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POPULATION GENETICS OF THE SAND CRAB, *EMERITA ANALOGA* Stimpson, IN SOUTHERN CALIFORNIA

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Abstract: Genetic variation at 25 enzymatic loci was studied by means of starch and acrylamide gel electrophoresis in six samples of the sand crab, *Emerita analoga* Stimpson, from southern California. Average heterozygosity was 0.07 ± 0.03 (SE) and five loci were polymorphic. Allele frequencies at a malate dehydrogenase locus were significantly different among samples. Genotype frequencies at an esterase locus and a phosphoglucosylase locus showed significant deviations from Hardy-Weinberg equilibrium in some samples. The activity of phosphoglucosylase was reduced in samples taken from the vicinity of the San Onofre Nuclear Generating Station (SONGS) and from Cabrillo Beach near Los Angeles Harbor. It has not been determined whether this is due to a decrease in the amount of the enzyme present, an inhibition of its activity or some other cause.

Key words: population genetics; sand crab; *Emerita analoga*; electrophoresis

INTRODUCTION

This study reports on the population genetics of the sand crab, *Emerita analoga* Stimpson, in southern California. Siegel & Wenner (1984) presented evidence that sand crab populations in the vicinity of the San Onofre Nuclear Generating Station (SONGS) had a high proportion of females carrying egg masses with empty egg shells. They suggested that some natural or man-made factor, centered near SONGS, was responsible for the abnormal reproduction of sand crabs. The present study examined genetic evidence for population differentiation and differential survival of genotypes, and was part of a larger study of sand crab interactions with SONGS.

The sand crab, one of the most abundant members of the intertidal sandy beach fauna of California, is widely distributed along the Pacific coast from Alaska to Chile. Larvae spend two to four or more months in the plankton before settling in the winter and spring (Barnes & Wenner, 1968; Efford, 1970). Adults are found in patches that move up and down the beach along with the tides (Efford, 1965) and may move along with sediment drift (Dillery & Knapp, 1970). The above evidence would suggest little population differentiation. Post-settlement selection and differentiation, however, have been documented in several intertidal organisms (Koehn *et al.*, 1973, 1976). The presumed stress on the populations in the vicinity of SONGS might then be detected as an alteration

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of typical allele or genotype frequencies at one or more enzymatic loci. Samples were taken at sites near SONGS, shown by Siegel & Wenner (1984) to be under stress, and at other sites in southern California thought to be more normal. One sample was taken north of the major faunal break at Point Conception, but no attempt was made to survey differentiation over the entire range of the species.

MATERIAL AND METHODS

Sand crabs were collected by personnel of Marine Ecological Consultants (MEC), Encinitas, California between 2 and 6 August, 1983 (Fig. 1). All animals came from a

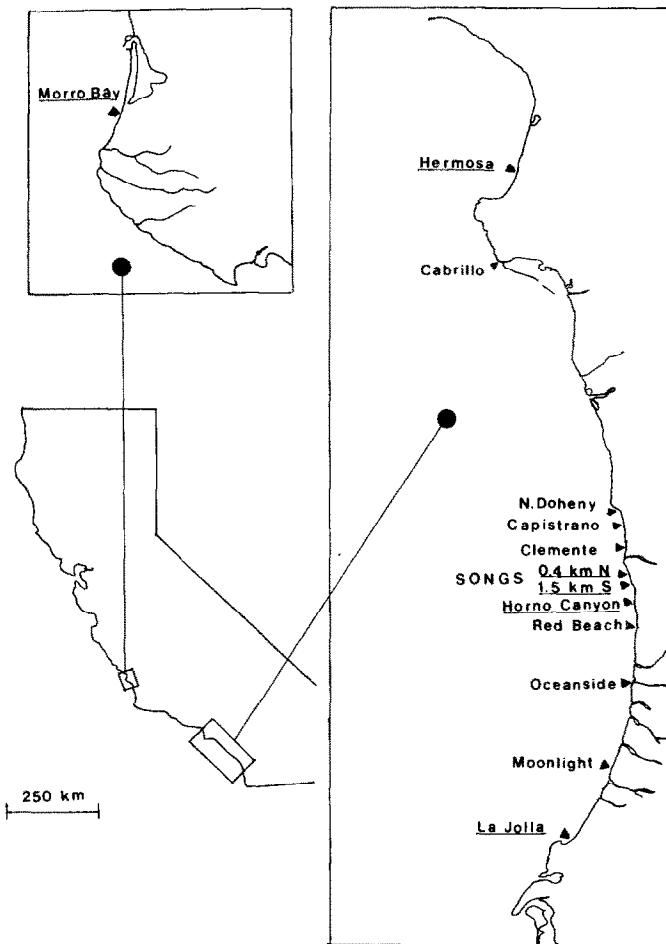


Fig. 1. Location of all sample sites for *Emerita analoga* in southern California: the underlined names designate those samples that were completely analyzed for genetic variation; other locations were only sampled for activity at the PGM locus.

single patch, where possible or from adjacent patches. Animals were washed free of sand while still alive, then frozen on dry ice. Samples were kept as whole animals at -20°C until used. Larger animals (> 7.5 mm carapace length) were dissected, the gut removed, and the body musculature and viscera homogenized separately. Smaller animals were homogenized in one piece, after the gut and carapace were removed.

Procedures for starch gel electrophoresis followed Nelson & Hedgecock (1980) with the following exceptions. All stains that used glucose-6-phosphate dehydrogenase were modified according to Buth & Murphy (1980). Glucosephosphate isomerase was assayed by the method of Corbin (1977). Malate dehydrogenase, mannosephosphate isomerase and phosphoglucomutase were assayed by the method of Selander *et al.* (1971). Starch gels were 10% (w/v) Sigma starch (Lot No. 23F-0712). Esterase, glucose-6-phosphate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase, as well as general proteins were more easily resolved after electrophoresis on 6% (w/v) polyacrylamide gels. Esterase was assayed using the eserine method of Selander *et al.* (1971) with alpha-naphthylbutyrate and beta-naphthylacetate as substrates. Table I gives

TABLE I

Electrophoretic buffers, tissue source and number of loci scored, for *Emerita analoga*: buffer systems: POUL = discontinuous borate-tris-citrate, "Poulik" (Selander *et al.*, 1971); TC7 = Tris-citrate-EDTA, pH 7.0 (Ayala *et al.*, 1973); TBE 9.2 = Tris-borate-EDTA, pH 9.2 (Ayala *et al.*, 1973); TBE 8.2 = Tris-borate-EDTA, pH 8.2 (Maniatis *et al.*, 1982); enzymes marked with an asterisk were run on polyacrylamide gels; tissue: M = muscle, V = viscera (with intestine removed).

Enzyme	Abbreviation	E.C. no.	Buffer	Tissue	No. of Loci
Acid phosphatase	ACP	3.1.3.2	TC7	M or V	2
Esterase*	EST	-	TBE 8.2	V	3
General proteins*	GP	-	TBE 8.2	M and V	6
Glucosephosphate isomerase	GPI	5.3.1.9	POUL	M	1
Glucose-6-phosphate dehydrogenase*	G-6-PDH	1.1.1.49	TBE 8.2	V	1
Glutamate dehydrogenase	GDH	1.4.1.2	TBE 9.2	M or V	1
Glyceraldehyde-3-phosphate dehydrogenase*	GAPDH	1.2.1.12	TBE 8.2	M or V	1
Lactate dehydrogenase	LDH	1.1.1.27	POUL	M	1
Leucine aminopeptidase	LAP	3.4.1.1	POUL	V	1
Malate dehydrogenase	MDH	1.1.1.37	TC7	M	2
Mannosephosphate isomerase	MPI	5.3.1.8	TBE 9.2	M or V	1
Phosphoglucomutase	PGM	2.7.5.1	POUL	M	2
Superoxide dismutase	SOD	1.15.1.1	TBE 9.2	V	2
Tetrazolium reductase	TR	-	TBE 9.2	V	1

the electrophoretic buffer and tissue used for each enzyme as well as the number of loci resolved. Alleles were named in terms of their mobility relative to the tracking dye xylene cyanol. Previously scored individuals were re-run on all gels to insure proper allelic identification. In the absence of breeding data, all genotypes were inferred from the electrophoretic pattern, compared with the subunit structure of the enzyme, if known.

Not all individuals were analyzed for variation at all loci. An initial survey determined that five loci were sufficiently polymorphic to be informative in an analysis of population differentiation (*Est-3*, *Gpi*, *Mdh-2*, *Mpi*, and *Pgm-2*). These loci were analyzed in six samples: Morro Bay ($N = 50$), Hermosa Beach ($N = 40$), SONGS 0.4 km north ($N = 60$), SONGS 1.5 km south ($N = 33$), Horno Canyon ($N = 47$), and La Jolla ($N = 40$). Most of the data analysis was performed using the BIOSYS-1 package of computer programs (Swofford & Selander, 1981).

RESULTS

Genetic variation was measured at 25 presumed genetic loci. Certain enzymes, as well as general proteins, exhibited multiple, invariant zones. These were scored as separate loci. Although it is possible that some of these zones were sub-bands produced by post-translational modification, the separation of the zones on the gel, the tissue distribution, and the patterns of staining intensity make this unlikely. The minimum number of loci would be 18. The mean heterozygosity per locus, calculated from Hardy-Weinberg expectation, was 0.07 ± 0.03 (SE, based on 25 loci), and five loci were polymorphic (frequency of the most common allele < 0.95). Allele frequencies at the polymorphic loci are given in Table II.

There was no obvious pattern of population subdivision in southern California. Even the sample from Morro Bay, taken from north of Point Conception, was not unique. Only a slight indication of genetic differentiation existed among the six samples. A G -test for independence of allele frequencies (Sokal & Rohlf, 1969) yielded significant heterogeneity ($P < 0.05$), due entirely to differentiation at the *Mdh-2* locus (Table III). When all pairs of samples were compared for allele frequency differences at the *Mdh-2* locus, two comparisons were significant: SONGS 0.4 km north vs Hermosa ($G = 7.74$, 1 d.f., $P < 0.01$) and SONGS 0.4 km north vs Horno Canyon ($G = 11.41$, 1 d.f., $P < 0.005$).

Genotype frequencies in each sample were tested for deviations from Hardy-Weinberg expectation using an exact probability test. Rare alleles were pooled into one synthetic allele, and expected frequencies were corrected for small sample size (Levene, 1949). The following samples had genotype frequencies significantly different from Hardy-Weinberg equilibrium: Horno Canyon, heterozygote excess at the *Pgm-2* locus ($P = 0.007$), heterozygote deficit at the *Est-3* locus ($P = 0.03$). La Jolla, heterozygote deficit at *Est-3* ($P = 0.001$); and Hermosa Beach, heterozygote deficit at *Est-3* ($P = 0.001$).

Analysis of *Pgm-2* and *Gpi* variability in some samples was complicated by an unexpected difficulty. In samples from SONGS 0.4 km north and 1.5 km south, *Pgm-2* and *Gpi* genotypes could not be resolved in most individuals. Numerous experiments, running and re-running individuals from several samples on the same gels, gave repeatable results. The *Pgm-2* or *Gpi* alleles (when they could be seen) were identical in electrophoretic mobility to those of other samples: either the amount of the enzyme

TABLE II

Allele frequencies for *Emerita analoga*: MB = Morro Bay; HB = Hermosa Beach; SN = SONGS 0.4 km north; SS = SONGS 1.5 km south; HC = Horno Canyon; LJ = La Jolla.

Locus	Population					
	MB	HB	SN	SS	HC	LJ
<i>Est-3</i>						
<i>N</i>	43	37	59	33	43	39
141	0.035	0.095	0.034	0.030	0.023	0.051
149	0.721	0.595	0.627	0.727	0.535	0.590
156	0.221	0.311	0.339	0.242	0.430	0.346
167	0.023	0.000	0.000	0.000	0.012	0.013
<i>Gpi</i>						
<i>N</i>	38	38	1	4	44	37
15	0.816	0.855	1.000	1.000	0.886	0.905
37	0.158	0.145	0.000	0.000	0.114	0.095
52	0.026	0.000	0.000	0.000	0.000	0.000
<i>Mdh-2</i>						
<i>N</i>	37	37	48	32	46	37
11	0.257	0.392	0.198	0.281	0.424	0.297
18	0.743	0.608	0.802	0.719	0.576	0.703
<i>Mpi</i>						
<i>N</i>	50	40	48	33	47	39
84	0.020	0.025	0.000	0.000	0.032	0.013
95	0.950	0.925	0.927	0.970	0.926	0.987
104	0.030	0.050	0.073	0.030	0.043	0.000
<i>Pgm-2</i>						
<i>N</i>	39	39	5	15	45	37
99	0.000	0.000	0.000	0.000	0.056	0.041
110	0.333	0.397	0.438	0.433	0.411	0.338
119	0.628	0.590	0.563	0.567	0.533	0.622
127	0.038	0.013	0.000	0.000	0.000	0.000

TABLE III

G-test for independence of allele frequencies among samples of *Emerita analoga*: rare alleles pooled;
* = $P < 0.05$; ** = $P < 0.025$.

Locus	d.f.	G
<i>Est-3</i>	5	10.16
<i>Gpi</i>	5	5.54
<i>Mdh-2</i>	5	14.95**
<i>Mpi</i>	5	6.49
<i>Pgm-2</i>	5	1.65
Totals	25	38.79*

or its activity was altered. The possibility of a silent allele present at high frequency was discounted because all genotype classes stained with varying intensity. Modifying the conditions of homogenization, extraction, electrophoresis or enzyme staining did not alter the observations. GPI activity, however, declined in all samples when whole animals were held at -20°C for >90 days. Small samples from seven other locations were analyzed only for activity at the *Pgm-2* locus: Cabrillo ($N = 12$), N. Doheny ($N = 17$), Capistrano ($N = 12$), Clemente ($N = 12$), Red Beach ($N = 12$), Oceanside ($N = 8$), Moonlight ($N = 8$). Of these, *Pgm-2* activity was judged to be low (scorable in less than half of the individuals) in three: Cabrillo, N. Doheny and Red Beach.

DISCUSSION

The lack of obvious genetic differentiation among samples of *E. analoga* is perhaps to be expected, given the dispersal abilities of the organisms and the restricted geographic range studied. The Morro Bay site is, however, usually considered to be in a different biogeographic province (Briggs, 1974), yet it fell within the range of values for the samples from southern California. The greatest observed differences in allele frequency were between samples separated by <7 km. This suggests that the differences are due to local selection. The alternative explanation of drift coupled with restricted gene flow, although it cannot be excluded, implies only local recruitment of larvae after 2 to 4 months in the plankton. The observed deviations from expected Hardy-Weinberg genotype frequencies could also be due to differential survival of genotypes; however, non-random mating or non-random dispersal of larvae cannot be excluded (Smith *et al.*, 1981). Samples from the vicinity of SONGS did not form a unique group. There was no evidence for differential mortality in these samples, either in terms of allele frequencies or genotype frequencies.

The reduction in PGM and GPI activity seen in some samples is puzzling, with a number of alternative explanations. The simplest is that the samples were mis-handled in some way: either allowed to thaw before being analyzed or improperly stored. The observed instability of GPI during storage allows this interpretation for that enzyme, but not for PGM, which was stable. Other possibilities include a reduced amount of the enzyme present or inactivation of the enzyme in some way. Inactivation could be due to an environmental pollutant forming a complex with the enzyme. Nevo *et al.* (1983) reviewed the effects of marine pollution on allozyme polymorphisms. They found selection for different PGM or GPI alleles of marine crustaceans and snails after heavy metal pollution. Pontecorvo *et al.* (1983) found that heavy metal concentration influenced the activity of some PGM allozymes in the shrimp *Palaemon elegans*. Nevo *et al.* (1978) presented evidence for selection acting on allozymes after petrochemical pollution.

Further speculation as to the cause of reduced enzyme activity is premature. The only sample away from the vicinity of SONGS that had reduced activity was from Cabrillo

Beach, near the Los Angeles Harbor. Since this is an area of heavy industrial impact, pollution in some form is a possible environmental factor. Siegel & Wenner (1984) discussed the possible sources of pollution in the vicinity of SONGS. They suggested "(1) run off of agricultural pesticides; (2) release of metals from corroding cooling pipes, and/or (3) increased turbidity of the nearshore waters."

Barnett & Green (unpubl. technical report to the Marine Review Committee of the California Coastal Commission) studied sand crab biology in relation to SONGS during the 1983 season. They came to the following conclusions. (1) There was a decrease in abundance of sand crabs within 12 km of SONGS during June; this was attributed to late recruitment. (2) There was a marked decrease in the percentage of reproductive females near SONGS in August, the peak reproductive period elsewhere in southern California; this was attributed to an early cessation of breeding. (3) There was an increase in the percentage of aborted embryos within developing egg masses of sand crabs near SONGS; this was also seen, to a lesser degree, at many sites north of SONGS. Using multivariate techniques, they concluded that most of the biological variations were due to differences in sediment type and food availability among sites. They also noted that Cabrillo Beach was poor for sand crabs and seemed to be polluted.

The observed differences in reproductive output of sand crabs from different beaches in southern California are not reflected in genetic differentiation at any of the allozyme loci investigated. Reduced levels of PGM activity may be associated with lowered reproductive output but the causal connection, if any, remains obscure.

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