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GENETIC VARIABILITY AND SIMILARITY IN TWO FAMILIES OF CRABS

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ABSTRACT

Serum Proteins and esterase isozymes of crabs: Eriphia spinoforms (red); Eriphis spinoforms (green) Family xanthidae and Portunus pelagicus; portunus arcuatus, Carcinus mediterraneus and Charybdis helleri Family Portunidae were studied electrophoretically. SDS polyacrylamide gel was used to estimate the molecular weight of the variable protein between the two families. The genetic distance was calculated for the two families.

The results indicated that Eriphia spinoforms (red) and Eriphia spinoforms (Green) appeared as two species. Portunus pelagicus, Carcinus mediterraneus and Carybdis helleri were closely related to each other and constitute one group; while Portunus arcuatus constitutes another group, loosely related to the first. Esterase isozymes patterns showed genetic variability in the different species of Crabs.

The molecular weight of the variable proteins between the two families ranged between 140,000 D and 18,000 D.

The genetic distance indicated also that portunus pelagicus; Carcinus mediterraneus and Carybdis helleri were closely related to each other and Portunus arcuatus was loosely related to this group. Eriphia spinoforms (red) and Eriphia spinoforms (Green) were two related species.

INTRODUCTION

Crabs are the most commercially important edible Crustaceans. They are classified in Order decapoda, Suborder Reptania, Section Brachura. Its fishery has grown consideraply and are now commercially well exploited.

The study of protein variation enables us to evaluate the amounts of genetic variation in populations of economically important species that cannot yet be bred in culture and later to document the genetic changes brought by their domestication. Furtthermore, identification of biochemical genetic markers and their distribution in natural populations facilitate the development of quantitative genetics research and breeding programs as control over reproduction is gained.

The biochemical genetics, therefore, is being recognized as an immediate and efficient approach to generate much of the basic genetic information crucial to the development of aquaculture (Utter et al., 1974).

The study of gene protein variation is accomplished by straight forward of electrophoresis. All electrophoretic methods have proved useful to varying degrees in characterizing specific organisms. In particular, acrylamide gel electrophoresis has been extensive used because of its excellent resolving power (Brewer, 1970).

Few of these procedures were described adequately for use with crustaceans (Redified and salini, 1980).

Accordingly, the present study includes preliminary observation concerning the applicability of protein and esterase isozymes to evaluate the amount of genetic variation in six species of crabs collected from Mediterranean Sea at Alexandria.

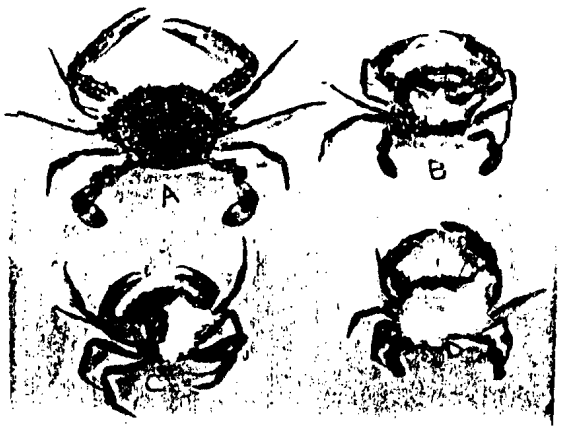
MATERIALS AND METHODS

The present investigation was carried out on crabs: portunus pelagicus (P.P), portunus arcuatus (P.a), Carcinus mediteraneus(C.m), Charybdis helleri (C.h) (Family Portunidae), Eriphia spiniforms red (ESR) and Eriphia spiniforms green (ESG) Family Xanthidae (Fig. 1). The samples were collected from Mediterranian Sea at Alexandria. Flesh was removed from alive samples and used for analysis immediately or after storage at 4°C. A crude aqueous extract of soluble proteins is obtained by homogenizing tissues and centrifuging for 20 minutes at 3000 rpm. The supernatants were vtilized to determine serum proteins by vertical polyacrylamide gel electrophoresis. The procedures were modified from Davis (1964).

Esterase isozymes were determined for the six species in the serium proteins by vertical polyacrylamide gel electrophoresis and the staining method was modified from Stordeur (1976) and Shaw and Prasad (1970).

SDS polyacrylamide gel electrophoresis was used to study serum proteins of Eriphia spinoforms red (Fam. Xanthidae) and Portunus pelagicus (Fam. Portunidae according to Stegemann et al. (1987). The molecular weight of the separated proteins was calculated from Table 1 which represents the molecular weight of the used marker proteins.

The genetic distances were calculated for each species of the two families from serum protein according to Sokal and Sneath (1963).



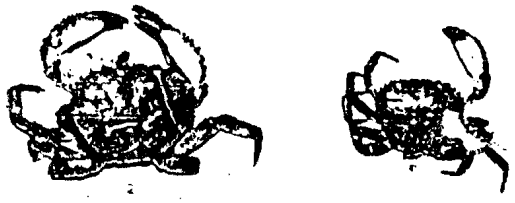


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FIG. 1
Family Portunidae
A= Portunus Pelagicus B= Portunus arcuatus
C= Carcinus mediterrneus D= Charybdis helleri

Family Xanthidae :
Eriphia spinoforms (red), Eriphia spinoforms (red)

TABLE 1
Marker proteins and their molecular weights.

Proteins as	Producer, Order number	Mol.Weight of Protomers
Immunoglobulin, Normal Human IgG, Purif.	Byk-Mallinokrodt, Nordic Immunology, D-6051 Dietzenbach-Steinberg	(150,000 D) (50,000 D 23,500 D
Phosphorylase b (rabbit muscle) lyophilized (40 mg= 5 mg protein)	Boehringer, 108 275	97,400 D
Albumin (bovine serum), dry puriss	Behringwerke, oRHD	67,000 D
Fumarase (pig heart) Crystal suspension	Boehringer, 104 957	49,000 D
Alcohol-dehydrogenase (yeast),(ADH) Lyophilized (50 mg=30 mg protein)	Boehringer, 102 709	37,000 D
Chymotrypsinogen A (bovine), 6 x Cryst., puriss.	Serva, 17 200	25,700 D
Lysozyme from egg white, puriss.	Serva, 28.260	14,300 D

RESULTS AND DISCUSSION

Since successful genetic improvement either through selection or hybridization depends on the amount of genetic variation within and between populations, this study has concerned with the quantification of genetic variation in natural populations by using protein and esterase electrophoresis in the species of crabs. Fig. 2 shows the serum protein patterns of Fam. Xanthidae: Eriphia spiniforms (red) and Eriphia spiniforms green. It is clear that 15 proteins migrated towards the anode. No apparent differences could be observed between the two species in major proteins except protein No. 13 and 14, but the differences between the two species appeared in the minor proteins at No. 1, 6, 9, 10, 14 and 15.

Figure 3 shows the serum proteins patterns of four species of Family Portunidae: Portunus pelagicus, portunus arcuatus, Carcinus mediterraneus and Charybdis helleri.It is clear that no two patterns are exactly alike in the four species. However, this family have been characterized by the presence of proteins 1, 2, 6, and 9 in the four species.

Shaw (1970) mentioned that closely related species which occur in the same genus, differ at about 50-80% of their genes. From Figs. 2 and 3, it is possible to state that in these samples the serum protein patterns are specific for any one of species and constitute a single by which these two families may be identified. The results also indicated that Eriphia spinoforms (red) and Eriphia spinoforms (green) are related to each other and appeared as two species.

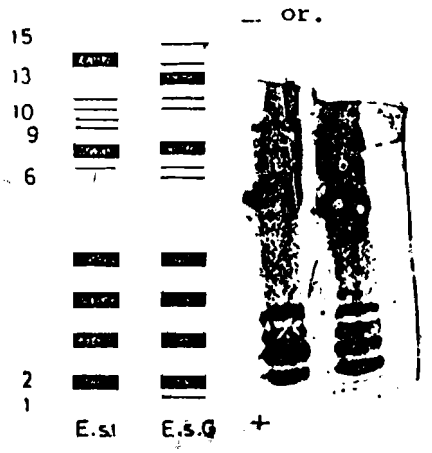

FIG. 2
Serum proteins of
Fam: Xanthidae

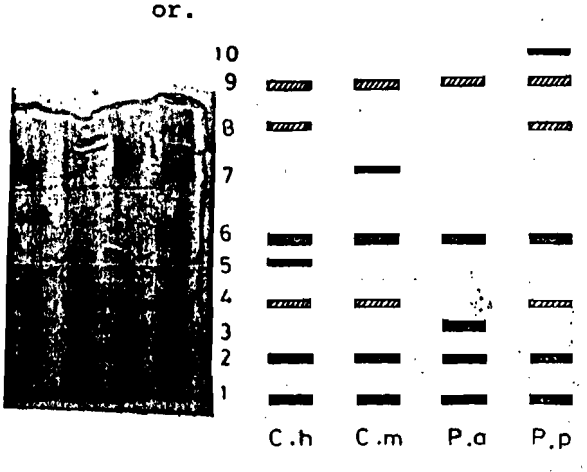

FIG. 3
Serum proteins
of Fam: Portunidae

Conventional systematics usually depend to a larger extent on mortphological characters. But morphological differences in congenetic animal species probably depend on relatively few genes. It can therefore not be ruled out that special selection pressure might have been exerted predominantly on this small fraction of the genome only, which mainly determines morphological characters, Morphological dissimilarities therefore may falsely imply in some cases considerable evolutionary divergence. Conversely it has already been shown that slight morphological and/or ecological dissimilarities exist between sibling species cannot be taken as evidence of little genetic differentiation. Sibling species of the Drosophila willistoni group are morphologically very similar but genetically very different (Ayala et al., 1971).

Figures 4 and 5 show the esterease isozymes of the different species of crabs. It is clear that four isozymes of esterases at Eriphia spp. and the differences between the two species appeared at E_2, E_3 and E_4. But in family Portunidae, it is appeared three isozymes of esterases and the differences between the four species in the three isozymes.

Kirpichnikov, (1973) said that esterases are inherited codominantly without formation of hybrid rings. As a rule, the esterase molecule is a homopolymer (probably a dimer) and therefore, the number of isozymes is not considerable for this enzymes. In some fishes, however, it is large (Holmes and Whitt, 1970).

Since electrophoresis examins only the structural genes which comprise approximately 1% of the genome. Hypothetically two proteins of different molecular weights may migrate toward the anode at the same rate if their size differences are balanced by compensating charge differences. For this reason acrylamide gel electrophoresis may not be used to gain information about molecular weight of a protein. A second restriction placed on electrophoretic techniques concerns the number of species observed on the gel molecules which are tightly but not covalently bound together and not usually separted from one another during electrophoresis.

Shapiro et al. (1967) attempted to surmount these problems by separate a mixture of proteins in the presence of sodium dodecyl sulfate (SDS), an anionic detergent. The binding of SDS introduces one negative charge per bound molecule of SDS on the protein molecule. At neutral pH the total charge of the protein SDS complex is almost entirely dependent upon the charge of the SDS molecules. It has been found that the charge per unit mass is approximately constant and therefore the electrophoretic mobility of the complex is mainly dependent on the molecular weight of the protein.

FIG. 4
Eeterase isozymes of
Fam. Xanthidae.

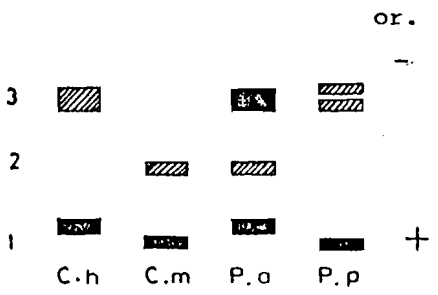

FIG. 5
Esterase isozymes of
Fam: Portunidae

To gain information about the molecular weight of proteins in the two families. Portunus pelagicus (Fam. Portunidae) edible performance species and Eriphia spiniforms red, the higher growth rates have been chosen to study the variation between the two species at the molecular weight (Fig. 6). The molecular weight of the variable proteins in the two species has been calculated from the standard curve (Fig. 7).

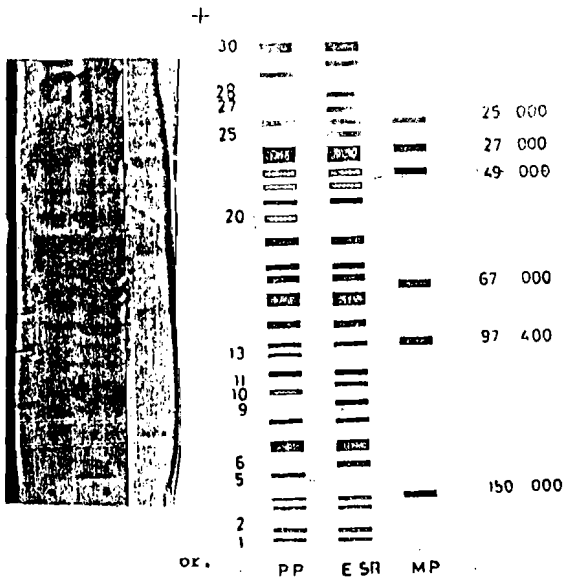

FIG. 6
SDS poly acrylamide gel
of P.P, ESR and marker
proteins.

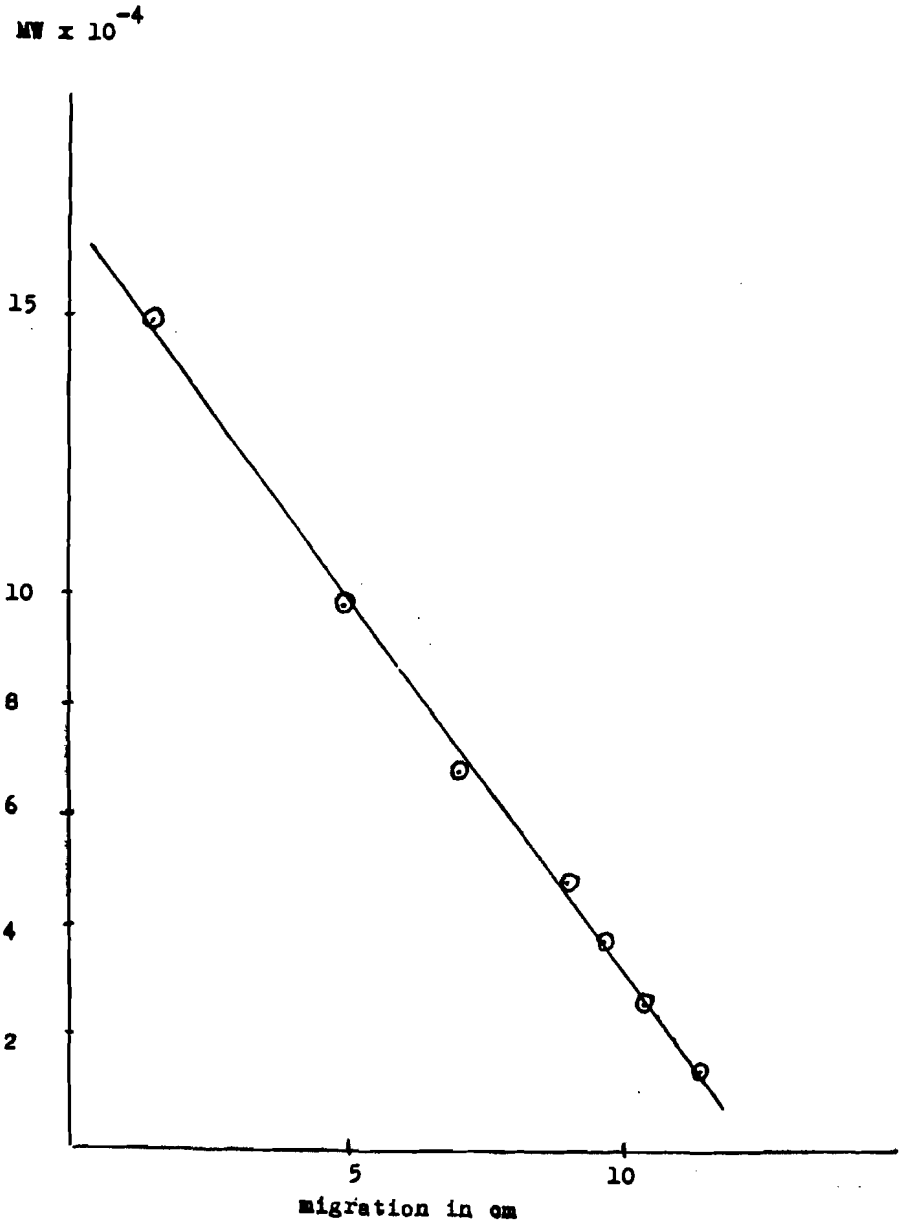

FIG. 7
Standard curve relating Protein molecular weight in Daltons and band migration in "Cm".

Table 2 represents the variable proteins in the two species and their molecular weights. It is clear that the variation between the two species is localized at 12 proteins and their molecular weight ranges between 140,000 D and 18.000 D.

For the development of inbreeding and cross breeding programs, biochemical genetics permits the evaluation of the degree of homozygosity and the genetic similarity of populations making designed crossing more likely to be productive.

TABLE 2
The molecular weight of the variable proteins in Portunus pelagicus and Eriphia spinoforms red.

Protein	Species		Mobility	Molecular weight MW 10^{-4}
	P.P	ESR		
5	+	-	2.1	14.0
6	-	+	2.3	13.7
9	-	+	4.0	11.5
10	+	-	4.2	11.2
11	-	+	4.4	11.0
13	+	-	4.9	10.1
20	+	-	8.0	6.0
25	-	+	9.9	4.5
27	-	+	10.4	2.5
28	-	+	10.6	2.4
29	+	-	10.8	2.1
30	-	+	11.0	1.8

The genetic distance was calculated from serum proteins of the two families at 12 genetic loci in Fam. Xanthidae and at 6 genetic loci in Family Portunidae. It is clear from Tables 3, 4 and Fig. 8 that the distance between ESR and ESG is 0.05 while the genetic distance in Family Portunidae range from 0.0054 to 0.125, and (P.P), (C.m) constitute one group closely related to each other while (P.a) constitutes another group loosely related to the previous group. The distance between the group (P.P), (C.m) and Eriphia spp. is nearly the same distance between (P.a) and the group (p.p, cm. c. h).

TABLE 3

Genetic distances between members of Family:Portunidae.

	P.P	P.a	C.m	C.h
P.P	—	0.078	0.0054	0.0062
P.a		—	0.1250	0.0890
C.m			—	0.0064
C.h.				—

Table 4

The genetic distances between ESr, ESr, ESG
Fam.: Xanthidae and C.h. Fam.: Portunidae.

	Ch	ESr	ESG
Ch	—	0.115	0.117
ESr		—	0.050
ESG			—

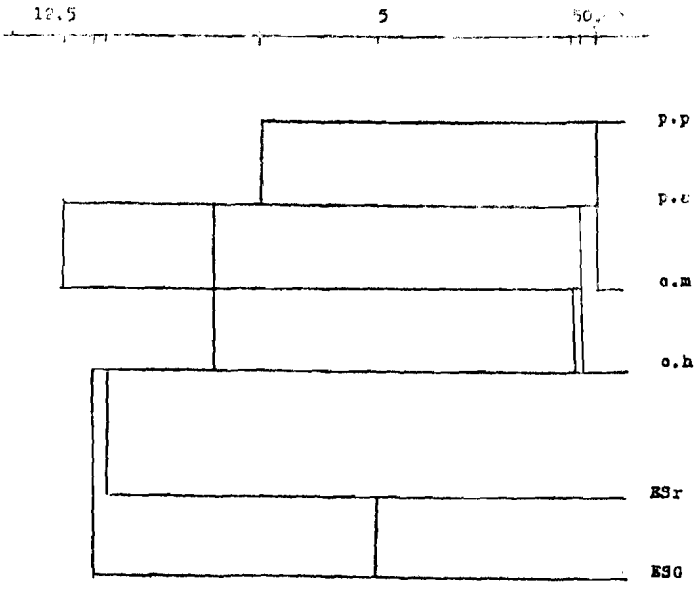

FIG. 8

Genetic distances of the Family: Portunidae and Family: Xanthidae.

Hedgecock et al.(1976) reported that genetic distance has enabled to measure the amount of genetic divergence between the American and European Lobsters (H. americanus and H. gammarus), Decapoda; Crustacia, using Nei's measure of genetic distance D. The statistic for this inter specific comparison D= 0.103 is 10 times that among different populations of American lobsters, D=0.006. Hybridization of the European and American species appears feasible and will result in highly heterozygous offspring. By measuring the performance of these lobsters, hybrid vigor can be correlated with heterozygosity and the practically of the hybridization program may by evaluated.

REFERENCE

Ayala, F.J.; J.R. Powell, and T. Dobzhansky, 1971. Polymorphism in continental and island populations of **Drosophila willistoni**. **Proc. Nat. Acad. Sci.** 68: 2480-2483.

Brewer, G.J., 1970. **An introduction to isozyme techniques.** New York; Academic Press.

Davis, B.J., 1964. Disc electrophoresis. II- Methods and application to human serum proteins. Ann. N.Y. Acad. Sci., 121: 404-407.

Hedgecock, D., R.A. Shleser and K. Nelson, 1976. Applications of Biochemical Genetics to Aquaculture. **J. Fish Res. Board Can.** 33: 1108-1119.

Holmes, R.S. and G.S. Whitt, 1970. Developmental genetics of the esterase isozymes of **Fundulus heteroclitus**. **Biochem. Genet.** 4 (4): 471-480.

Klyashtorin, V.S., 1973. **Biochemical polymorphism and microevolution processes in Fish.** Genetic and mutagenesis of fish. Edited by J.H. Schroder. Springerverlage.

Redfied, J.A.and J.P. Sellers, 1989. **Techniques of starch gel electrophoresis of penaeid prawn enzymes, *Penaeus esc.* and Metapenaeus spp.** Csiro Aust. Div. Fish Oceanogr. Rep. 116 (1989).

Shapiro, A.L.; E. Vinuela and J.V. Maizel, 1967. Molecular weight estimation of polypeptide chains py electrophoresis in SDS polyacrylamide Gels. **Biochem. Biophys. Res. Commun.**, 28: 815-820.

Shaw, C.R. (1970). how many genes evolve? **Biochem. Genet.** 4: 275-283.

Shaw, C.R. and R. Prasad, 1970 Starch gel electrophoresis of enzymes. A compilation of recipes. **Biochem. Gent.**, 4: 707- 718.

Sokal, R.R. and P.H. Sneath, 1963. **Principles of numerical taxonomy.** W.H. Freeman, San Francisco.

Stegemann, H.; W. Burgermeister and A. Shan, 1987. **Electro focusing in tubes.** Institut Fur Biochemie, Biologische, Bundesanstalt,Messweg, 11, D-330 Braunschweig (west Germany).

Stordeur, E., 1976. Esterase in the mosquito Culex Pipiens pipiens L: Formal genetic and polymorphism of adult esterase. **Biochem. Genet.**, 14: 481-493.

Utter, F.M.; H.O. Hodings and F.W. Allendrof. 1974. Biochemical genetics. Studies of Fishes: polentialities and limitations,P.213-238. In : J.K. Sargeant (ed.) **Biochemical and Biophysical Preapectives in Marine Biology.** Vol. 1. Academic Press,Inc., San Francisco Calif.