utilizing the Pharmacia Gel Electrophoresis apparatus GE-2/4 (Anderson and Thorpe, 1980; Sammons ***et al.***, 1981).

Agar-Starch-Polyvinyl Pyrolidine (P.V.P.) gel electrophoresis was carried out according to the procedures described by Shaw and Kaen (1967); El-Metainy ***et al.*** (1977) and Sabrah and El-Metainy (1985).

According to the results of esterase electrophoretic patterns, gene frequencies of alleles segregating at each locus and in combined organ tissues were estimated.

The identity of genes and genetic distance were calculated by using a BASIC computer program which was described by Green (1979).

RESULTS

A gel electrophoretic pattern of esterase isozymes from the organs; heart, liver and kidney of sole fishes is shown in Fig. (1). This figure declares that five anodal, and two cathodal loci were controlling esterase isozymes. Also, Est-2A & Est-3A loci were the only ones that expressed in all studied samples, and the locus Est-3A showed higher activities in all samples, than other loci.

Figure (2) presents the zymogram of electrophoretic profiles of heart, liver and kidney esterase isozyme of sole fishes from different water sources. It was found that Est-3A, as well as, Est-2A were the only loci invariably expressed in all organ tissues for ***Solea vulgaris*** from Abu-Kir Bay and ***Solea aegyptiaca*** from Abu-Kir Bay and Lake Qarun. Other loci showed variable expression in relation to organ and/or species analyzed. As for the specificity of loci expression, it was noted that the Est-5A locus was limited to ***Solea vulgaris*** profiles for kidney tissues only, while Est-4A locus to ***Solea aegyptiaca*** from Lake Qarun and ***Solea vulgaris*** of Abu-Kir Bay of Alexandria in all organ tissues. Est-1A locus was expressed in the heart and kidney of ***Solea aegyptiaca*** from Abu-Kir Bay profiles, but for ***Solea aegyptiaca*** from Lake Qarun, Est-1A was only shown in liver, and in the kidney of ***Solea vulgaris*** samples. Specificity was represented by the Est-1C, which was absent in heart and kidney tissues of ***Solea aegyptiaca*** from Lake Qarun only, as for Est-2C was absent in all organ tissues of ***Solea aegyptiaca*** from Lake Qarun. On the other hand, Est-2C was absent also in heart and liver for ***Solea vulgaris*** and in heart of ***Solea aegyptiaca*** from Abu-Kir Bay.

With regard to the gene frequency estimations for the studied alleles in different organ tissues (Tables 1 to 4), it was found that Est-3A locus showed a tendency of high polymorphism regarding to all studied organ tissues.

Estimates of genetic identity and Nei's genetic distance between pairs among ***Solea vulgaris*** from Abu-Kir Bay and ***Solea aegyptiaca*** from each Abu-Kir Bay and Lake Qarun are presented in Table 5. Table (from 1 to 3) represents gene frequency estimates derived from a separate organ, and Table (4) indicates the estimates from the data of combined organ tissues, while the last one (Table 5) shows Nei's estimates for the mean values of each of the three organs. Also this table presents these values as combined data from the three loci and the three organ tissues for each species or population.

Table 1. Gene frequency estimates for alleles segregating at different loci coding for **heart** esterase isozymes of the Solea species from Abu-Kir Bay of Alexandria and Lake Qarun.

Habitat	Loci / Species	Est-5A		Est-4A		Est-3A		Est-2A		Est-1A		Est-1C		Est-2C	
		+	-	+	-	F	S	+	-	+	-	+	-	+	-
Abu-Kir Bay	*Solea vulgaris*	--	--	0.229	0.771	0.571	0.429	0.314	0.686	--	--	0.057	0.943	--	--
	Solea aegyptiaca	--	--	--	--	0.554	0.446	0.508	0.492	0.138	0.862	0.092	0.908	--	--
Lake Qarun	*Solea aegyptiaca*	--	--	0.139	0.861	0.514	0.486	0.708	0.292	--	--	--	--	--	--

Table (2): Gene frequency estimates for alleles segregating at different loci coding for **liver** esterase isozymes of the Solea species from Abu-Kir Bay of Alexandria and Lake Qarun.

Habitat	Loci / Species	Est-5A		Est-4A		Est-3A		Est-2A		Est-1A		Est-1C		Est-2C	
		+	-	+	-	F	S	+	-	+	-	+	-	+	-
Abu-Kir Bay	*Solea vulgaris*	--	--	0.057	0.943	0.529	0.471	0.429	0.571	--	--	0.486	0.514	--	--
	Solea aegyptiaca	--	--	--	--	0.531	0.469	0.328	0.672	--	--	0.344	0.656	0.094	0.906
Lake Qarun	*Solea aegyptiaca*	--	--	0.181	0.819	0.521	0.479	0.194	0.806	0.028	0.972	0.028	0.972	--	--

Table (3): Gene frequency estimates for alleles segregating at different loci coding for **kidney** esterase isozymes of the Solea species from Abu-Kir Bay of Alexandria and Lake Qarun.

Habitat	Loci / Species	Est-5A		Est-4A		Est-3A		Est-2A		Est-1A		Est-1C		Est-2C	
		+	-	+	-	F	S	+	-	+	-	+	-	+	-
Abu-Kir Bay	*Solea vulgaris*	0.297	0.703	0.486	0.514	0.500	0.500	0.459	0.541	0.405	0.595	0.757	0.243	0.216	0.784
	Solea aegyptiaca	--	--	--	--	0.508	0.492	0.377	0.623	0.066	0.934	0.279	0.721	0.049	0.951
Lake Qarun	*Solea aegyptiaca*	--	--	0.208	0.792	0.542	0.458	0.236	0.764	--	--	--	--	--	--

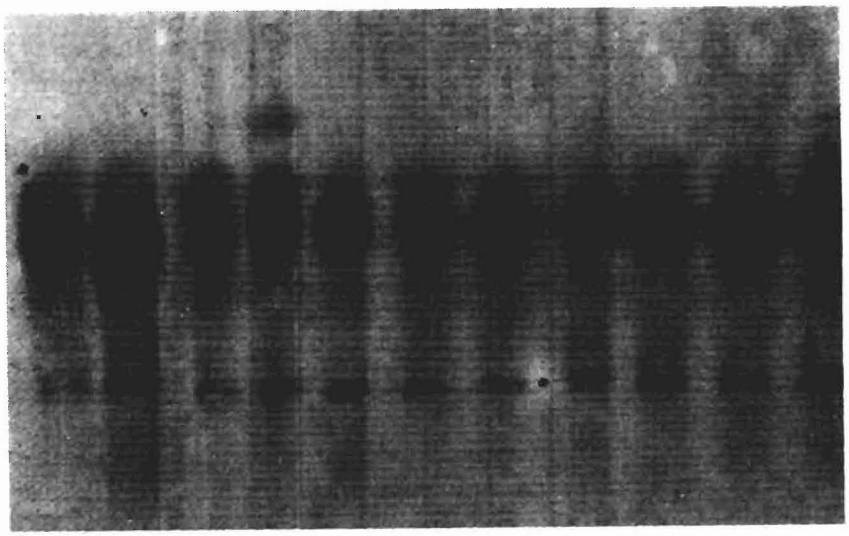
Table (4): Gene frequency estimates for alleles segregating at different loci coding for esterase isozymes in **combined organ tissues** (heart, liver and kidney) of the Solea species from Abu-Kir Bay of Alexandria and Lake Qarun.

Habitat	Loci / Species	Est-5A		Est-4A		Est-3A		Est-2A		Est-1A		Est-1C		Est-2C	
		+	-	+	-	F	S	+	-	+	-	+	-	+	-
Abu-Kir Bay	*Solea vulgaris*	0.103	0.897	0.262	0.738	0.533	0.467	0.402	0.598	0.132	0.868	0.439	0.561	0.071	0.929
	Solea aegyptiaca	--	--	--	--	0.531	0.469	0.405	0.595	0.068	0.932	0.237	0.763	0.047	0.953
Lake Qarun	*Solea aegyptiaca*	--	--	0.176	0.824	0.526	0.474	0.379	0.621	0.009	0.991	0.009	0.991	--	--

Table (5): Estimates of genetic identity and genetic distance between pairs among the Solea species for heart, liver, kidney and combined for loci and organs.

Species	*S. vulgaris* (Abu-Kir Bay)				*S. aegyptiaca* (Abu-Kir Bay)				*S. aegyptiaca* (Lake Qarun)			
	heart	liver	kidney	combined	heart	liver	kidney	combined	heart	liver	kidney	combined
S. vulgaris (Abu-Kir Bay)	---	---	---	---	0.020	0.019	0.156	0.025	0.460	0.142	0.291	0.101
S. aegyptiaca (Abu-Kir Bay)	(0.980)	(0.981)	(0.856)	(0.975)	---	---	---	---	0.341	0.055	0.247	0.024
S. aegyptiaca (Lake Qarun)	(0.632)	(0.867)	(0.747)	(0.904)	(0.711)	(0.946)	(0.781)	(0.976)	---	---	---	---

The genetic identity are given in parentheses, and the genetic distance are given without parentheses.



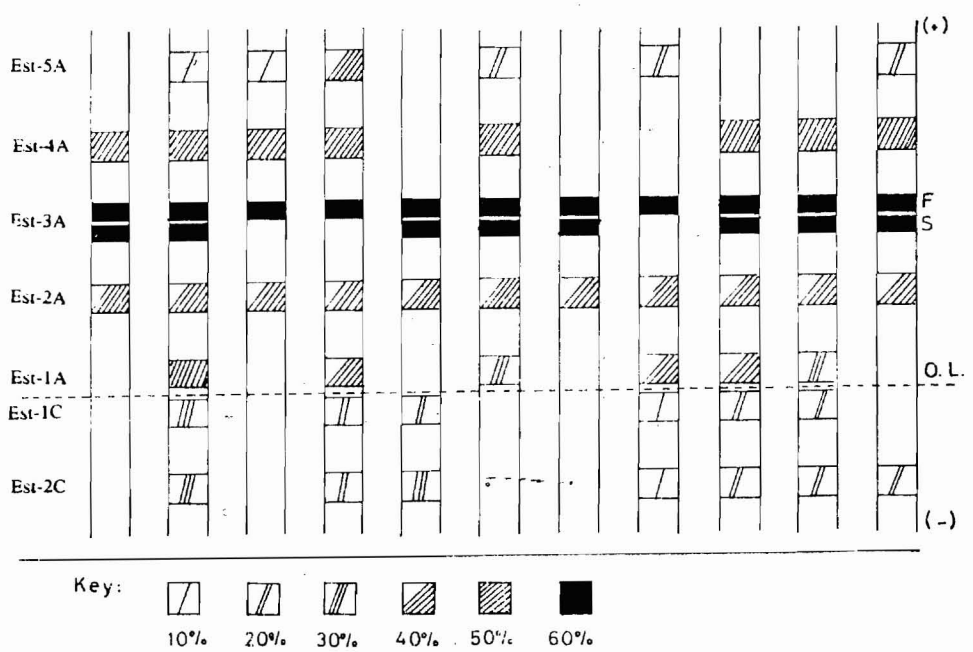


Figure 1. A photograph and a zymogram showing electrophoretic pattern and loci coding for esterases.

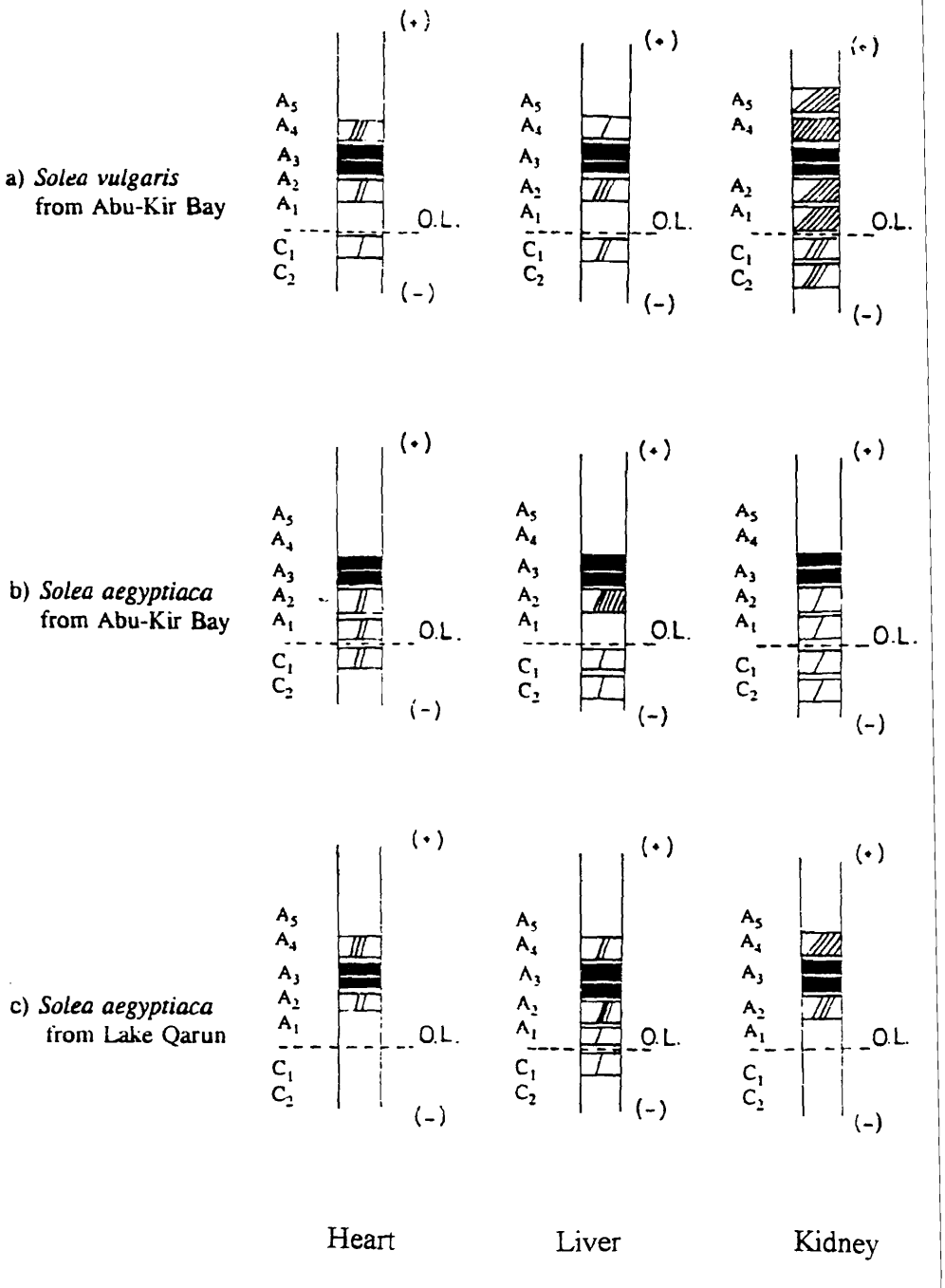


Figure 2. Zymogram showing electrophoretic profiles of **heart**, **liver** and **kidney** esterase systems of different sole fishes.

Furthermore, phylogenetic relationships based on genetic distances are better illustrated through a diagram and a dendrogram using the mean of the three organs together (Figure 3, a-b). The figure reveals a different magnitudes of similarities between pairs of ***Solea*** species and populations under study.

Distinct genetic distances derived from heart tissues was evident, and the closed genetic distance was observed between ***Solea vulgaris*** and ***Solea aegyptiaca*** from Abu-Kir Bay (D = 0.020) followed by that between the two ***Solea aegyptiaca*** population that had different habitat (D = 0.341) and the largest value was between ***Solea vulgaris*** from Abu-Kir Bay and ***Solea aegyptiaca*** from Lake Qarun (D = 0.460), which are different genetically and also have different environmental conditions for many years.

The data of genetic identity and genetic distance derived from liver tissues indicated that genetic identity varies in a wide range. Genetic distance between the two different species, ***Solea vulgaris*** and ***Solea aegyptiaca*** form Abu-Kir Bay, was equal to 0.019, but the relation between the two populations of ***Solea aegyptiaca*** from Abu-Kir Bay and Lake Qarun, was equal to 0.055, while the largest interval was observed between ***Solea vulgaris*** from Abu-Kir Bay and ***Solea aegyptiaca*** from Lake Qarun (D = 0.142).

Data derived from kidney tissues showed a similar case as shown in previous organs. Genetic distance between ***Solea vulgaris*** and ***Solea aegyptiaca*** from Abu-Kir Bay was equal to D = 0.156, but the relation between ***Solea aegyptiaca*** from Abu-Kir Bay and Lake Qarun was equal to D = 0.247, and the interval between ***Solea vulgaris*** from Abu-Kir Bay and ***Solea aegyptiaca*** from Lake Qarun was observed to be the largest D = 0.291.

In order to summarize the phylogenetic relationships among the ***Solea*** species and populations under study, the data of the chosen loci in all organ tissues were combined together. This over-all genetic identity and genetic distance may be considered as key parameters expressing phylogenetic relationships between the species and populations under study. An equal maximal magnitudes of genetic identity estimates were observed between ***Solea vulgaris*** and ***Solea aegyptiaca*** from Abu-Kir Bay and also between ***Solea aegyptiaca*** population from Abu-Kir Bay and Lake Qarun (I = 0.97). Minimal value for these estimates was observed, between ***Solea vulgaris*** and ***Solea aegyptiaca*** from Abu-Kir Bay and Lake Qarun, respectively (I = 0.9).

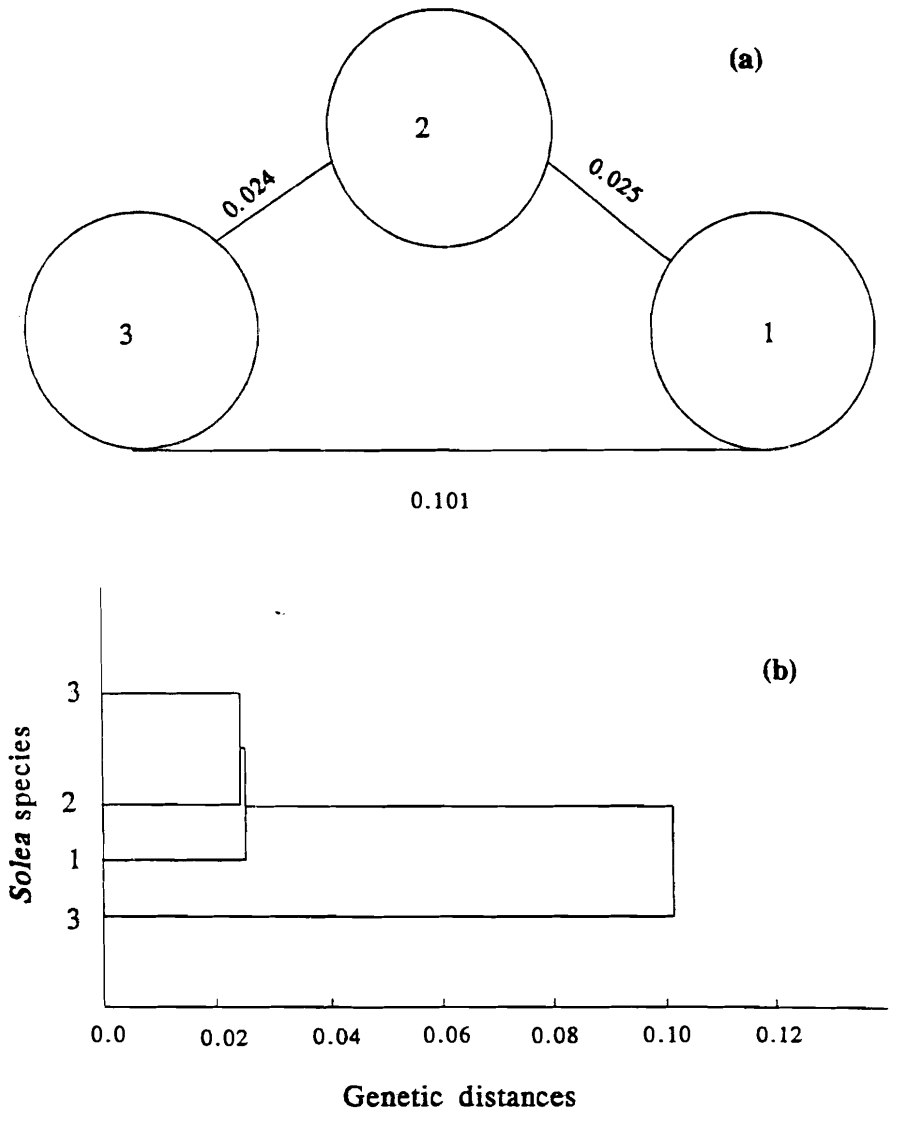


Figure 3. (a) A diagram; and (b) A dendrogram of Nei's genetic distances combined for loci from three organ tissues among the different solea species.

1, ***Solea vulgaris*** from Abu-Kir Bay;
2, ***Solea aegyptiaca*** from Abu-Kir Bay;
3, ***Solea aegyptiaca*** from Lake Qarun.

Considering genetic distance estimates presented in Table (5), the minimal values for these estimates were found between both ***Solea vulgaris*** and ***Solea aegyptiaca*** from Abu-Kir Bay and also between ***Solea aegyptiaca*** populations at the two habitats (D = 0.025, and D = 0.024), respectively. Maximal genetic distance, expressing higher divergence, was observed between ***Solea vulgaris*** from Abu-Kir Bay and ***Solea aegyptiaca*** from Lake Qarun (0.101). This result indicates that there is a high divergence between ***Solea vulgaris*** from Abu-Kir Bay and ***Solea aegyptiaca*** from Lake Qarun.

DISCUSSION

A presence of two common sympatric species of Genus ***Solea*** in Mediterranean off Alexandria has an urgent need to study their phylogenetic relationship based on genetic distance between these two species.

In the present study, the gene frequency and genetic distance are estimated for alleles segregating at different loci coding for esterase isozymes in three organs; heart, liver and kidney of the ***Solea vulgaris*** and ***Solea aegyptiaca*** from Abu-Kir Bay. The specificity of isozymes to organ and species was indicated by the Est-5A locus which its presence was restricted to kidney profiles in ***Solea vulgaris*** from Abu-Kir Bay.

The Nei's genetic distance between the two species based on 6 alleles at 3 loci for all organ tissues, ranged from 0.025 to 0.101. The great genetic divergence between ***Solea vulgaris*** and ***Solea aegyptiaca*** from Abu-Kir Bay revealed two distinct characteristic species. This finding is in accordance with that reported by Quignard ***et al.*** (1984) who demonstrated that two distinct species *of* ***Solea vulgaris*** and ***Solea aegyptiaca*** from the Gulf of Lion are found using biochemical genetics and biometric studies, they showed that esterases were expressed, in kidney and liver tissue organs, as three loci in the two species, where, Est-2 was only expressed in kidney, and the three loci were varied in the electrophoretic mobility. The Est-1 in both species are monomorphic. The allelic frequencies observed in the Est-2 in different region area for ***Solea vulgaris*** are not significant, but those of Est-2 in ***Solea aegyptiaca*** are significant in between regions. The two species are different from each other and Nei's genetic distances between the two species are ranged from 0.721 to 0.860.

Further, isozymic variation was used to determine genetic variability between transplanted sole in Lake Qarun and Mediterranean. Segregating alleles at different loci coding for esterase isozymes in heart, liver and kidney are found to be specific to population. The specificity was represented by the absence of Est-4A and Est-2C in all organ tissues of ***Solea aegyptiaca*** from Abu-Kir Bay and Lake Qarun, respectively. These results agree with that of Grant ***et al.*** (1984) who found differences in the number of esterase bands in different habitats. Moreover, gene frequency estimates for alleles segregating in each of these loci revealed that Est-3A locus was highly polymorphic and the allele frequencies did not vary significantly between samples from different locations which agrees with the results of Mork and Haug (1983). On the other hand the population genetic distance of ***Solea aegyptiaca*** from Abu-Kir Bay and Lake Qarun is 0.024. This value shows a markedly genetic variability between sole fish populations from these different habitats which was previously confirmed by using biometric analysis (Abd El-Gawad ***et al.***, 1995).

As a whole, the electrophoretic study gives evidence of the great genetic divergence between ***Solea vulgaris*** and ***Solea aegyptiaca*** from Abu-Kir Bay. On the other hand the high value of genetic distance of ***Solea aegyptiaca*** populations from Abu-Kir Bay and Lake Qarun showed a markedly genetic variability which may be induced as the result of the environmental differences between these two habitats for many years, which exhibited contrasting selection pressures on fish genome for inducing genetic variations which seem to reflect adaptation to local conditions. However, this degree of biogenetic variations indicated that the sole fish from Lake Qarun became a distinct population.

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