Genetic Differentiation of the Semi-terrestrial Amphipod *Orchestia traskiana* in an Expanded Habitat on Santa Cruz Island

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INTRODUCTION

One of the frequently observed characteristics of species on islands is expansion of the range of habitat compared with that of their mainland counterparts. This has most often been attributed to release from biological pressures due to the absence of mainland competitors or predators in the generally depauperate island biota (MacArthur 1972).

Habitat expansion, however facilitated, is presumably the critical precursor of adaptive radiation, a phenomenon commonly seen in island situations (Carlquist 1974). The underlying hypothesis supposes that species arriving on islands move into empty niches (ones they did not occupy on the mainland) and, by natural selection, may become sufficiently adapted to their new habitats to form new species. This end result would be preceded by a stage at which populations in divergent habitats would have accumulated enough genetic differences to distinguish them as ecological races or "ecotypes," but not enough for the establishment of reproductive isolation.

Orchestia traskiana Stimpson, a semi-terrestrial talitrid amphipod, is found on both the Pacific mainland coast and the California Channel Islands. Both the diversity and the number of occupied habitats are much greater on Santa Cruz Island than on the adjacent mainland coast in Santa Barbara County. Populations of *O. traskiana* on the island were found in environments ranging from marine cobble beaches to freshwater stream banks several miles inland and as much as 500 feet above sea level. Furthermore, nearly every island cobble beach, brackish pond, freshwater stream, or cliff-bottom seep that I examined had a population of these animals.

These observations led to the question: How is this species able to exist in such a wide range of habitats; are the animals incredibly ecologically plastic, or are populations in fact genetically adapted to their specific habitats?

Starch gel electrophoresis was the technique chosen to investigate possible genetic variation in *O. traskiana*. Several electrophoretic studies have shown correlations between allele frequency patterns and various ecological variables (Johnson *et al.* 1969, Tomaszewski *et al.* 1973, Rockwood-Sluss *et al.* 1973, McKechnie *et al.* 1975), but only a few have explored genetic variation between populations in specific microhabitats (Tsuno 1975, Saul *et al.* 1978).

The distribution of *O. traskiana* on Santa Cruz Island thus provided a unique opportunity to study both the genetic variation of a species which had apparently undergone habitat expansion and, possibly, adaptive radiation on an island, and the nature and intensity of genotype-microhabitat variation within a species.

MATERIALS AND METHODS

Six populations of *O. traskiana* were chosen, five of them representing three habitats from Santa Cruz Island, and one from the mainland. The locations of the island sample sites are indicated in Figure 1. These include: two cobble beach sites, Prisoners Harbor Cobbles (PHC)

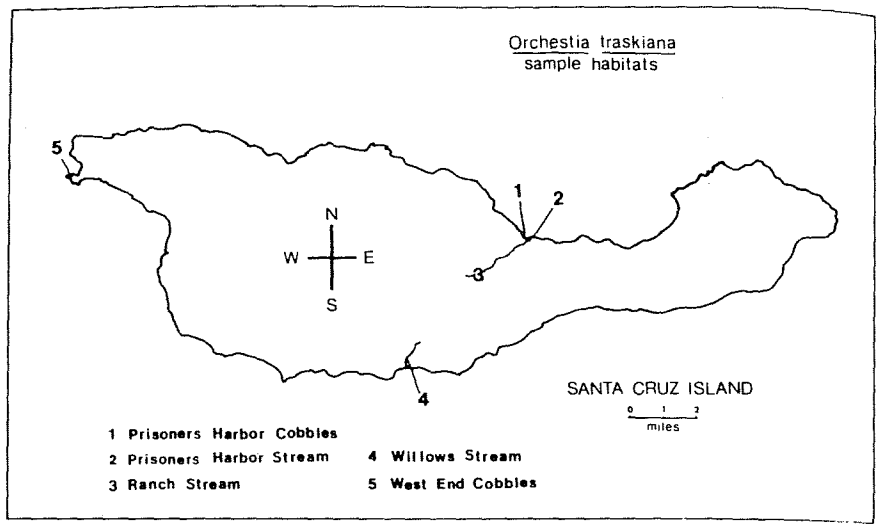

FIGURE 1. *Five sampling sites representing three divergent habitat types for* Orchestia traskiana *on Santa Cruz Island. Sites 1 and 5 are cobble beach habitats, 2 and 4 are variable brackish pond habitats, and 3 is a freshwater habitat.*

and West End Cobbles (WE); two variable brackish ponds fed by freshwater streams, Prisoners Harbor Stream (PHS) and Willows Stream (W); and a freshwater stream, Ranch Stream (RS). The mainland site was located along the border of the Lagoon Pond of the University of California, Santa Barbara campus, a habitat similar to the PHS island site.

The island sites at PHC, PHS, and RS were chosen, first, because they represented three strikingly different but representative habitat types, and, second, because they were all found along the same drainage. Gene exchange between populations was at least potentially possible. The populations at PHC and PHS are separated by a sand dune only a few meters wide; certainly these populations could exchange individuals. The remaining island sites, W and WE, are similar to PHS and PHC, respectively, but are completely isolated from any other population of *O. traskiana*. Thus, the pattern of genetic variation in isolated habitats could be compared with that found in non-isolated populations from similar microhabitats, and, if the pattern were the same for both, the hypothesis that the animals were being selected on the basis of suitability for survival in a particular habitat would seem rather well supported.

The mainland Lagoon Pond (L) habitat, though not totally analogous to any of the habitats on the island, was chosen to provide information concerning the magnitude of habitat differences in relation to island-mainland differences.

These six populations were surveyed using the technique of starch gel electrophoresis as described in Ayala *et al.* (1972). Two buffer systems and two enzymes were used, as described below.

Buffer systems:

a) Poulik bridge and gel buffers (system A in Ayala *et al.* 1972).

b) Borate bridge and gel buffers as follows:
Electrode buffer: 60.0 g/l boric acid, 60.6 g/l Tris HCl, and 6.0 g/l EDTA;
Gel buffer: 100 ml electrode buffer, 900 ml distilled water.

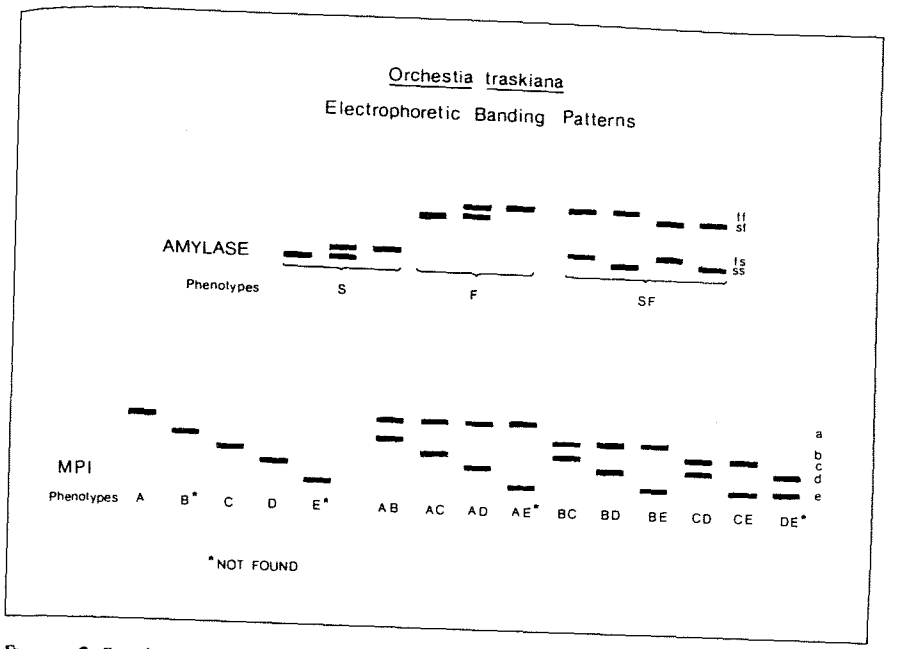

FIGURE 2. *Banding pattern of electromorphs for the enzymes amylase and mannose phosphate isomerase found in* Orchestia traskiana *using starch gel electrophoresis.*

Enzyme assays:

Amylase.—This enzyme required no stain since it digested holes in the starch gel. The reaction was allowed to develop in 50 ml of 0.05 M sodium acetate, pH 4.8, for 30 to 45 minutes. Gels were run on borate buffer.

Mannose phosphate isomerase (MPI).—The stain contained 30 mg mannose-6-phosphate, 12.5 mg NADP, 10 mg MTT, 2.5 mg PMS, 15 units phosphoglucose isomerase, and 20 units glucose-6-phosphate dehydrogenase dissolved in 50 ml 0.05 M Tris HCl pH 8.0. The reaction was incubated at 37°C in the dark. Gels were run on Poulik buffer.

RESULTS

Amylase.—This enzyme showed two banding areas approximately four millimeters apart. If run on a regular thick gel, the enzyme appeared to have only two bands, a fast one and a slow one. If run on a gel one-half the thickness of the regular gel, it became evident that each of these bands was, in turn, composed of one or two bands, a fast-fast and slow-fast, and a fast-slow and slow-slow (Fig. 2). No animal ever had more than two bands, and the pattern suggested a two-allele, single-locus system. Gel results were not consistent enough so that these four bands could always be distinguished. Therefore, animals were scored according to fast or slow *categories*. Sample location, sample size, allele frequencies, and significance of the chi-square test for goodness-of-fit to the Hardy-Weinberg distribution are listed in Table 1. Only one sample showed a significant departure from Hardy-Weinberg equilibrium, which I attribute to sampling error.

It is immediately obvious that there are differences in gene frequencies between these

TABLE 1. Allele frequencies for two enzymes found in *Orchestia traskiana*.

		Amylase		
Sample site	*n*	Slow	Fast	χ^2
L	78	0.654	0.346	NS
RS	78	0.994	0.006	NS
PHS	72	0.910	0.090	*
W	84	0.821	0.179	NS
PHC	79	0.044	0.956	NS
WE	78	-0-	1.00	NS

			MPI			
n	A	B	C	D	E	χ^2
82	0.079	0.049	0.860	0.006	0.006	NS
84	-0-	0.012	0.982	-0-	0.006	NS
67	-0-	0.007	0.963	0.015	0.015	NS
84	-0-	-0-	1.00	-0-	-0-	NS
62	0.226	0.032	0.621	0.121	-0-	NS
70	0.307	0.014	0.564	0.115	-0-	NS

*$0.01 < P < 0.05$.
NS = not significant.

populations. It is also obvious that some correlation with habitat type exists. The two beach populations both have high frequencies of the fast allele category and very low frequencies of the slow category. In fact, the fast "allele" appears to be fixed in the WE population. The nonbeach populations, including the mainland sample, show high frequencies of the slow "allele," which is nearly fixed in the RS population. In populations from brackish water, a larger proportion of heterozygotes and homozygotes for the fast allele was found. The mainland population, which lives in the most variable and most saline of the brackish habitats, shows the highest incidence of the fast allele.

MPI.—Five alleles were found at this locus (Fig. 2). No animal had more than two alleles, which indicated that this, too, was a single-locus system. Sample size, gene frequencies, and significance of the chi-square test for Hardy-Weinberg equilibrium are given in Table 1.

As shown for amylase, there is a distinction between habitat types for MPI. Allele C was always the most common allele, but was present in a much larger proportion in the stream and brackish pond populations. It is apparently fixed in the isolated population at Willows. Allele A, which was not found in any island fresh or brackish water population and found only in very low frequencies for the mainland population, was present in higher frequencies for both the cobble beach populations. Alleles B and D were present in very low frequencies in all populations, but allele E is apparently absent from both cobble beach populations (and from the Willows population, which is fixed for C).

Figure 3 shows bar graphs with 95 per cent confidence intervals for allelic distributions at both the amylase and MPI loci. There is a clear congruence in the pattern of differences between these populations for both enzymes. Though the enzymes are involved in unrelated metabolic functions—amylase is a digestive enzyme and MPI is involved in glucose metabolism (Christ-

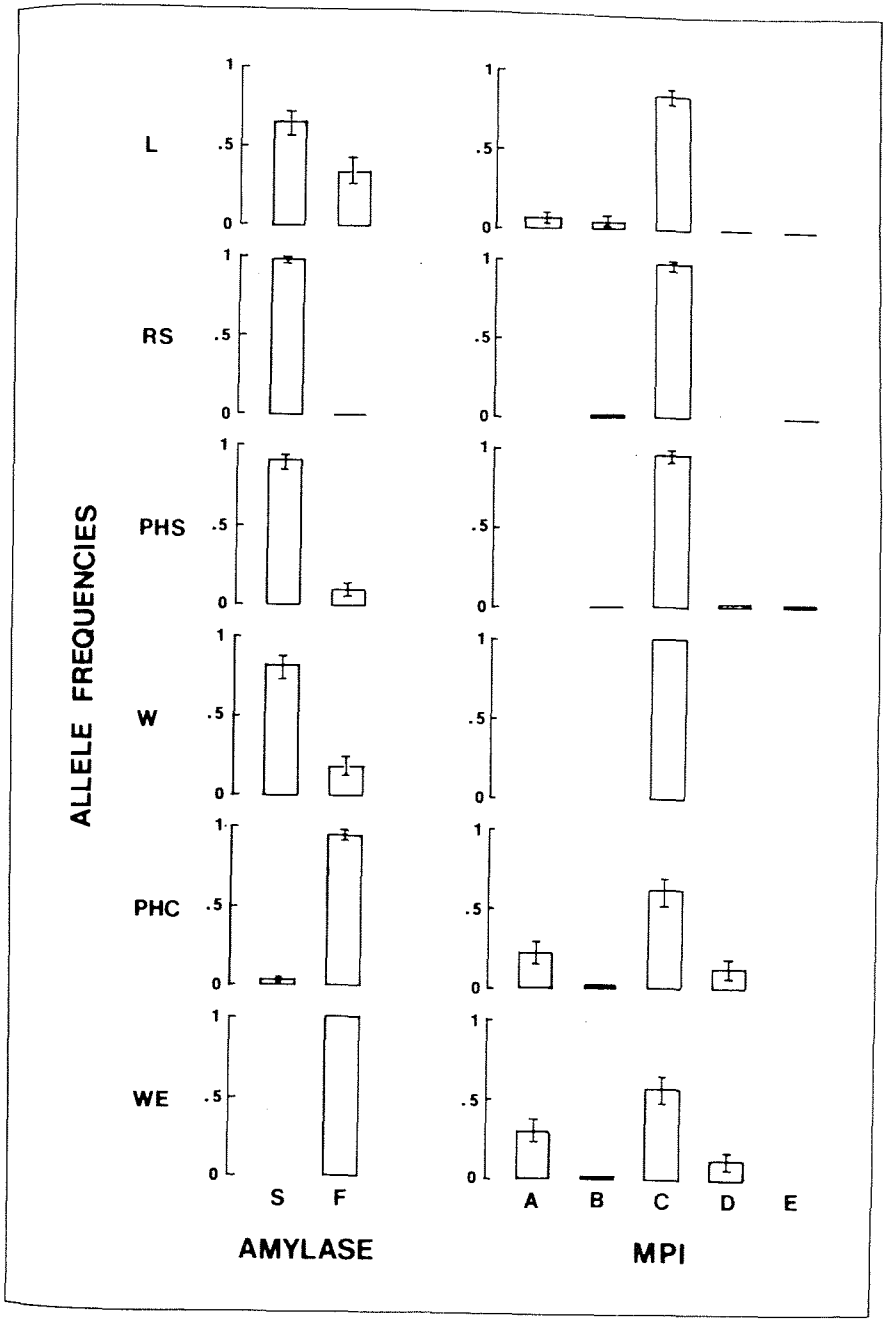

FIGURE 3. *Bar graphs with 95 per cent confidence intervals for allele frequencies at the amylase and MPI loci found in populations of* Orchestia traskiana *from different habitats.*

ensen *et al.* 1974, Nichols *et al.* 1973)—they show the same pattern of habitat differences.

It seems clear that the ecological diversity of *O. traskiana* is due in great part to genetic differences and that these genetic distinctions reflect a selective response to at least the large divergence between beach and nonbeach habitats.

DISCUSSION

The results indicate that populations of *O. traskiana* on Santa Cruz Island can be characterized as either beach or nonbeach "races" which are readily distinguishable electrophoretically. In allele frequencies, the freshwater population is essentially identical to brackish island populations. The mainland population shows a larger proportion of both the fast amylase alleles and allele A at the MPI locus. Whether this is due to geographic isolation between the island and mainland or to the nature of the habitat is not evident. However, the same loci and, except for total absences, the same alleles are present in all populations, which is consistent with the interpretation that they represent a single species.

Hurley (1959) reported that, in a multitude of instances, supralittoral species of *Orchestia* in the Indo-Pacific have given rise to terrestrial endemics on isolated islands. There are approximately 50 recorded terrestrial (by which he means capable of completing the life cycle away from the ocean or other body of water) members of the family Talitridae; almost all of these have very localized distributions. In this region, the forest leaf mold layer descends to the supralittoral zone, so that the transition from beach to land is relatively easy. Terrestrial talitrids apparently have not invaded via fresh water (Hurley 1968).

O. traskiana on Santa Cruz Island showed clear genetic differentiation only between beach and nonbeach (or "terrestrial," in Hurley's terminology) populations. The situation appears analogous to that found in Indo-Pacific island species of *Orchestia* except that: (1) the terrestrial invasion has been confined to the leaf mold margin of ponds and streams in the absence of the tropical forest; and (2) the ecotypes have not differentiated to the point of speciation. Other investigations of this species revealed a similar pattern of ecological variation and absence of reproductive isolating mechanisms, at least prior to maturity of the offspring (Busath 1979). It thus appears that *O. traskiana* may represent an example of incipient speciation enhanced by the unique properties of the island setting.

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