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Genetic differentiation between *Penaeus kerathurus* and *P. japonicus* (Crustacea, Decapoda)

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ABSTRACT: Genetic differentiation and variability data have been obtained by electrophoretic analysis of 2 species of prawns (*Penaeus kerathurus* and *P. japonicus*). Both are fished commercially and used in either commercial or experimental aquaculture systems. Genetic distance calculated according to Nei's method (D = 0.887) shows the 2 species to be very different genetically. Similar values have frequently been found in other animal groups between congeneric species that are phylogenetically distant or between species in different genera. The contribution of different loci to the genetic distance, with respect to their metabolic role, is briefly discussed. Variability estimates for the 2 species differ considerably. In *P. japonicus*, mean heterozygosity expected at equilibrium ($\overline{He}$) is 0.121; mean number of alleles per locus (A) is 1.484, and frequency of polymorphic loci (P) is 0.387. Respective values for *P. kerathurus* are $\overline{He}$ = 0.055, A = 1.265 and P = 0.265. Stochastic and deterministic factors that may possibly explain high heterozygosity in *P. japonicus* are hypothesized. Future research requires natural populations to be examined.

INTRODUCTION

Decapod crustaceans have recently been the object of electrophoretic studies (Lester, 1979; Mulley and Latter, 1980; Nelson and Hedgecock, 1980). However, only a few studies are concerned with commercially important decapod species (Hedgecock et al., 1976; Malecha et al., 1980), although they have been well studied both ecologically and physiologically. In the Mediterranean, *Penaeus kerathurus* (Forskäl) has been considered for aquaculture since 1970 (Lumare, 1976). More recently, farming of *P. japonicus* Bate has been the object of initial research programmes (Lumare, 1980; Lumare and Palmegiano, 1980).

This paper reports initial data on genetic divergence and variability obtained by electrophoretic analysis of *Penaeus kerathurus* and *P. japonicus* enzymes and proteins. *P. kerathurus* is found in Mediterranean and Eastern Atlantic regions (from Portugal to Angola). Adults live in muddy-bottom shelf regions in shallow-water environments (40 to 45 m deep) during winter; in summer they migrate to reproduce in coastal areas with low-salinity waters. *P. japonicus* has a wide geographical distribution in the Indo-West Pacific region and Mediterranean coastal waters of Turkey, Israel and Cyprus. Adults live on sand bottoms in shallow-water environments during winter. During spring-summer they migrate to reproduce in estuarine waters. Both species are eurythermic and euryhaline and show nocturnal activity.

Our study sets out to establish the degree of genetic differentiation between *Penaeus kerathurus* and *P. japonicus* in view of possible hybridization programmes in which they might be involved. In addition, we have attempted to ascertain genetic variability levels, which could, in principle, be associated with the different physiological adaptive features shown by the 2 species.

MATERIALS AND METHODS

Samples of *Penaeus kerathurus* and *P. japonicus* were obtained from Italy's National Research Council's Istituto per lo Sfruttamento Biologico delle Lagune (Institute for the Biological Exploitation of Lagoons) at

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Lesina. The sample of *P. kerathurus* was wild-caught from the Adriatic Sea, near Termoli, whereas the study sample of *P. japonicus* was obtained from Japan as hatchery-reared postlarvae from wild parents. After arrival, prawns were released into the lagoon of Lesina, allowed to grow for a time and then recaught.

Electrophoresis was carried out using 12% starch gels from Connaught Laboratories. The set of proteins studied, the tissues analyzed, the routine electrophoretic systems used, the staining techniques and the abbreviations used for the corresponding genetic loci are detailed in Table 1. The electrophoretic systems are designated as follows: A – discontinuous tris citrate pH 8.6 (Poulik, 1957); B – tris versene borate pH 9.1 (as buffer B of Ayala et al., 1973); B1 – as buffer B but with $NADP^+$ added; C – continuous tris citrate pH 8.4 (modified from buffer C of Ayala et al., 1972); D – tris maleate pH 7.4 (Brewer and Sing, 1970); F – continuous tris citrate pH 7.1 (modified from buffer C of Ayala et al., 1973); G – borate pH 8.0 (Brewer and Sing, 1970); H – tris versene borate pH 8.0 (Brewer and Sing, 1970).

RESULTS

In *Penaeus kerathurus* 34 gene loci were examined; in *P. japonicus* 31. Inheritance patterns were not confirmed by breeding tests, but genotypic frequencies at most polymorphic loci agreed closely with Hardy-Weinberg expectations; 16 loci were found to be monomorphic in both species: *Acph, Aldo-1, Ao-1, Aph, Fk, Got, G6pd-1, G6pd-2, Me, Tpi, Xdh, Pt-1, Pt-2, Pt-3, Pt-7, Pt-9*; 8 loci were found to be monomorphic in *P. kerathurus* only: *Ao-2, Ca-1, Ca-2, Ca-3, Est-2, Lap, Mdh-1*; 3 loci were found to be monomorphic in *P. japonicus* only: *Est-3, Ldh, Pt-8*; 9 loci were found to be fixed for alternative alleles in the 2 species: *Acph, Aldo-1, Fk, Got, G6pd-1, Me, Tpi, Pt-7, Pt-9*. At 2 polymorphic loci, *Phi, Pt-8*, the 2 species did not share any allele in common. At 8 loci the 2 species were found to differ greatly in the frequency of the most common allele: *Ca-1, Ca-2, Ca-3, Est-2, Est-3, Mdh-1, Mpi, Pgm*. Gene frequency estimates for polymorphic loci are presented in Tables 2 and 3. Only those loci in which the commonest variant had a frequency less than 0.99 were considered polymorphic.

Starting from allele frequencies, genetic identity (I) and genetic distance (D) between the 2 species were calculated according to Nei's method (1972, 1975). Coefficient of genetic identity ranges from 0 (no alleles in common) to 1 (the same alleles at identical frequencies).

DISCUSSION

Genetic distance

Genetic distance value between *Penaeus kerathurus* and *P. japonicus* is 0.887, whereas genetic identity is 0.412. These values show the 2 species to be very

Table 1. Tissues and electrophoretic systems used for each of the 34 proteins

Protein	E.C. No	Tissue*	Electrophoretic system	Staining techniques	Gen. locus	N. loci scored
Acid phosphatase	3.1.3.2	m	A	Tracey et al. (1975)	*Acph*	1
Aldeyde oxidase	1.2.3.1	c	B	Ayala et al. (1974a)	*Ao*	2
Aldolase	4.1.2.13	c	B1	Ayala et al. (1972)	*Aldo*	2
Alkaline phosphatase	3.1.3.1	c	A	Ayala et al. (1972)	*Aph*	1
Carbonic anhydrase	4.2.1.1	c	G	Brewer and Sing (1970)	*Ca*	3
Esterase	3.1.1.2	m	A	Ayala et al. (1972)	*Est*	2
Fructokinase	2.7.1.4	c	H	Brewer and Sing (1970)	*Fk*	1
Glucose-6-phosphate dehydrogenase	1.1.1.49	m	B1	Ayala et al. (1974c)	*G6pd*	2
Glutamic oxaloacetic transaminase	2.6.1.1	m	B1	Ayala et al. (1975)	*Got*	1
Lactate dehydrogenase	1.1.1.27	m	A	Selander et al. (1971)	*Ldh*	1
Leucine amino peptidase	3.4.11.1	m	A	Ayala et al. (1972)	*Lap*	1
Malate dehydrogenase	1.1.1.37	m	A	Ayala et al. (1972)	*Mdh*	2
Malic enzyme	1.1.1.40	m	F	Ayala et al. (1972)	*Me*	1
Mannose phosphate isomerase	5.3.1.8	c	F	Harris et al. (1977)	*Mpi*	1
Phosphohexose isomerase	5.3.1.9	m	C	Brewer and Sing (1970)	*Phi*	1
Phosphoglucomutase	2.7.5.1	m	D	Brewer and Sing (1970)	*Pgm*	1
Protein	–	m	A, C	Ayala et al. (1973)	*Pt*	9
Triosephosphate isomerase	5.3.1.1	c	B1	Ayala et al. (1972)	*Tpi*	1
Xanthine dehydrogenase	1.2.1.37	c	A	Selander et al. (1971)	*Xdh*	1

* c = cephalotorax m = muscle

Table 2. *Penaeus kerathurus*. Allele frequencies at polymorphic loci

Locus and allele designations	Sample size and allele frequencies	Locus and allele designations	Sample size and allele frequencies
Aldo-2	N = 24	*Mpi*	N = 24
102	0.042	102	0.167
100	0.958	100	0.833
Ho	0.083	Ho	0.333
d	0	d	0.150
Est-3	N = 48	*Pgm*	N = 68
100	0.854	102	0.176
95	0.146	100	0.824
Ho	0.125	Ho	0.235
d	−0.509	d	−0.202
Ldh	N = 56	*Phi*	N = 26
100	0.982	103	0.115
98	0.018	100	0.885
Ho	0.036	Ho	0.231
d	0	d	0.087
Mdh-3	N = 68	*Pt-8*	N = 18
100	0.809	100	0.944
94	0.191	97	0.056
Ho	0.206	Ho	0.111
d	−0.344	d	0

N = number of genes sampled; Ho = observed heterozygosity; d = an estimate of the deviations from the Hardy-Weinberg expectations. d = (Ho − He)/He; negative values of d indicate a deficiency of heterozygosity, positive values an excess. d parameter has never been found significant at the probability level : $P < 0.1$

different genetically. Similar values have frequently been found in other animal groups between congeneric species that are phylogenetically distant or between species in different genera (Nei, 1975; Ayala, 1975; Avise, 1976). On the grounds of the electrophoretic results it would appear unlikely that programmes for the hybridization of *P. kerathurus* and *P. japonicus* would be successful despite their morphological similarity. However, in other organisms F1 hybrids have been occasionally recorded even from parental species with higher values of D (Bullini et al. 1978).

Genetic distance, D, may be considered as an approximate measure of the mean number of electrophoretically detectable substitutions per locus accumulated by 2 populations since they form a single ancestral population. Sarich (1977) has proposed that there are 2 main groups of electrophoretically studied proteins which evolve at different rates: plasma proteins and some enzymes not involved in complex metabolic pathways appear to accumulate amino acid substitutions 10 times more rapidly than do those enzymes which utilize specific metabolically produced substrates. Loci encoding for the first group of enzymes have been called 'fast' loci, while loci encoding for the second group of enzymes have been called 'slow' loci. According to Sarich, at an early stage of speciation (assuming neutral evolution of protein polymorphism) fast loci would accumulate substantial gene differences, while slow loci would contribute later; this dichotomy biases a single genetic distance calculation. In addition, rapidly substituting loci would seem to be polymorphic more often for neutral situations.

Recently, attempts have been made to relate enzyme-specific polymorphism levels to molecular structure (Zouros, 1976; Harris et al., 1977; Koehn and Eanes, 1977, 1978; Ward, 1977). Enzymes may also be subdivided into 2 groups on the grounds of their metabolic function, which appears to be correlated with variability levels at corresponding loci. Group I includes regulatory and variable substrate enzymes. Group II includes non-regulatory enzymes (Johnson, 1974; Powell, 1975). We consider Sarich's 'fast' enzymes as those belonging to Group I and his 'slow' enzymes as those belonging to Group II (Table 4).

Genetic distance calculated between *Penaeus kerathurus* and *P. japonicus* on fast loci is 0.826, whereas D coefficient calculated on slow loci is 0.988. Corresponding values for I_{fast} and I_{slow} are 0.438 and

Table 3. *Penaeus japonicus*. Allele frequencies at polymorphic loci

Locus and allele designations	Sample size and allele frequencies	Locus and allele designations	Sample size and allele frequencies
Aldo-2	N = 16	*Lap*	N = 34
100	0.938	100	0.618
98	0.062	98	0.382
Ho	0.125	Ho	0.294
d	0	d	−0.396
Ao-2	N = 26	*Mdh-1*	N = 34
100	0.962	102	0.735
98	0.038	100	0.265
Ho	0.077	Ho	0.412
d	0	d	0.027
Ca-1	N = 16	*Mdh-3*	N = 28
107	0.500	100	0.679
102	0.375	92	0.321
100	0.125	Ho	0.500
Ho	0.625	d	0.105
d	−0.013		
		Mpi	N = 20
Ca-2	N = 16	100	0.050
102	0.250	92	0.950
100	0.375	Ho	0.100
97	0.375	d	0
Ho	0.750		
d	0.071	*Pgm*	N = 34
		100	0.059
Ca-3	N = 16	98	0.941
95	0.938	Ho	0.118
93	0.062	d	0.031
Ho	0.125		
d	0	*Phi*	N = 22
		96	0.955
Est-2	N = 28	94	0.045
104	0.036	Ho	0.091
102	0.714	d	0
100	0.250		
Ho	0.429		
d	−0.030		

N = number of genes sampled; Ho = observed heterozygosity; d = an estimate of the deviations from the Hardy-Weinberg expectations. d = (Ho − He)/He; negative values of d indicate a deficiency of heterozygotes, positive values an excess. d parameter has never been found significant at the probability level : $P < 0.1$

0.372. Fast and slow loci give similar contributions to genetic differentiation. In addition, both high and low values of I are represented in each of the 2 classes (Table 4, Fig. 1). These data may be useful for comparisons with other situations: in fact data from previous studies on genetic differentiation of local populations, sibling species and morphologically differentiated species of amphipods, beetles and fruit-flies (Ayala and Tracey, 1973; Ayala et al., 1974a, b; Sbordoni et al., 1979; Delay et al., 1980; Sbordoni, 1982) show that the same locus may give a different contribution to genetic distance, at comparable stages of evolutionary divergence, depending considerably on the taxonomic group (Sbordoni et al., unpubl.).

Genetic variability

Several estimates of genetic variability have been calculated for the 2 examined species of *Penaeus*. In *P. kerathurus*, mean heterozygosity expected at equilibrium ($\overline{He}$) is 0.055, average observed heterozygosity

Table 4. *Penaeus kerathurus* and *P. japonicus*. Genetic identity (I) and genetic distance (D) for non-regulatory and variable substrate plus regulatory enzyme loci. After the classifications of Johnson, 1974, and Powell, 1975

Group I*			Group II**		
Regulatory and variable substrate enzyme loci	I	D	Non-regulatory enzyme loci	I	D
Acph	0	∞	*Aldo-1*	0	∞
Ao-1	1	0	*Aldo-2*	0.997	0.003
Ao-2	0.999	0.001	*Ca-1*	0.196	1.629
Aph	1	0	*Ca-2*	0.640	0.447
Est-2	0.330	1.108	*Ca-3*	0	∞
Est-3	0.170	1.770	*Gof*	0	∞
Fk	0	∞	*Ldh*	0.999	0.001
G6-pd-1	0	∞	*Mdh-1*	0.339	1.081
G6-pd-2	1	0	*Mdh-3*	0.880	0.128
Lap	0.851	0.162	*Tpi*	0	∞
Me	0	∞			
Mpi	0.051	2.965			
Pgm	0.061	2.794			
Phi	0	∞			
Xdh	1	0			

* Equivalent to 'fast' enzymes after Sarich (1977)
** Equivalent to 'slow' enzymes after Sarich (1977)

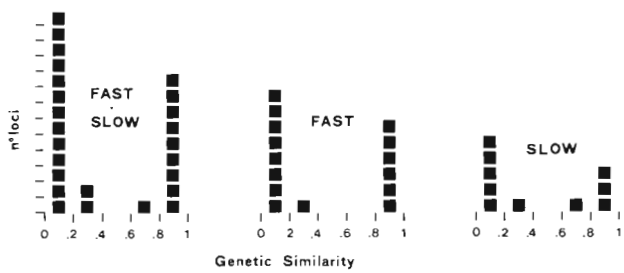

Fig. 1. *Penaeus kerathurus* and *P. japonicus*. Distribution of loci with respect to genetic identity. The same U shape, typical in comparisons between well differentiated species, is represented either in the distribution of all loci (left), or in the distribution of fast loci (in between) and/or of slow loci (right)

($\overline{H}o$) is 0.049, mean number of allele per locus (A) is 1.265, frequency of polymorphic loci (P) is 0.265. Respective values for *P. japonicus* are: $\overline{H}e = 0.121$, $\overline{H}o = 0.118$, A = 1.484, P = 0.387. All these estimates account for a different degree of genetic variability in *P. kerathurus* and *P. japonicus*.

Table 5 shows estimates of genetic variation in the 2 species studied here and variability estimates of 16 species of American and Australian penaeid prawns studied by Lester (1979) and Mulley and Latter (1980) respectively. Overall the level of heterozygosity is extremely low in all species except in *Penaeus japonicus*.

Table 5. Genetic variation in penaeid prawns. $\overline{H}e$ = average frequency of heterozygous individuals per population expected at Hardy-Weinberg equilibrium; P = proportion of polymorphic loci

Species	$\overline{H}e$	P	Species	$\overline{H}e$	P
P. kerathurus[1]	0.055	0.26	*P. plebejus*[3]	0.022	0.24
P. japonicus[1]	0.121	0.39	*P. latisulcatus*[3]	0.032	0.13
P. setiferus[2]	0.089	–	*P. longistylus*[3]	0.006	0.08
P. atzecus[2]	0.076	–	*M. macleayi*[3]	0.026	0.17
P. duorarum[2]	0.092	–	*M. bennettae*[3]	0.020	0.20
P. merguiensis[3]	0.008	0.16	*M. endeavouri*[3]	0.030	0.20
P. semisulcatus[3]	0.017	0.22	*M. ensis*[3]	0.013	0.20
P. monodon[3]	0.008	0.09	*M. insolitus*[3]	0.010	0.10
P. esculentus[3]	0.033	0.24	*M. eboracensis*[3]	0.019	0.17

[1] Present paper; [2] Lester (1979); [3] Mulley and Latter (1980)

In a study of enzyme polymorphism distribution in 44 species of decapod crustaceans, Nelson and Hedgecock (1980) found this group generally to have lower heterozygosities (0.05 to 0.06) than other groups of invertebrates (Nevo, 1978). Only 2 species, *Emerita analoga* (Anomura, Hippidae) and *Callinectes arcuatus* (Brachyura, Portunidae), have heterozygosity values comparable to those found in *P. japonicus*. In particular, 6 loci in this species are exceptional in showing a comparatively high degree of genetic variation ($Ho > 0.25$): *Ca-1, Ca-2, Est-2, Lap, Mdh-1, Mdh-3*.

The relatively high level of heterozygosity found in our sample of *Penaeus japonicus* could be associated with one of the following factors:

(1) Large effective population size (as roughly indicated by the wide range of species). This hypothesis may be realistic although other studied species of *Penaeus*, such as *P. monodon* and *P. latisulcatus* (Mulley and Latter, 1980) are characterized by low levels of genetic variability and occupy extensive geographic areas (Racek, 1970). However, these data are not to be considered complete, as they depend on analysis of only 1 population for each species.

(2) Heterogeneity of the sample studied, due to any possible genetic admixture by different geographical populations prior to tank breeding. This hypothesis has not been supported by the analysis of genotypic frequencies at polymorphic loci which closely agree with Hardy-Weinberg equilibrium (Table 3).

(3) Particular adaptive strategy at species or population levels. The amount of genetic variation of a population or species may be regarded as an adaptive strategy for increasing population or species fitness (Levins, 1968; Valentine, 1976). From a survey of 44 species of decapod crustaceans, Nelson and Hedgecock (1980) propose with Selander and Kaufman (1973) that animals 'choosing' a fine-grain adaptive strategy are generally larger and more mobile, with better physiological and behavioural means of regulation; and further, at least among the decapods, that they are more likely to be trophic generalists. The authors hypothesize a hybrid 'environmental heterogeneity-trophic diversity' model where fine-grained decapods will generally be found to have relatively low heterozygosities for enzymes involved in processing internally generated metabolites. Enzymes with broad substrate specificities involved in processing substances derived from external environment, would seem to show high heterozygosities.

In *Penaeus japonicus* heterozygosities at single loci, except for *Mdh-1* and *Mdh-3*, fit well with this model. *Emerita analoga* has also been found to be highly heterozygous at *Mdh* locus, while the same locus is not variable at all in *Callinectes arcuatus*. *P. japonicus*, with its wide distribution, could well be a trophic generalist, with 'local' enzymatic specializations.

To test all these hypotheses, samples from different natural populations should be studied further.

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