Genetic structure of two commercial penaeids (*Penaeus californiensis* and *P. stylirostris*) from the Gulf of California, as revealed by allozyme variation

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Abstract—Two commercial shrimp species (*Penaeus californiensis and P. stylirostris*) were sampled along the Gulf of California and crude extracts were assayed electrophoretically to assess allozyme variation and population genetic structure. *Penaeus californiensis*, a more oceanic species, displayed a 12.5% polymorphism (P_{95}) and a 0.023–0.037 expected heterozygosity (H_e) in three sampled populations, whereas *P. stylirostris*, a more coastal species, showed a north–south clinelike pattern in its genetic variability parameters: P_{95} from 15.63% to 31.25% and H_e from 0.038 to 0.086. Differences between species in levels of genetic variation and genotype distribution may be related to differences in habitat during important life cycles stages which reflect the remarkable changes of environmental conditions of coastal lagoons in the Gulf of California. *Penaeus stylirostris* subpopulations appeared more structured (F_{st}=0.372) than those of *P. californiensis* (F_{st}=0.182). A number of private alleles and alternation of the most common allele in several loci account for the outstanding high results of both species. Nei's genetic similarities were computed within species (*P. californiensis* subpopulations, I=0.988–0.997; *P. stylirostris* subpopulations, I=0.929–0.954) and between species (*P. californiensis* *P. stylirostris,* I=0.674). A dendrogram generated from Nei's genetic similarities segregated the upper Gulf populations of both species from the other two populations (middle Gulf and mouth of the Gulf). This segregation may be the result of the "Island Barrier" hypothesized as segregating other decapods inhabiting the Gulf of California.

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Penaeid shrimp fisheries and hatcheries have undergone an accelerated development during the past three decades (Rosenberry, 1996). Yields of such productive activities are highly appreciated worldwide and have resulted in the economic development of prawn farming in several eastern (e.g. Thailand, Indonesia, Vietnam, China, India, etc.) and Latin American (e.g. Ecuador, Mexico, Colombia, Peru, Panama, etc.) countries.

Business based on penaeid shrimp trading may be considered as having two components: 1) heavy producers, countries, such as those mentioned above, which are mainly exporters, and 2) heavy importers, countries such as those of Europe and the United States of America, where the consumption of imported shrimp has long exceeded landings from their domestic fisheries (Lightner et al., 1997). Moreover, in the way of production (fishing on wild populations vs. rearing in hatcheries), three kinds of producer countries can be recognized: 1) countries that base their shrimp production mainly on their fishery yields (for example, the United States, where farmed shrimps make up only 1% of its production); 2) countries that have focused their shrimp production efforts almost exclusively on hatcheries (for example, Ecuador, where farmed shrimps make up 95% of its shrimp production); and 3) countries that produce shrimps in similar percentages from both wild stocks and hatcheries (for example, Mexico, China, India, and Indonesia) (Lightner et al., 1997).

Shrimp-producing countries, no matter their main way of production, need increasingly to take into account the resource's genetic structure and the variability of fisheries or hatcheries management (Allendorf et al., 1987). Two key questions must be outlined

when a resource is desired to be characterized genetically: 1) What amount of genetic variation is present across its populations? 2) Is the variation homogeneously distributed? This line of research has impelled a number of studies on populations of shrimp species to ensure adequate exploitation and optimal rearing (for a review see Rodríguez-Romero and Rosa-Vélez, 1993).

Our study aimed to characterize the genetic variability and structure of two wild populations of shrimp species, to recognize the actual genetic pool currently segregating in them, and to render information to design rearing strategies based on existing genetic variability.

Penaeus (*Farfantepenaeus) californiensis* Holmes is an oceanic species distributed in the eastern Pacific, San Francisco Bay, U.S.A., to Sachura Bay, Peru and Galapagos Islands, Ecuador (Rodriguez de la Cruz, 1976). Adults are found up to 220 m deep, although the peak of abundance occurs at 55 m on silt-clay or sand-silt bottoms (Rodríguez de la Cruz and Rosales, 1970). *Penaeus (Litopenaeus) stylirostris* Stimpson is a more coastal species distributed from the upper Gulf of California, Mexico, to Tumbes, Peru. Adults are found in shallow waters around the mouth of coastal lagoons, up to 40 m deep (CICTUS, 1985), where they are more abundant.

Life cycles are similar for both species but there is one very distinctive difference: *P. californiensis* can complete its whole life cycle in the marine environment, whereas *P. stylirostris* must spend part of its life cycle (postlarval and juvenile stages) as an inhabitant of coastal lagoons. In brief, for both species, females deposit eggs in demersal zones where they undergo total segmentation in 12–15 hours. The outcome is a planktonic larva that metamorphoses through five naupliar, three protozoean, and three mysis stages, before reaching the semibenthic postlarval stage. Once the rostral form is accomplished, the animal is considered a juvenile and is totally benthic. The complete cycle may take12–17 days (Rodríguez de la Cruz and Rosales, 1970).

In our study we ascertained different levels of genetic variation between species, a clinelike pattern of genetic variability in the more coastal species, and significant genetic structure among subpopulations of both species.

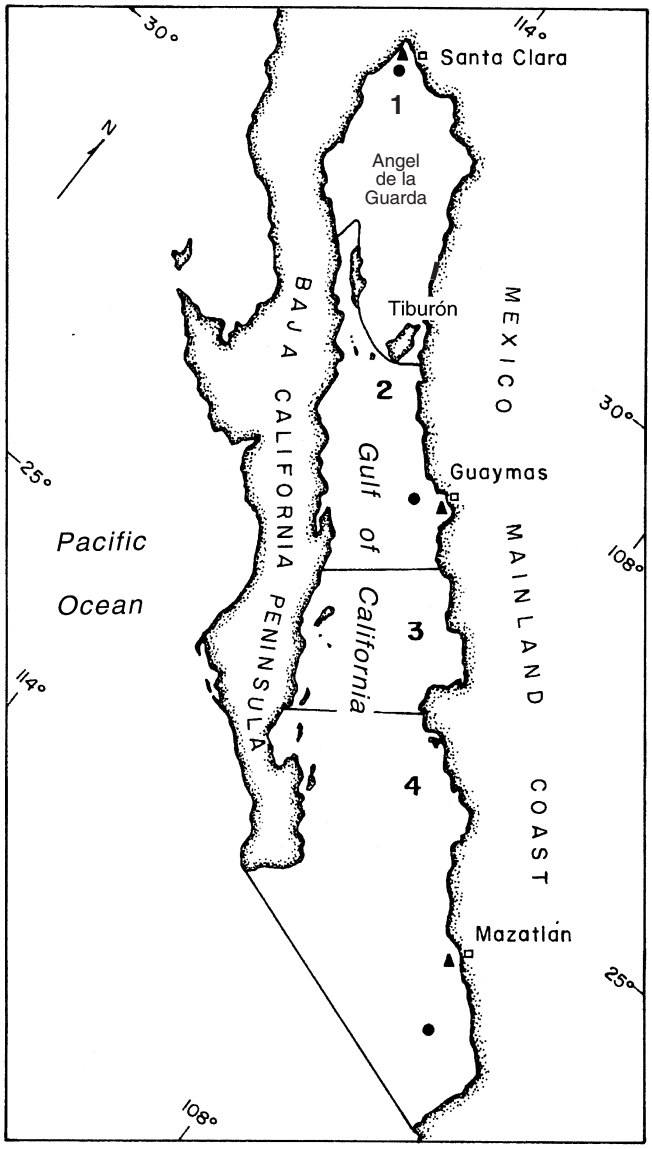

Figure 1

Map of the Gulf of California showing its subdivision (after Round, 1967): 1 = upper Gulf; 2 = middle Gulf; 3 = lower Gulf; and 4 = mouth of the Gulf; and the sampling locations: ▲ = *Penaeus stylirostris,* and ● = *Penaeus californiensis.*

Materials and methods

Samples of *Penaeus californiensis* were obtained in November 1995 by means of bottom trawling nets operated from commercial shrimp fishing vessels performing standard catching efforts (average trawling time: 1 hour; trawling speed: 2 knots), in the following locations: south of Santa Clara Port (31°34′N, 114°19′W); west of Guaymas Port (27°50′N, 111°05′W); and southwest of Mazatlan Port (22°27′N, 106°44′W). Samples of *P. stylirostris* were caught in September 1995 with a cast net thrown from small boats in the following shallow coastal areas: off Santa Clara Port (31°44′N, 114°19′W); off Guaymas Port (27°54′N, 110°55′W); and off Mazatlan Port, (23°12′N, 106°30′W). The samples covered three out of four distinctive areas (called here "upper Gulf, middle Gulf, lower Gulf, and mouth of the Gulf") according to Round (1967; Fig. 1). Samples were frozen (–20°C) and shipped to the laboratory, where they were stored at –75°C until dissection.

Soft tissues from head and abdominal muscle from adult specimens were separated and homogenized in 1 to 2 volumes of cold buffer prepared with 0.1 M Tris-HCl, pH 7.00 + NAD^+ + $NADP^+$ + PVP (10:1:1:100; v:p:p:p). Homogenates were spun (12,000 g) at 4°C, for 20 min. Supernatants were stored at –75°C until needed.

Electrophoretic assays were performed on 12.5% starch gels (Sigma Chemical Co., St. Louis, MO) in a horizontal apparatus that was placed in the refrigerator (4°C). Three

buffer systems were used to resolve 16 enzymatic systems and muscular protein: continuous tris-citrate buffer, pH 8.0 (TC8) following Selander et al. (1971), 150 V, 6 h; discontinuous tris-citrate, pH 8.3-sodium borate buffer, pH 8.65 (POU) according to Poulik (1957), 250 V, 5 h; and continuous tris-EDTA-borate, pH 8.0 (TEB) following Shaw and Koen (1968), 200 V, 6 h. Tissue sources, electrophoresis systems employed to resolve each enzyme or muscular protein, and number of loci resolved in each enzyme system are listed in Table 1. Staining procedures were accomplished according to the methods of Schaal and Anderson (1974), Shaw and Prasad (1970), Shaw and Koen (1968), Abreu-Grobois (1983), and Rosa-Vélez (1986).

Allelomorphs were named A to F depending on their anodal mobility, A being the fastest one. Zymogram interpretation was achieved following recommendations by Utter et. al (1987). In those cases where more than one zone of activity was present in the gel, genetic hypotheses were constructed to ensure correct interpretation. The more complex pattern was displayed by the group of esterases, which was interpreted by standardizing procedures. These involved gel staining with each of the esters used in the staining mixture (a-naphtyl acetate [black bands] and b-napthyl acetate [red bands]), but in separate assays, followed by a joined assay of the same sample, as Laubier et al. (1984) suggested.

Swofford's (1989) BIOSYS-1 software was fed with raw genotypic data from electropherograms to obtain allelic frequencies, proportion of polymorphic loci at the 95% level, observed and unbiased expected (Nei, 1978) heterozygosity, chi-square goodness-of-fit test for testing conformity to the Hardy-Weinberg equilibrium (H-W equilibrium) of variable loci, and Nei's (1978) unbiased genetic similarity and distance.

The same set of raw data was used with GENEPOP (versus 1.2) (Raymond and Rousset, 1995), to compute an unbiased estimate of the *P*-value of an F_{st}-based exact test for the distribution of genotypes by means of the Markovian chain method. PHYLIP's software (versus 3.5) was used to perform bootstrap resampling of gene frequencies to obtain a genetic distance UPGMA (unweighted pair-group method with arithmetic averaging) dendrogram. A Bonferroni correction was applied where multiple tests were carried out.

Results

A total of 32 loci were resolved from 16 enzyme systems and muscular protein. Twenty loci (*Acph-1, Aph-2, Gdh, Got-1, Got-2, G3pd, Gpd, Idh, Ldh, Mdh-1, Mdh-2, Me, Pt-1, Pt-2, Pt-4, Pt-5, Pt-6, Sdh-1, Sdh-2,* and *Xdh*) were monomorphic across all populations sampled. Protein polymor-

Table 1

Electrophoretic conditions, tissues assayed and number of loci resolved in the allozymic variation study of *Penaeus californiensis* and *P. stylirostris.* E.C. = Enzyme Commission.

Enzyme	E.C. no.	Tissue[1]	Buffer system[2]	Staining recipe[3]	Genetic locus	No. of loci scored
Acid phosphatase	3.1.3.2	C	B	SP	*Acph*	2
Alkaline phosphatase	3.1.3.1	C,M	A	SK	*Aph*	3
Esterase	3.1.1.1	C	C	SP	*Est*	5
Glucose-6-phosphate dehydrogenase	1.1.1.49	M	C	SA	*G6pd*	1
Glutamate dehydrogenase	1.4.1.3	M	C	SA	*Gdh*	1
Glutamic oxalacetic transaminase	2.6.1.1	M	A	SA	*Got*	2
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	M	C	SA	*G3pd*	1
α-Glycerophosphate dehydrogenase	1.1.1.8	M	C	SA	*Gpd*	1
Isocitrate dehydrogenase	1.1.1.42	M	C	AG	*Idh*	1
Lactate dehydrogenase	1.1.1.27	M	C	SP	*Ldh*	1
Leucinamino peptidase	3.4.11.1	C	A	SP	*Lap*	1
Malate dehydrogenase	1.1.1.37	M	C	SA	*Mdh*	2
Malic enzyme	1.1.1.40	M	A	SA	*Me*	1
Phosphoglucomutase	5.4.2.2	M	A	SA	*Pgm*	1
Total protein	—	C,M	B	RV	*Pt*	6
Sorbitol dehydrogenase	1.1.1.14	M	A	SA	*Sdh*	2
Xantine dehydrogenase	1.2.1.37	M	A	SA	*Xdh*	1

[1] C = cephalothorax; M = muscle.

[2] A = tris-citrate, pH 8.0 (Selander et al., 1971); B = tris-citrate, pH 8.3-sodium borate, pH 8.65 (Poulik 1957; C = Tris-EDTA-borate; pH 8.0 (Shaw and Koen, 1968).

[3] SA = Schaal and Anderson (1974); SP = Shaw and Prasad (1970); SK = Shaw and Koen (1968); AG = Abreu-Grobois (1983); RV = Rosa-Vélez (1986).

phism ($P \leq 0.95$) was 0.125 for *P. californiensis* in the three populations sampled along the Gulf of California, unlike *P. stylirostris* that showed a clinelike pattern with increasing polymorphism from 0.156 in the upper Gulf population to 0.312 in the mouth of the Gulf. The genetic diversity, reported as expected heterozygosity, was quite homogeneous along the surveyed distribution of *P. californiensis* and showed a gradual northern–southern increase in *P. stylirostris*. (Table 2).

After the Bonferroni correction for multiple tests was applied ($\alpha' = 0.00142$), only *Est-2* was out of H-W equilibrium in at least one population of each species, and *Pgm* and *Pt-3* displayed the same pattern in *P. stylirostris*, all of them accounting for heterozygote deficiency (Table 2).

Four loci (*Aph-3, Est-1, Est-4,* and *Est-5*) in the upper population of *P. stylirostris* showed $D = -1.000$ values (see Table 2); however, four loci had to be considered monomorphic because the polymorphism criterion applied was the most conservative (P_{95}; a locus is considered polymorphic only when the frequency of the most common allele is ≤0.95). In most of these cases only one or two individuals were scored as homozygotes for the alternative allele. Such a low frequency may not be significant for the evolutionary process owing to the high probability of disappearance through random processes in just a few generations.

On the other hand, in spite of the high frequencies of both alleles ($p \approx 0.5$), only one heterozygous individual was scored for the diallelic locus *Aph-1* in the upper population of *P. stylirostris*, resulting in a highly significant heterozygote deficiency.

Standardized variance (F_{st}) analysis was achieved among subpopulations within species (Table 3). *Penaeus californiensis* showed a lesser degree of genotypic differentiation among its populations ($F_{st} = 0.182$) than *P. stylirostris* ($F_{st} = 0.372$). This result is possibly related to the mean frequency of private alleles (*sensu* Barton and Slatkin, 1986) in their populations ($p = 0.096$ in *P. californiensis*; and $p = 0.214$ in *P. stylirostris*), even though both figures were notably high. Three loci accounted for differences in the subpopulations of *P. californiensis*: *Aph-3, Est-1,* and *Est-2*. The higher genetic variability displayed by *P. stylirostris* produced a more complex pattern of genotypic differentiation where ten loci accounted for significant differences among subpopulations (*Acph-3, Est-1, Est-2, Est-3, Est-4, Est-5, G6pd, Lap, Pgm, and Pt-3*).

Genetic similarities among subpopulations showed a similar pattern in both species (Table 4); there was a closer resemblance between the middle Gulf and the mouth of the Gulf populations of both species than with the upper Gulf population. This pattern can be visualized in the dendrogram (Fig. 2), where the upper Gulf population of both species segregates from the other two, which are clustered together.

Furthermore, a different level of similarity among subpopulations within species is clearly noticeable (Table 4). *Penaeus californiensis* showed a higher level of similarity among its subpopulations (I range: 0.988–0.997) than *P. stylirostris* (I range: 0.929–0.954). Genetic similarities between species rendered a clustering level of 0.674 (Fig. 2, Table 4).

Discussion

Two results are of particular interest: a north–south clinelike gradual increase in genetic variability in the more coastal species and the finding of a heterogeneous distribution of genotype frequencies in both assayed species. Most of the previous studies on *Penaeus* species around the world have depicted a general pattern of low homogeneous genetic variability. For example, Mulley and Latter (1980) reported heterozygosity values ranging between 0.006 and 0.033 in Australian penaeids (four species of *Metapenaeus* and six of *Penaeus*). These data were confirmed by subsequent studies: Richardson (1982) found an average heterozygosity of 0.028 in six populations of *P. latisculatus,* and Tam and Chu (1993) reported an observed heterozygosity range of 0.007–0.049 in some species of *Penaeus* and *Metapenaeus* from the South China Sea. *Penaeus japonicus* exhibited one of the highest heterozygosity values ($H_0 = 0.047 \pm 0.029$) among the species surveyed in that study. However there are other reports of even greater heterozygosity in populations of that species transported to European hatcheries as broodstock ($H_0 = 0.102$, Sbordoni et al., 1986; $H_0 = 0.071$, Laubier et al., 1984).

For penaeid species occurring in the western hemisphere, Lester (1979) reported heterozygosities between 0.070 and 0.089 for three commercial penaeids of the Gulf of Mexico. Very similar data for the same species were later reported by Labacena et al. (1994). Lester (1983) also studied one population of each species, *P. vannamei* (from Chomes, Costa Rica) and *P. stylirostris* (from Guaymas, México), which dwell along the northeastern Pacific coast, and reported heterozygosity values of 0.02 and 0.06, respectively. Sunden and Davis (1991) reported heterozygosity values for *P. vannamei* samples from Mexico, Panama, Ecuador, and one farmed population at a Texas hatchery, which were 0.0173, 0.0172, 0.0208, and 0.0111, respectively.

Levels of genetic variability of the species that we studied were not out of the range of those of previous estimations. It must be noticed, in addition, that *P. californiensis* showed a lower, narrower heterozygosity range (0.023 ±0.014–0.037 ±0.012) than *P. stylirostris* (0.038 ±0.021–0.086 ±0.027). The latter might be explained by the different habitats that each species occupies during its life cycle. This important difference may also be related to the clinelike pattern of the heterozygosity values in *P. stylirostris*, whereas the genetic variability of *P. californiensis* could be evidence of the more stable oceanic conditions that this species experiences during its life span. *Penaeus stylirostris* appears to reflect the environmental variability of the coastal lagoons, which it penetrates during a critical stage of its life cycle. Latitudinal variability of hydrological, ecological, and productivity conditions characterize coastal lagoons along the eastern coast of the Gulf of California. The upper zone's coastal lagoons (Fig. 1) are located in an arid region where vegetation is scarce; around the lagoons, some halophytes and sea grasses predominate. Productivity in these basins depends almost exclusively on microalgae (phytoplankton and microphytobenthos) (Contreras, 1985). To the south, through the

Table 2

Genetic variation of six naturally occurring populations of *Penaeus californiensis* (3) and *P. stylirostris* (3) throughout the Gulf of California (upper, middle and mouth of Gulf). Population names correspond to designated zones of the Gulf (see Fig. 1); n = the number of genes assayed, H = the frequency of observed heterozygotes, D = Selander's coefficient of deviation. Significances of goodness-of-fit chi-square tests (after Bonferroni correction) are given as usual (*=$P \leq 0.05$; ***=$P \leq 0.001$).

			Penaeus californiensis			*Penaeus stylirostris*		
Locus		Allele	Upper	Middle	Mouth	Upper	Middle	Mouth
Acph-3	*n*		76	44	92	96	42	28
		A	1.000	1.000	1.000	1.000	1.000	0.429
		B	0.000	0.000	0.000	0.000	0.000	0.321
		C	0.000	0.000	0.000	0.000	0.000	0.250
	H		0.000	0.000	0.000	0.000	0.000	0.429
	D		0.000	0.000	0.000	0.000	0.000	–0.365
Aph-1	*n*		90	48	96	96	48	32
		A	1.000	1.000	1.000	0.510	0.250	0.406
		B	0.000	0.000	0.000	0.490	0.750	0.594
	H		0.000	0.000	0.000	0.021	0.417	0.438
	D		0.000	0.000	0.000	–0.959***	0.088	–0.121
Aph-3	*n*		90	34	90	92	48	32
		A	0.067	0.000	0.021	0.978	1.000	1.000
		B	0.889	0.765	0.947	0.022	0.000	0.000
		C	0.044	0.000	0.032	0.000	0.000	0.000
		D	0.000	0.235	0.000	0.000	0.000	0.000
	H		0.178	0.235	0.022	0.000	0.000	0.000
	D		–0.136	–0.365	–0.662	–1.000***	0.000	0.000
Est-1	*n*		76	38	96	84	36	28
		A	0.737	0.342	0.156	0.976	0.389	0.643
		B	0.171	0.632	0.646	0.024	0.500	0.214
		C	0.092	0.026	0.198	0.000	0.111	0.143
	H		0.237	0.211	0.333	0.000	0.444	0.286
	D		–0.443	–0.576	–0.365	–1.000***	–0.263	–0.471
Est-2	*n*		82	48	96	88	44	32
		A	0.024	0.000	0.000	0.000	0.000	0.000
		B	0.012	0.104	0.083	0.000	0.000	0.000
		C	0.061	0.229	0.052	0.398	0.023	0.344
		D	0.573	0.667	0.854	0.602	0.909	0.625
		E	0.329	0.000	0.010	0.000	0.068	0.031
	H		0.244	0.625	0.292	0.159	0.182	0.125
	D		–0.569*	0.243	0.107	–0.672*	0.055	–0.753
Est-3	*n*		72	38	96	74	44	32
		A	0.083	0.053	0.052	0.203	1.000	1.000
		B	0.889	0.947	0.948	0.797	0.000	0.000
		C	0.028	0.000	0.000	0.000	0.000	0.000
	H		0.111	0.105	0.104	0.351	0.000	0.000
	D		0.458	0.028	0.044	0.072	0.000	0.000
Est-4	*n*		76	42	94	86	34	24
		A	1.000	1.000	1.000	0.953	0.853	0.292
		B	0.000	0.000	0.000	0.047	0.147	0.708
	H		0.000	0.000	0.000	0.000	0.176	0.250
	D		0.000	0.000	0.000	–1.000***	–0.317	–0.420

continued

mouth of the Gulf, subtropical conditions prevail; abundant fringing vegetation, dominated by four species of mangroves, contributes a huge amount of organic matter that triggers a complementary source of organic production within the detritus chain. Biodiversity indices rise and trophic resources become diversified (González-Farias and Mee, 1988; Flores-Verdugo, 1990). Hence, it is plausible to assume that complexity of ecological webs increases from the northern to the southern coastal zone of the Gulf of California. A parallel gradual increment in heterozygosity in the populations of *P. stylirostris* may be related to that ecological feature. However, Burton (1983) has stated that a direct relation between high heterozygosity and high ecological complexity is very difficult to demonstrate,

Table 2 (continued)

			Penaeus californiensis			*Penaeus stylirostris*		
Locus		Allele	Upper	Middle	Mouth	Upper	Middle	Mouth
Est-5	*n*		96	46	96	94	46	30
		A	1.000	1.000	1.000	0.957	0.022	0.767
		B	0.000	0.000	0.000	0.043	0.717	0.200
		C	0.000	0.000	0.000	0.000	0.261	0.033
	H		0.000	0.000	0.000	0.000	0.130	0.133
	D		0.000	0.000	0.000	–1.000***	–0.694	–0.653
G6pd	*n*		96	48	96	90	38	30
		A	1.000	1.000	1.000	1.000	1.000	0.767
		B	0.000	0.000	0.000	0.000	0.000	0.100
		C	0.000	0.000	0.000	0.000	0.000	0.133
	H		0.000	0.000	0.000	0.000	0.000	0.267
	D		0.000	0.000	0.000	0.000	0.000	–0.329
Lap	*n*		96	48	96	92	48	32
		A	1.000	1.000	1.000	0.272	0.458	0.688
		B	0.000	0.000	0.000	0.728	0.542	0.313
	H		0.000	0.000	0.000	0.543	0.500	0.500
	D		0.000	0.000	0.000	0.358	–0.014	0.127
Pgm	*n*		96	48	94	88	48	32
		A	1.000	1.000	1.000	0.125	0.104	0.094
		B	0.000	0.000	0.000	0.795	0.292	0.281
		C	0.000	0.000	0.000	0.000	0.188	0.531
		D	0.000	0.000	0.000	0.000	0.333	0.094
		E	0.000	0.000	0.000	0.034	0.083	0.000
		F	0.000	0.000	0.000	0.045	0.000	0.000
	H		0.000	0.000	0.000	0.136	0.208	0.313
	D		0.000	0.000	0.000	–0.613*	–0.728*	–0.513
Pt-3	*n*		96	48	96	96	48	32
		A	1.000	1.000	1.000	0.000	0.250	0.063
		B	0.000	0.000	0.000	1.000	0.750	0.938
	H		0.000	0.000	0.000	0.000	0.083	0.000
	D		0.000	0.000	0.000	0.000	–0.782*	–1.000
Number of loci studied			32	32	32	32	32	32
Mean number of individuals per locus			45.8 ±0.6	22.3 ±0.7	46.2 ±1.5	44.7 ±1.1	22.0 ±0.6	15.7 ±0.2
Mean number of alleles per locus			1.31 ±0.16	1.19 ±0.09	1.25 ±0.13	1.38 ±0.12	1.44 ±0.16	1.53 ±0.16
Polymorphism (P_{95})			12.50	12.50	12.50	15.63	25.00	31.25
H_0			0.024 ±0.012	0.037 ±0.021	0.023 ±0.014	0.038 ±0.021	0.067 ±0.025	0.086 ±0.027
H_e			0.044 ±0.023	0.046 ±0.024	0.030 ±0.018	0.075 ±0.027	0.109 ±0.038	0.145 ±0.041

and therefore, correlation should not be taken as conclusive evidence for causation.

Several reports have stated a small probability of encountering genetically "differentiated" stocks in *Penaeus* species, mainly due to their low variability and the apparent homogeneous allele distributions among the subpopulations (Lester, 1979, 1983; Mulley and Latter, 1981a, 1981b; Richardson, 1982). However, Sunden and Davis (1991) could trace a slight geographic differentiation across the range of *P. vannamei.* They detected at least one unique allele in each wild population. Tam and Chu (1993) attributed the higher genetic similarity shown between the species pair *P. merguiensis* and *Metapenaeus ensis* to the genetic differentiation among populations of the same species. In addition, some allozyme variation was observed among populations of *Metapenaeus benettae* (Salini, 1987) in Australia and *P. kerathurus* in the Mediterraenean (Mattocia et al., 1987). From later studies, it was noticed that when the allozymes surveys were performed over larger geographical scales, substantially higher significant variation and differentiation among populations was found. Such is the case for the wild populations of *P. monodon* along the Australian coast (Benzie et al., 1992), where expected heterozygosities have ranged between 0.053 and 0.103, and significant genetic differences have existed among Australian populations.

The use of more variable markers such as mtDNA genes have confirmed the allozymic findings on the structuring of *P. monodon* populations in Australia (Benzie et al., 1993), and *P. notialis* and *P. schmitti* in the South American Atlantic coast (Machado et al., 1993).

From our data, subpopulations of both species appear genetically differentiated in terms of significant values of standardized variance of their genotypic distributions (Table 3). Two reasons seem to account for such a result: 1) private alleles exist in about every polymorphic locus (75% in both species), and 2) the most frequent allele alternates among subpopulations of the same species in several loci (see Table 2).

Heterogeneity among subpopulations is commonly explained as a lack of genetic flow (see Slatkin, 1985, for a review). Nonetheless, penaeids can be considered vagile species because they have two dispersal phases, the planktonic larvae and the vagile adult. Lester (1979) imputes this characteristic to the high levels of genetic similarity among the penaeids populations of the Gulf of Mexico.

Although penaeids are capable of being displaced, geographical barriers must prevent their movement. The Gulf of California is now considered as a much more complex ecosystem than it was formerly believed: Santamaría-Del Angel et al. (1995) proposed 14 biogeographic subdivisions in the Gulf on the basis of satellite images describing concentrations of photosynthetic pigments. Likewise, González-Farias et al. (1995) described complicated patterns of carbon turn-over throughout the Gulf from the analysis of organic matter and heterotrophic bacteria. The upper Gulf region is a very unique environment where the Colorado River drained until about fifty years

Table 3

Wright's standardized variance (F_{st}) of populations within species. Statistical significance in brackets (ns=not significant; **=$P \leq 0.01$; ***=$P \leq 0.001$).

Loci	*P. californiensis*	*P. stylirostris*
Acph-3	—	0.510(***)
Aph-1	—	0.054(ns)
Aph-3	0.094(***)	–0.022(ns)
Est-1	0.278(***)	0.384(***)
Est-2	0.134(***)	0.128(**)
Est-3	–0.005(ns)	0.756(***)
Est-4	—	0.514(***)
Est-5	—	0.650(***)
Gdh-1	—	–0.009(ns)
G6pd	—	0.207(***)
Lap	—	0.149(***)
Pgm	—	0.264(***)
Pt-3	—	0.210(**)
Mean values	0.182(***)	0.372(***)

Table 4

Nei's genetic similarity (above diagonal *****) and distance (below diagonal *****) among six populations of two penaeid species, *Penaeus californiensis* and *P. stylirostris,* throughout the Gulf of California (upper, middle, and mouth of Gulf).

		Penaeus californiensis			*Penaeus stylirostris*		
	Population source	Upper	Middle	Mouth	Upper	Middle	Mouth
Penaeus californiensis	Upper	*****	0.991	0.988	0.690	0.654	0.691
	Middle	0.009	*****	0.997	0.632	0.656	0.686
	Mouth	0.012	0.003	*****	0.672	0.655	0.679
Penaeus stylirostris	Upper	0.371	0.383	0.397	*****	0.929	0.936
	Middle	0.425	0.422	0.423	0.074	*****	0.954
	Mouth	0.370	0.377	0.387	0.067	0.047	*****

ago, when the Hoover Dam was constructed up river and flow was severely restricted. Presently, hypersaline conditions prevail in the upper Gulf region. This dynamic and extreme environment is topographically bounded to the south by a submarine range. Tiburón and Angel de la Guarda Islands are the heights of this submarine range (Fig. 1); south of these islands the Gulf increases in depth to around 3000 m in the Guaymas Basin. Thus, the islands may be considered a geographical or ecological boundary that reduces the free flow of penaeids. Correa-Sandoval and Carvacho-Bravo (1992) came to a similar conclusion when they described the distribution pattern of brachyuran crabs in the Gulf of California.

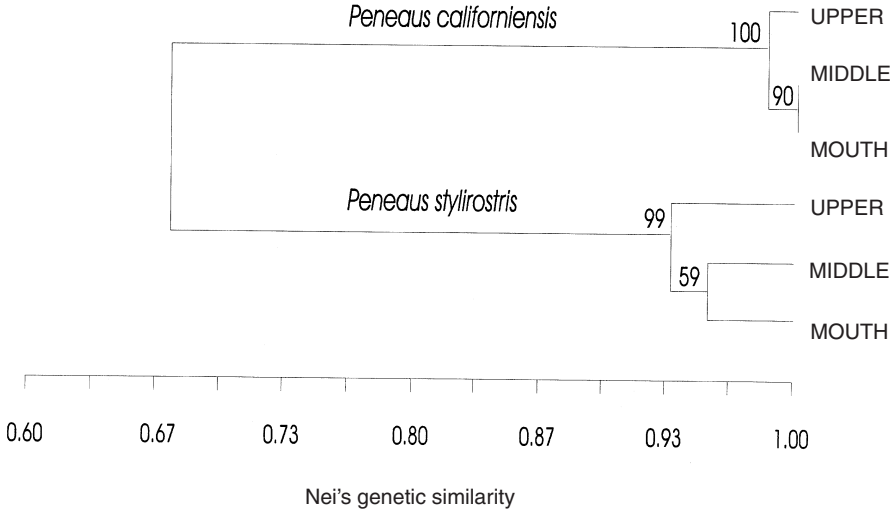

Figure 2

Bootstrapped dendrogram generated from a Nei's (1978) genetic similarities matrix (UPGMA algorithm) between species and their subpopulations. Data based on 32 loci.

In some populations of both species, three loci (*Aph-1*, *Est-2,* and *Pgm*) were found to be out of H-W equilibrium according to the chi-square goodness-of-fit test. Two of the common causes of heterozygote deficiency, as discussed by Zouros and Foltz (1984), can be invoked here: 1) the Wahlund effect, because some genetic structure has been demonstrated in both species, at least in the geographic range that we studied; thus, different genetic compositions taking part in reproductive events, will yield such a pattern; and 2) selection against heterozygotes, a hypothesis that is difficult to prove, but is feasible owing to very recent environmental modification (ca. half a century). Homozygous genotypes may be selected if they perform better under extreme conditions with no drastic gene erosion during the little time elapsed since environmental alterations began. For example, *Aph-1* displays a ca. 50% frequency of the two segregating alleles, which allows us to expect high heterozygote frequency. However, the sample lacked these genotypes almost completely. With no additional evidence to discard either of the above, a third one, i.e. the presence of a null allele segregating in this population, may also be invoked. Further evidence from breeding experiments among individuals of this population is needed to evaluate this supposition.

Additional evidence of the divergence of populations (remarkably, populations of both species dwelling in the upper Gulf) is given by Nei's genetic similarity (Table 4; Fig. 2). It is evident from the dendrogram that 1) there is a clear subdivision in both within-populations dendrograms that distinguishes the populations inhabiting the upper Gulf (hence, it is not only the previously discussed characteristic of heterozygote deficiency that segregates these populations from the rest, but the distribution of their genes too) and 2) a subdivision between species where the degree of genetic similarity is similar to those previously reported in other penaeid species. Average genetic similarities among species within *Penaeus* genera were 0.65 ±0.08, and 0.69 ±0.08 within *Metapenaeus* (Mulley and Latter, 1980). Similar values (I=0.66) were found between two forms of brachyuran freshwater crabs from South Africa (Stewart and Cook, 1998).

The characteristic genetic features we found provide additional support for the recent scheme proposed by Pérez-Farfante and Kensley (1997). Their proposition, regarding the reorganization of American shrimps in the family Penaeidae, involves the promotion of an existing subgenus to genus, i.e. *Penaeus californiensis* would become *Farfantepenaeus californiensis*, and *Penaeus stylirostris* would change to *Litopenaeus stylirostris*. However, more evidence must be compiled to support this reclassification.

Complexity of the Gulf of California ecosystem, as well as the biological features described for each species, may account for the greater degree of genetic structure in the species that inhabit it. Fisheries and hatcheries managers can take advantage of such information, for example, by defining stocks or selecting the more variable populations to be submitted to artificial selection programs in the aquaculture scheme.

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