GENETIC VARIATION AND POPULATION STRUCTURE IN A SPINY LOBSTER, *PANULIRUS MARGINATUS*, IN THE HAWAIIAN ARCHIPELAGO[1]

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ABSTRACT

Samples of the commercially important spiny lobster, *Panulirus marginatus*, were collected from localities throughout the Hawaiian Archipelago and subjected to starch gel electrophoretic analysis of protein variation. The amount and pattern of genetic variation exhibited by specific enzymes was determined and analyzed to see whether or not there was evidence that the species was composed of multiple stocks or subpopulations throughout its range.

The lobster exhibited polymorphisms at 7 loci (Est-3, Umb, Gpi, Mpi, Pep-1, Pep-2, and Pgm) out of the 46 enzyme-coding loci screened. However, genetic variability in the species was quite low, the average heterozygosity for all loci was 0.021. Observed genotype distributions at the variable loci agreed with Hardy-Weinberg expectations. Allele-frequency distributions for each locus were remarkably similar across localities and statistical tests failed to reveal clear patterns of genetic differentiation within the Archipelago. The results are consistent with the existence of a single panmictic stock of *Panulirus marginatus* throughout the Hawaiian Archipelago.

The rational management of any fisheries resource, whether directed at exploitation, conservation, or some other goal, requires many different types of information about the species in question, and its interaction with environmental and biological factors in its environment. Data on basic biology (taxonomy, distribution and abundance, food habits, behavior, etc.), ecological requirements, reproductive characteristics, and population dynamics are all relevant to management decisions. Although the above types of information are necessary to any meaningful management plan, they are not sufficient. Information concerning the stock or subpopulation structure of the species is also of critical importance to the formulation of any comprehensive, long-term management program (MacLean and Evans 1981).

Subpopulations or stocks are generally considered to be self-sustaining subunits of a species which are more-or-less reproductively isolated from other such groups. It is reasonable to assume that as a result of random processes and local selection pressures, these subpopulations (stocks) will become genetically differentiated from one another. For this reason, the electrophoretic analysis of genetic characteristics provides one of the most direct, and therefore theoretically powerful, approaches to the problem of defining subpopulation structure. However, it should be emphasized that all tests of stock structure, including electrophoretic ones, are really one-sided. It is actually only possible to establish the existence of multiple differentiated stocks by falsifying the null hypothesis of a single, widespread, panmictic stock. It is not possible to prove that only a single panmictic population exists although the data (be they genetic, morphological, behavioral, or whatever) may be consistent with this hypothesis.

In the last decade, a substantial, multispecies, commercial fishery has developed in the Northwestern Hawaiian Islands (NWHI). This fishery is directed almost exclusively at demersal species and is dominated by catches of spiny lobsters (Palinuridae), snappers (Lutjanidae), and groupers (Serranidae). Because of the largely unknown and previously unexploited nature of this fishery, a coordinated, large-scale, multidisciplinary study involving personnel from the National Marine Fisheries Service Honolulu Laboratory, the U.S. Fish and Wildlife Service, the Hawaii Division of Fish and Game, and the University of Hawaii was initiated to describe, analyze, and model the major components of the NWHI ecosystem (Grigg and Pfund 1980). The genetic analysis

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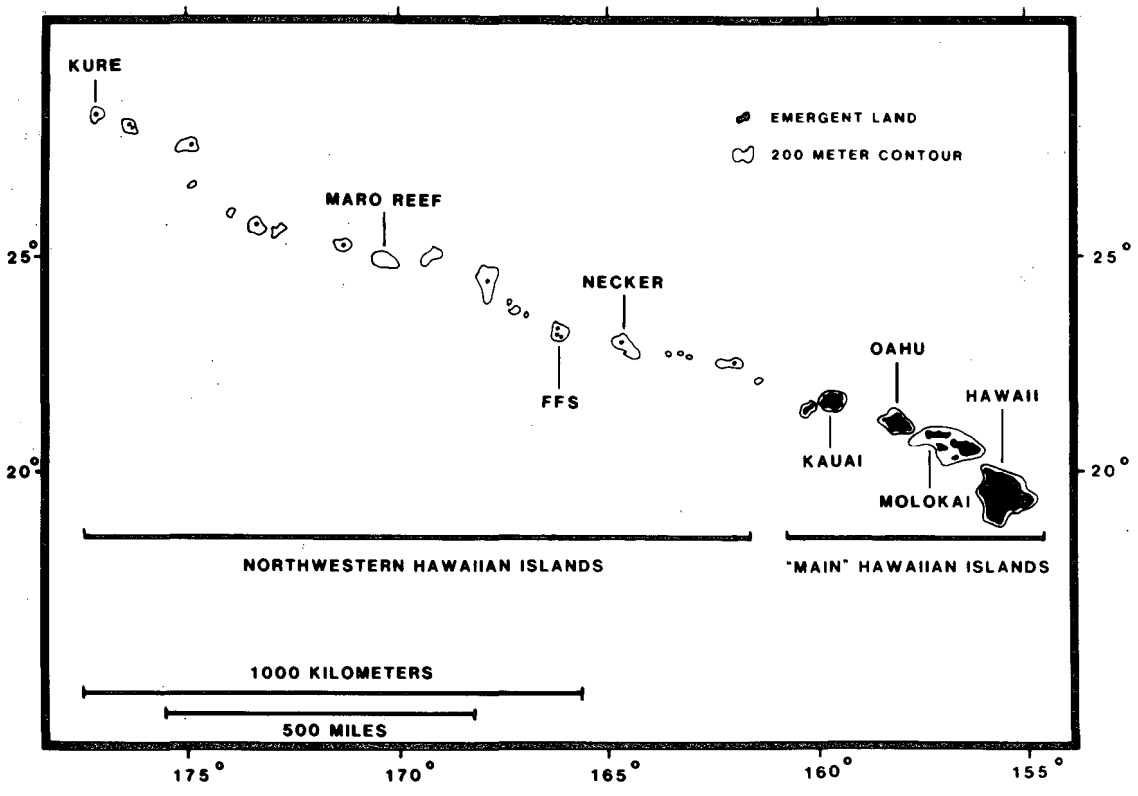
of stock structure of the lobster detailed in this report was one part of this overall program (Shaklee and Samollow 1980).

Two general questions regarding lobster subpopulation structure were asked in the present study. First, was there any detectable stock heterogeneity within the entire Hawaiian Archipelago? Second, and specifically relating to the potential impact of the emerging fishery in the NWHI on the existing fishery in the main islands, was there evidence that populations in the main islands were differentiated, and thus independent, from populations in the NWHI?

The spiny lobster, *Panulirus marginatus*, is endemic to the Hawaiian Archipelago where it occurs in large numbers from Hawaii in the southeast to Kure Atoll in the northwest (Fig. 1). Highest apparent abundances are localized at Necker Island and Maro Reef (Uchida et al. 1980), the two localities where the lobster fishery is presently concentrated. *Panulirus marginatus* is generally found in waters deeper than 10 m. *Panulirus marginatus* has an annual fecundity of from 125,000 to 450,000 eggs per female (Honda 1980). After mating, females carry one or more spermatophores ventrally on the thorax until the eggs are extruded and fertilized. Embryonic development in this species takes about 30 d during which time the embryos remain attached to the pleopods of the female (Morris 1968). Based on studies of related species it appears that, after hatching, the larvae are planktonic for a period of 6-12 mo passing through 8-12 phyllosoma larval stages (Johnson 1956, 1968; Johnson and Knight 1966; Inoue 1978). The larvae metamorphose into puerulus postlarvae. The postlarvae settle from the plankton and assume the benthic lifestyle characteristic of the adults. Based on tag-recapture studies at Kure Atoll and French Frigate Shoals (MacDonald[4]) and similar studies at Oahu (Morris 1968), adults appear to be relatively sedentary, not exhibiting large-scale movements.

[4]C. MacDonald, Zoology Department, University of Hawaii, Honolulu, HI 96822, pers. commun. May 1982.

FIGURE 1.—Map of the Hawaiian Islands showing sampling localities. Note the 200 m depth contours.



MATERIALS AND METHODS

Sample Collection and Processing

A total of 1,869 spiny lobsters from the five localities was collected over a 2½-yr period (Fig. 1). Lobsters from Kure Atoll, Oahu, and Hawaii were collected by hand by divers using scuba. Lobsters from Maro Reef and Necker Island were collected using standard, wire mesh lobster traps. The sampling periods and numbers of lobsters collected for each of the five localities are Kure: October 1978-January 1979 (N = 21), June 1979-September 1979 (N = 136), August 1979-June 1980 (N = 249), and June 1980-January 1981 (N = 176); Maro Reef: October 1978 (N = 60), November 1979 (N = 213), and September 1980 (N = 145); Necker: October 1978 (N = 97), March 1979-June 1979 (N = 421), and December 1980 (N = 148); Oahu: May 1979-January 1980 (N = 53), July 1980-December 1980 (N = 71), and March 1981 (N = 30); and Hawaii: April 1980-March 1981 (N = 49). Because not all individuals were sexed and measured (in some cases one or both types of data were unavailable for entire collections), sex ratios and size compositions could not be accurately calculated. However, all animals included in the data analyses were adults. Some samples were frozen at −20°C in the field (as whole animals, as carcasses minus tails, or as isolated pereiopods—walking legs) and shipped frozen to the laboratory. Other samples were transported live to Honolulu where they were dissected and sampled. In the initial screening for polymorphic loci, samples of pereiopod muscle, abdominal muscle, digestive gland, green gland, eye, heart, and gills were dissected from whole, frozen lobsters. Each of these samples was homogenized at 4°C in an equal volume of 0.1 M Tris-HCl pH 7.0 buffer (containing 1×10^{-3} M EDTA and 5×10^{-5} M $NADP^+$) using a loose fitting, motorized pestle. Homogenates were centrifuged at 4°C for at least 20 min at a minimum of 20,000 × g. Digestive gland supernatants were routinely centrifuged a second time to minimize lipid content. The resulting supernatants were transferred to individually labeled glass vials which were capped and stored at −75°C until the electrophoretic analyses were completed. Since all variable enzymes could be analyzed in pereiopod muscle extracts (and digestive gland extracts for peptidase-1 (PEP-1)) only these tissues were examined in the subsequent analyses.

Electrophoresis

The supernatants were analyzed by a combination of vertical and horizontal starch gel electrophoresis (Selander et al. 1971; Shaklee et al. 1973). Each enzyme system surveyed in the initial screening for genetic variation was electrophoresed on 2-10 different buffer systems, using extracts of several different tissues. Following electrophoresis, isozyme patterns were visualized using standard recipes (modified from Shaw and Prasad 1970; Selander et al. 1971; Siciliano and Shaw 1976). Esterases of the lobster were detected using α-naphthyl acetate (EST) or 4-methylumbelliferyl acetate (Umb = Est-D) as substrates, both variable peptidases were stained using leucylleucylleucine as substrate.

Gel Scoring and Data Analysis

Patterns of enzyme variation that were consistent with the subunit structure of presumably homologous proteins of other species (when known), and with simple genetic models, were scored and recorded as genotypes. Names of enzymes and Enzyme Commission numbers follow the recommendations of the Commission on Biochemical Nomenclature (1973). For multilocus enzyme systems, loci were consecutively numbered beginning with the most anodal isozyme. Alleles at each locus were designated according to the relative electrophoretic mobilities of the homomeric isozymes they encode. The most common allele at a locus was designated "100" and all other alleles at that locus were numbered according to the electrophoretic mobilities of their products relative to that of the product of the 100 allele. Negative numbers were given to alleles encoding isozymes with cathodal migration. In the case of the glucosephosphate isomerase (GPI) system of the spiny lobster, subbanding anodal to the 100 isozyme was often quite pronounced, especially in older samples. Because we could not be confident of scoring allelic variation in this region, no attempt was made to score isozymes having an electrophoretic mobility greater than that of the most common (100) isozyme. Many samples were reelectrophoresed with controls of known mobility to verify the identity of rare alleles for each enzyme system.

Despite that all alleles were initially identified and assigned numerical designations as described above, in many cases data summaries and statistical analyses employed fewer electromorph (allelic)

classes. This pooling was necessary because many of the alleles were extremely rare. The distribution of genotypes at each locus in each sample was examined for internal consistency with the Mendelian inheritance model by chi-square tests of observed genotype ratios with those expected for a single random mating population in the absence of differential selection among alleles. The expected ratios were calculated from observed allele numbers using Levene's (1949) unbiased method for small samples. The heterozygosity at each locus (h) was calculated as $h = 1 - \Sigma X_i$ where X_i is the frequency of the ith allele. Average heterozygosity (H) was calculated as the unweighted arithmetic mean of h (the individual locus heterozygosity) over all loci examined. A locus was considered polymorphic if the frequency of the most common allele was <0.99.

Three levels of analysis were employed to examine genetic differentiation (stock heterogeneity) among the population samples. First, for each polymorphic locus, contingency chi-square tests of all possible pairwise combinations of localities were conducted. Second, contingency tests comparing pooled samples representing the main Hawaiian Islands and the Northwestern Hawaiian Islands were conducted for all loci. Finally, large samples of spiny lobsters collected from three localities allowed the analysis of year-to-year variability within and among these localities.

RESULTS

Forty-six presumed gene loci encoding 28 different enzyme systems were surveyed for genetic variation in *Panulirus marginatus*. Thirty-nine of these were either monomorphic in the first 100 or more animals screened or exhibited only rare variants ($P < 0.01$) These enzymes which were not studied further were aspartate aminotransferase (2 loci), acid phosphatase (2 loci), adenylate kinase, alkaline phosphatase (3 loci), alanine aminotransferase, arginine kinase, esterase (3 loci), glyceraldehyde-3-phosphate dehydrogenase, glycerol-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, hexokinase, isocitrate dehydrogenase (2 loci), lipoamide dehydrogenase (= diaphorase), lactate dehydrogenase (2 loci), malate dehydrogenase (2 loci), malate dehydrogenase-$NADP^+$ (= malic enzyme) (2 loci), monophenolmonooxygenase (= tyrosinase), naphthyl amidase (= leucine aminopeptidase), peptidase (3 loci), peroxidase (2 loci) pyruvate kinase, superoxide dismutase (2 loci), and triosephosphate isomerase (2 loci). The remaining seven loci were polymorphic (frequency of the most common allele <0.99 in at least one population) and the conditions for their analysis are summarized in Table 1. Thus, *P. marginatus* exhibits a $P_{.99} = 0.152$.

TABLE 1.—Polymorphic enzymes in the spiny lobster, *Panulirus marginatus*: Characteristics and conditions for analysis. M = muscle, DG = digestive gland, V = vertical starch gel, H = horizontal starch gel.

Enzyme (EC Number)	Locus	Subunit structure[1]	Tissue	Gel	Buffer
Esterase (3.1.1.-)	Est-3	monomer	M	V	EBT[2]
Glucosephosphate isomerase (5.3.1.9)	Gpi	dimer	M	H	TC-1[3]
Mannosephosphate isomerase (5.3.1.8)	Mpi	monomer	M	V	EBT
Peptidase-1 (3.4.11.-)	Pep-1	monomer	DG	H	LiOH[4]
Peptidase-2 (3.4.11.-)	Pep-2	monomer	M	V	EBT
Phosphoglucomutase (2.7.5.1)	Pgm	monomer	M	H	TC-1
Umbelliferyl esterase	Umb	dimer	M	H	LiOH-2[5]

[1]Presumed structure based on enzyme banding pattern in heterozygotes (see text).
[2]EDTA-boric acid-Tris pH 8.6 buffer of Boyer et al. (1963).
[3]Tris-citric acid pH 7.0 buffer; buffer 1 of Shaw and Prasad (1970).
[4]Discontinuous lithium hydroxide pH 8.1 buffer (slightly modified buffer 2 of Selander et al. 1971).
[5]Continuous lithium hydroxide pH 8.1 buffer (modified buffer 2 of Selander et al. 1971 with stock "B" of gel buffer replaced with water).

Zymogram patterns for 6 of the 7 polymorphic enzymes are shown in Figure 2. The allele frequencies, heterozygosity per locus, and number of alleles successfully scored for each polymorphic locus at each locality are presented in Table 2. Although all seven loci in Table 2 are polymorphic at the 0.99 level in at least one sample, only two loci [Mpi (mannosephosphate isomerase) and Pep-1] exhibit a per locus heterozygosity of >0.1. As a result, the heterozygosity (H) averaged over all loci is only 0.021 for this species.

Several features of the data summarized in Table 2 warrant explanation. First, as mentioned in Materials and Methods, many of the allelic classes summarized in this table are heterogeneous containing two or more rare alleles. For example, the Est-3 (esterase) "fast" (Est-3^f) class contains alleles Est-3^{105} and Est-3^{103}, the "medium" class consists of allele Est-3^{100} only, and the "slow" class contains alleles Est-3^{97}, Est-3^{95}, and Est-3^{90}. Similar groupings were carried out for several other loci as indicated in Table 2. The allelic classes for Mpi, Pep-1, and Pep-2 were not detectably heterogeneous. Examples of phenotypes expressing several of the rare alleles contributing to these pooled allelic classes are shown and identified in Figure 2. Second, because

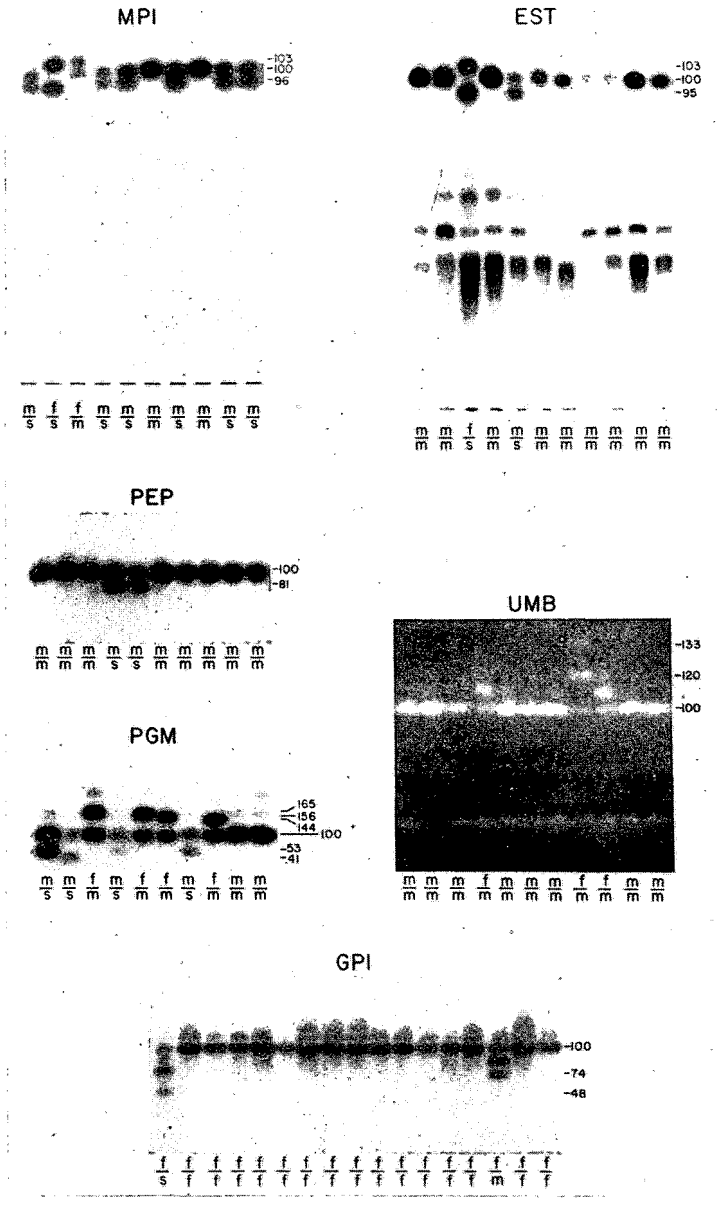


FIGURE 2.—Isozyme patterns of spiny lobster, *Panulirus marginatus*. MPI = mannosephosphate isomerase, EST = esterase-3, PEP = peptidase-2, PGM = phosphoglucomutase, UMB = umbelliferyl esterase (= EST-D), GPI = glucose-phosphate isomerase. Alleles are indicated at the right of each gel. Scoring of individual genotypes (by allelic class) is indicated at the bottom of each gel. Note that the sample origin is at the bottom and the anode is toward the top.

of the rarity of variant alleles, and therefore, of all but the common homozygote at all loci except Pep-1 and Mpi, tests of Hardy-Weinberg equilibrium were not very robust; most genotypic classes had to be pooled to obtain adequate numbers of expected genotypes. In all cases when there were three or more alleles, these were pooled into two groups; a group consisting of only the most common allele and a group including all other alleles. Furthermore, in the case of Mpi the unusual na-

TABLE 2.—Allele frequencies and heterozygosity values at seven polymorphic loci in the spiny lobster, *Panulirus marginatus*. N = number of individuals in total sample; h = per locus heterozygosity; number of genes successfully scored in parentheses; localities as in Figure 1.

		Locality						
Locus	Allelic class[1]	Kure $N = 582$	Maro Reef $N = 418$	Necker $N = 666$	Oahu $N = 154$	Hawaii $N = 49$	NWHI[2] $N = 1,666$	Main[3] $N = 203$
Est-3	f	0.004	0.003	—	0.003	0.010	0.002	0.005
	m	0.975	0.957	0.971	0.980	0.958	0.969	0.975
(h=0.059)	s	0.021	0.040	0.030	0.017	0.031	0.028	0.020
		(1,164)	(772)	(1,332)	(300)	(96)	(3,268)	(396)
Gpi	f	0.958	0.959	0.966	0.944	0.976	0.962	0.951
	m	0.037	0.039	0.033	0.049	0.024	0.036	0.043
(h=0.076)	s	0.005	0.002	0.001	0.007	—	0.003	0.005
		(1,058)	(640)	(1,070)	(288)	(82)	(2,768)	(370)
Mpi	f	0.008	0.011	0.007	0.019	0.010	0.008	0.017
(h=0.374)[4]	m+s	0.992	0.989	0.993	0.981	0.990	0.992	0.983
		(1,156)	(836)	(1,298)	(308)	(98)	(3,290)	(406)
Pep-1	f	0.784	0.725	0.785	0.638		0.763	0.638
(h=0.370)	s	0.216	0.275	0.215	0.362		0.237	0.362
		(190)	(284)	(284)	(58)		(758)	(58)
Pep-2	f	0.002	—	0.001	—	—	0.001	—
	m	0.985	0.988	0.984	1.000	0.969	0.985	0.994
(h=0.026)	s	0.013	0.012	0.015	—	0.031	0.014	0.006
		(600)	(670)	(850)	(266)	(64)	(2,120)	(330)
Pgm	f	0.013	0.007	0.007	0.003	—	0.009	0.003
	m	0.976	0.984	0.976	0.983	1.000	0.978	0.986
(h=0.042)	s	0.012	0.009	0.017	0.014	—	0.013	0.011
		(1,036)	(690)	(1,116)	(292)	(78)	(2,842)	(370)
Umb	f	0.006	0.001	0.011	0.010	—	0.003	0.008
	m	[5]0.983	[5]0.988	[5]0.980	0.983	1.000	0.991	0.986
(h=0.032)	s	.011	0.010	0.008	0.007	—	0.006	0.005
		(1,010)	(680)	(1,066)	(288)	(78)	(2,756)	(366)

[1]Pooled allelic classes as follows: Est-3^f includes alleles 105 and 103 while Est-3^s includes alleles 97, 95, and 90; Gpi^m includes alleles 80, 77, 74, 72, and 68 while Gpi^s includes alleles 65, 58, 54, 48, and 33; Pgm^f includes alleles 240, 186, 180, 165, 157, and 144 while Pgm^s includes alleles 82, 73, 53, 41, 35, and 25; Umb^f includes alleles 133, 120, 115, and 111 while Umb^s includes alleles 85, 80, and 75.

[2]Consisting of lobsters from Kure Atoll, Maro Reef, and Necker Island.

[3]Consisting of lobsters from Oahu and Hawaii.

[4]Subject to sex-restricted allele distribution (see text).

[5]Significantly different annual samples combined (see text).

ture of the distribution of alleles at this sex-linked locus (Shaklee 1983) effectively precluded Hardy-Weinberg analysis involving the two most common alleles. In spite of these caveats, out of 30 χ^2 tests (6 loci × 5 localities) only 1 significant deviation from Hardy-Weinberg expectation was observed; a heterozygote deficiency for Pep-1 at Necker Island ($\chi^2_1 = 7.63$ $P < 0.01$). Third, the data in Table 2 represent the pooled allele frequencies observed at each locality over the 2½-yr period of the study. In three cases (Umb at Kure Atoll, Maro Reef, and French Frigate Shoals), there was statistically significant year-to-year fluctuation in allele frequencies. The significance of this finding is discussed below. Fourth, because of the unusual relationship between MPI phenotype and sex in this species (Shaklee 1983), the medium and slow alleles had to be pooled into one class to prevent differences in sex ratio in each collection from biasing the allele-frequency analysis.

The first level of analysis, involving χ^2 tests of all pairwise comparisons, failed to reveal convincing evidence of stock heterogeneity in this lobster. Of the 66 comparisons, only three were significant: 1) Est, Kure vs. Maro ($\chi^2_1 = 4.76$ $P < 0.05$); 2) Pep-1, Kure vs. Oahu ($\chi^2_1 = 5.07$ $P < 0.025$); and 3) Pep-1, Necker vs. Oahu ($\chi^2_1 = 5.73$ $P < 0.025$). Given an $\alpha = 0.05$ level of significance, one would expect, on the basis of chance alone, about 3 significant outcomes from 60 tests. Additionally, given the basic linear arrangement of islands within the Hawaiian Archipelago (Fig. 1), population differentiation, if it were based upon isolation by distance (Wright 1943), would be expected to be most pronounced between widely separated localities. In contrast to these expectations, two of the three observed significant outcomes involve adjacent, not distant, localities.

The second statistical test compared the allelic composition of lobsters from the NWHI (Kure Atoll, Maro Reef, and Necker Island samples pooled) with that of lobsters from the main Hawaiian Islands (Oahu and Hawaii samples

pooled). Only one out of these seven contingency χ^2 tests was significant—Pep-1 ($\chi^2_1 = 4.50 P < 0.05$). Thus, in spite of the considerably larger sample sizes resulting from pooling, there is still no strong evidence of stock heterogeneity.

The third analysis involved the three cases of year-to-year differences in Umb allele frequency (Table 3). In each of the three cases, the frequency

TABLE 3.—Umbelliferyl esterase allele frequencies in *Panulirus marginatus* at three localities in successive years.

Locality (year)	Number of genes scored	Allelic class f	m	s
Kure Atoll A (June 1979-Sept. 1979)	232	0.000	0.996	0.004
Kure Atoll B (June 1980-Jan. 1981)	350	0.009	0.974	0.017
Maro Reef A (Nov. 1979)	284	0.000	0.996	0.004
Maro Reef B (Sept. 1980)	290	0.003	0.976	0.021
Necker Island A (Mar. 1979-June 1979)	662	0.006	0.986	0.008
Necker Island B (Dec. 1980)	290	0.028	0.962	0.010

of the two rare alleles (allelic classes) was higher in the 1980 collections than in those from 1979. When the rare alleles were pooled to allow χ^2 tests of the distributions, all three cases exhibited significant year-to-year changes: Kure Atoll A vs. B ($\chi^2_1 = 3.85$ $P < 0.05$), Maro Reef A vs. B ($\chi^2_1 = 4.44$ $P < 0.05$), and Necker Island A vs. B ($\chi^2_1 = 5.81$ $P < 0.025$). However, when allele distributions were compared among localities in either 1979 or 1980 (or in pooled 1979 + 1980) no significant differences between localities were observed. These results suggest annual fluctuations in Umb allele frequency within at least the NWHI. Unfortunately the sample sizes from the main Hawaiian Island localities were not large enough to be subdivided by year to see whether or not this annual fluctuation in Umb allele frequency occurred there also. Given that statistically significant annual fluctuations in Umb allele frequency were occurring at all three localities where this could be tested, it is important that they were parallel and did not lead to any significant differences in allele frequency between localities. This pattern of fluctuating allele frequency, common to the three NWHI localities, argues for the existence of a single panmictic lobster population throughout this region and suggests that a cohort having "unusual" Umb allele frequency was recruited into the fishery throughout the NWHI in 1980.

DISCUSSION

Electrophoretic studies of genetic variation have been reported for several decapod crustacean species. With the exception of *Panulirus argus* (Menzies 1981), all species that have been examined exhibit relatively little genetic diversity either within or between populations (Tracey et al. 1975; Mulley and Latter 1980; Nelson and Hedgecock 1980; Redfield et al. 1980; Smith et al. 1980; Hanley 1980). Nevertheless, several of the enzymes found to be polymorphic in *P. marginatus* in the present study have been shown to be variable in other decapods. Because of their relevance to the genetic interpretations in the present investigation, published accounts of genetic variation for these enzymes are summarized below.

Esterases (EST). Nearly all species of decapod crustaceans studied to date exhibit multiple esterases which hydrolyze naphthol esters. In many species, one or more esterase loci are reported to be polymorphic and, in virtually all published studies where banding patterns of heterozygotes have been described, the variable esterases appear to be monomeric proteins (Menzies and Kerrigan 1979b). Analysis of the mode of inheritance of variable esterases has only been accomplished for one esterase locus in decapods (Hedgecock et al. 1975). This locus exhibited simple Mendelian segregation of alleles. The genetic interpretation of EST-3 variation in *P. marginatus* (single- and double-banded phenotypes) is consistent with these findings. Since there have been no published reports of UMB (= EST-D) variation in decapod crustaceans, the observed variation in UMB in *P. marginatus* must stand on its own. However, as shown in Figure 2, the staining intensities of the three isozymes in presumed heterozygotes are approximately those expected for a dimeric enzyme (i.e., 1:2:1). Furthermore, as noted above, no deviations from Hardy-Weinberg expectations were observed for this enzyme.

Glucosephosphate Isomerase (GPI). The enzyme glucosephosphate isomerase is frequently variable in decapod crustaceans. Although as many as three loci have been reported in decapods, most investigations have focused on a single locus whose apparently dimeric protein product is predominant in muscle extracts. The inheritance of this enzyme in *Homarus americanus* follows a simple Mendelian pattern (Hedgecock et al. 1975). It would appear that the polymorphic GPI in *P.*

marginatus is homologous to this GPI locus of other decapods.

Mannosephosphate Isomerase (MPI.) Mannosephosphate isomerase is polymorphic in many crustaceans and behaves as a monomeric protein. The observed MPI phenotypes in *P. marginatus* are consistent with this presumed subunit structure (Fig. 2). Further, a more detailed analysis of the MPI variation in *P. marginatus* indicates that the MPI locus in this species is sex-linked and that, although there are three alleles segregating in the species, males always have at least one slow allele while females only very rarely carry this same allele (Shaklee 1983). Because of this restriction on segregation, the slow allele was pooled with the medium (100) allele (Table 2) for the statistical analyses of population structure.

Peptidases (PEP). Like esterases, the peptidases represent a family of enzymes sharing similar catalytic activities. However, unlike the esterases, there are a relatively small number of different peptidases, and they exhibit differential and somewhat characteristic substrate specificities (Frick 1983). Peptidases have been studied in both *P. argus* and *P. cygnus*. Hanley (1980) reported that two peptidases (using leucylglycylglycine and leucyltyrosine as substrates) are monomorphic in *P. cygnus*. Menzies and Kerrigan (1979a) and Menzies (1981) reported two monomorphic and three polymorphic peptidases in *P. argus*. One of these variable peptidases is a prolidase (stained with leucylproline or phenylalanylproline) which appears to be a dimer and exhibits three alleles. The other two variable peptidases (stained with leucylglycine or phenylalanyltyrosine) are apparently monomers. Whether or not the two variable peptidases in *P. marginatus* (which behave as monomers, Fig. 2) are homologous to any of those described in *P. argus* is not clear at this point.

Phosphoglucomutase (PGM). Essentially all species of decapod crustaceans exhibit one PGM in muscle. The enzyme is reported to be monomeric and it exhibits Mendelian inheritance in *H. americanus* (Hedgecock et al. 1975). The PGM of *P. marginatus* appears to be homologous to this PGM of other decapods.

Despite that direct inheritance testing of the presumed genetic variation observed in the Hawaiian *P. marginatus* was not attempted, the general agreement between the observed isozyme patterns and those reported for other decapod crustaceans, the data indicating the genetic basis for variation in EST, GPI, MPI, and PGM in other decapods, and the general agreement in *P. marginatus* between observed genotypic distributions and those expected assuming Hardy-Weinberg equilibrium, strongly support the assumption that the observed variation in the Hawaiian spiny lobster is under direct genetic control.

The present study of *P. marginatus* has revealed electrophoretic variation encoded by 7 gene loci. However, in spite of the fact that over 1,800 lobsters from five localities were analyzed, none of the statistical tests of stock structure provided convincing evidence of subpopulation differentiation within the Hawaiian Archipelago. Indeed, the overall impression is one of remarkable genetic uniformity throughout the range of this species. This outcome is perhaps not too surprising, since levels of detectable genetic variation are quite low in *P. marginatus*: hence, a robust test of stock structure using electrophoresis is difficult at best and requires very large sample sizes. Additionally, assuming that this species has larval life history characteristics similar to those of other species in the family, namely a series of planktonic phyllosoma stages lasting from 6 to 12 mo, dispersal and mixing of larvae throughout the Hawaiian Archipelago might be expected. However, this same current-driven transport and dispersal might also be expected to disperse larvae to other Pacific island groups near Hawaii (e.g., Johnston Atoll, the Line Islands, the Marquesas, the Tuamotus, the Gilbert Islands, the Marshall Islands, etc.). It is, therefore, somewhat of a paradox that, with the exception of one report of a single individual that was collected at Johnston Atoll (Brock 1973), *P. marginatus* is only found in the Hawaiian Islands.

The lack of demonstrable subpopulation differentiation in *P. marginatus* is not unusual for a decapod crustacean. Studies of other decapods have also generally failed to reveal stock heterogeneity (Lester 1979; Smith et al. 1980). Even in those cases where some genetic differences among stocks have been reported, the amount of differentiation is almost always very small (Tracey et al. 1975; Hanley 1980; Mulley and Latter 1981a, b). The only notable exception is the case of *P. argus* which exhibits substantial variation throughout the Caribbean (Menzies and Kerrigan 1979a; Menzies 1981). Hence, the Hawaiian spiny lobster fits the common decapod pattern of low heterozygosity and little subpopulation differentiation. At this point, all indications suggest

that the *P. marginatus* fishery in the Hawaiian Islands should be managed on a unit stock basis.

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LITERATURE CITED

BOYER, S. H., D. C. FAINER, AND E. J. WATSON-WILLIAMS.
1963. Lactate dehydrogenase variant from human blood: evidence for molecular subunits. Science (Wash., D.C.) 141:642-643.

BROCK, R. E.
1973. A new distributional record for *Panulirus marginatus* (Quoy and Gaimard, 1825). Crustaceana 25:111-112.

COMMISSION ON BIOCHEMICAL NOMENCLATURE.
1973. Enzyme nomenclature. American Elsevier Publ. Co., Inc., N.Y., 443 p.

FRICK, L.
1983. An electrophoretic investigation of the cytosolic di- and tripeptidases of fish: molecular weights, substrate specificities, and tissue and phylogenetic distributions. Biochem. Genet. 21:309-322.

GRIGG, R. W., AND R. T. PFUND (editors).
1980. Proceedings of the symposium on the status of resource investigations in the Northwestern Hawaiian Islands. Sea Grant Misc. Rep., UNIHI-SEAGRANT-MR-80-04, 333 p.

HANLEY, J. R.
1980. An investigation of the genetic relationships among stocks of the western rock lobster (*Panulirus cygnus* George 1962). Unpubl. Honours Thesis, Univ. Western Australia, Nedlands, W.A., 27 p.

HEDGECOCK, D., K. NELSON, R. A. SHLESER, AND M. L. TRACEY.
1975. Biochemical genetics of lobsters (*Homarus*). II. Inheritance of allozymes in *H. americanus*. J. Hered. 66:114-118.

HONDA, V. A.
1980. Preliminary results of studies on fecundity of the spiny lobster, *Panulirus marginatus*, in the Northwestern Hawaiian Islands. *In* R. W. Grigg and R. T Pfund (editors), Proceedings of the symposium on status of resource investigations in the Northwestern Hawaiian Islands, p. 143-148. Sea Grant Misc. Rep., UNIHI-SEAGRANT-MR-80-04.

INOUE, M.
1978. Studies on the cultured phyllosoma larvae of the Japanese spiny lobster, *Panulirus japonicus*—I: Morphology of the phyllosoma. [In Jpn., Engl. abstr.] Bull. Jpn. Soc. Sci. Fish. 44:457-475.

JOHNSON, M. W.
1956. The larval development of the California spiny lobster, *Panulirus interruptus* (Randall), with notes on *Panulirus gracilis* Streets. Proc. Calif. Acad. Sci. 29: 1-19.
1968. Palinurid phyllosoma larvae from the Hawaiian archipelago (Palinuridea). Crustaceana, Suppl. 2:59-79.

JOHNSON, M. W., AND M. KNIGHT.
1966. The phyllosoma larvae of the spiny lobster *Panulirus inflatus* (Bouvier). Crustaceana 10:31-47.

LESTER, L. J.
1979. Population genetics of penaeid shrimps from the Gulf of Mexico. J. Hered. 70:175-180.

LEVENE, H.
1949. On a matching problem arising in genetics. Ann. Math. Stat. 20:91-94.

MACLEAN, J. A., AND D. O. EVANS.
1981. The stock concept, discreteness of fish stocks, and fisheries management. Can. J. Fish. Aquat. Sci. 38:1889-1898.

MENZIES, R. A.
1981. Biochemical population genetics and the spiny lobster larval recruitment problem: an update. Proc. Gulf Caribb. Fish. Inst. 33:230-243.

MENZIES, R. A., AND J. M. KERRIGAN.
1979a. Implications of spiny lobster recruitment patterns of the Caribbean—A biochemical genetic approach. Proc. Gulf Caribb. Fish. Inst. 31:164-178.
1979b. Genetics of several esterase loci in the Caribbean spiny lobster (*Panulirus argus*). Isozyme Bull. 12:59.

MORRIS, D. E.
1968. Some aspects of the commercial fishery and biology of two species of spiny lobsters, *Panulirus japonicus* (De Siebold) and *Panulirus penicillatus* (Oliver) in Hawaii. M.S. Thesis, Univ. Hawaii, Honolulu, 82 p.

MULLEY, J. C., AND B. D. H. LATTER.
1980. Genetic variation and evolutionary relationships within a group of thirteen species of penaeid prawns. Evolution 34:904-916.
1981a. Geographical differentiation of eastern Australian penaeid prawn populations. Aust. J. Mar. Freshw. Res. 32:889-895.
1981b. Geographic differentiation of tropical Australian penaeid prawn populations. Aust. J. Mar. Freshw. Res. 32:897-906.

NELSON, K., AND D. HEDGECOCK.
1980. Enzyme polymorphism and adaptive strategy in the decapod crustacea. Am. Nat. 116:238-280.

REDFIELD, J. A., D. HEDGECOCK, K. NELSON, AND J. P. SALINI.
1980. Low heterozygosity in tropical marine crustaceans of Australia and the trophic stability hypothesis. Mar. Biol. Lett. 1:303-313.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON, AND J. B. GENTRY.
1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old field mouse (*Peromyscus polionotus*). Stud. Genet. IV, Univ. Texas Publ. 7103, p. 49-90.

SHAKLEE, J. B.
1983. Mannosephosphate isomerase in the Hawaiian spiny lobster *Panulirus marginatus*: a polymorphic, sex-linked

locus useful in investigating embryonic and larval sex ratios. Mar. Biol. (Berl.) 73:193-201.

SHAKLEE, J. B., K. L. KEPES, AND G. S. WHITT.
1973. Specialized lactate dehydrogenase isozymes: the molecular and genetic basis for the unique eye and liver LDHs of teleost fishes. J. Exp. Zool. 185:217-240.

SHAKLEE, J. B., AND P. B. SAMOLLOW.
1980. Genetic aspects of population structure of four species in the Northwestern Hawaiian Islands. *In* R. W. Grigg and R. T. Pfund (editors), Proceedings of the symposium on status of resource investigations in the Northwestern Hawaiian Islands, p. 264-277. Sea Grant Misc. Rep., UNIHI-SEAGRANT-MR-80-04.

SHAW, C. R., AND R. PRASAD.
1970. Starch gel electrophoresis of enzymes—A compilation of recipes. Biochem. Genet. 4:297-320.

SICILIANO, M. J., AND C. R. SHAW.
1976. Separation and visualization of enzymes on gels. *In* I. Smith (editor), Chromatographic and electrophoretic techniques. Vol. 2 Zone electrophoresis, p. 185-209. Halsted Press, N.Y.

SMITH, P. J., J. L. MCKOY, AND P. J. MACHIN.
1980. Genetic variation in the rock lobsters *Jasus edwardsii* and *Jasus novaehollandiae*. N.Z. J. Mar. Freshw. Res. 14:55-63.

TRACEY, M. L., K. NELSON, D. HEDGECOCK, R. A. SCHLESER, AND M. L. PRESSICK.
1975. Biochemical genetics of lobsters: genetic variation and the structure of American lobster (*Homarus americanus*) populations. J. Fish. Res. Board Can. 32:2091-2101.

UCHIDA, R. N., J. H. UCHIYAMA, R. L. HUMPHEREYS, JR., AND D. T. TAGAMI.
1980. Biology, distribution and estimates of apparent abundance of the spiny lobster, *Panulirus marginatus* (Quoy and Gaimard), in waters of the Northwestern Hawaiian Islands: Part 1. — Distribution in relation to depth and geographical areas and estimates of apparent abundance. *In* R. W. Grigg and R. T. Pfund (editors), Proceedings of the symposium on status of resource investigations in the Northwestern Hawaiian Islands, p. 121-130. Sea Grant Misc. Rep., UNIHI-SEAGRANT-MR-80-04.

WRIGHT, S.
1943. Isolation by distance. Genetics 28:114-138.