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REPEATED GEOGRAPHIC VARIATION AT THREE ENZYME LOCI IN THE AMPHIPOD *PLATORCHESTIA PLATENSIS*

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A correlation of allele frequency with an environmental factor is one of the most common forms of evidence for natural selection on allozyme loci. However, an apparent association of allele frequency with a single environmental gradient, such as a latitudinal cline correlated with temperature, may also result from random drift in isolated populations followed by migration. One way to test the drift hypothesis is to examine areas of repeated environmental variation for repeated patterns of genetic differentiation, since random processes would not produce a consistent association of a particular allele with a particular environment. Examples of such repeated patterns include six enzyme loci in *Drosophila melanogaster*, in which latitudinal clines of allele frequency are present on three continents (Oakeshott et al., 1981, 1982, 1983, 1984). At six loci in *Fundulus heteroclitus*, latitudinal clines are present in both Chesapeake Bay and on the Atlantic coast (Powers et al., 1986). In *Mytilus edulis*, clines at the *Lap* locus are present in Long Island Sound (Koehn et al., 1976) and two smaller estuaries (Boyer, 1974). Here I report repeated differentiation between different habitats in the amphipod *Platorchestia platensis* at loci coding for three polymorphic enzymes: alanine ami-

notransferase (ALAT, E.C. 2.6.1.2), glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), and mannose-6-phosphate isomerase (MPI, E.C. 5.3.1.8).

Horizontal starch gels containing 10.5% starch were used for electrophoresis. The pH 8.6 Tris-borate-EDTA buffer system of Nichols and Ruddle (1973) was used for all three enzymes. The stains used are those of Shaw and Prasad (1970) for GPI, Nichols and Ruddle (1973) for MPI, and the fluorescent glutamate pyruvate transaminase stain of Harris and Hopkinson (1976) for ALAT. Each amphipod was ground whole in an equal volume of 0.05 M Tris-HCl, pH 8. Amphipods were captured by hand from under rocks and seaweed in the upper intertidal area of beaches. The average sample size was 49 individuals.

Each locus had two common alleles. There were also two rare *Gpi* alleles, with mean frequencies over all locations of 0.031 and 0.011, and one rare *Mpi* allele with a mean frequency of 0.0005. The common allozyme which migrated furthest toward the anode was designated 100.

For each locus, several pairs of locations were surveyed initially. The locations within each pair differed from each other in a subjective assessment of habitat

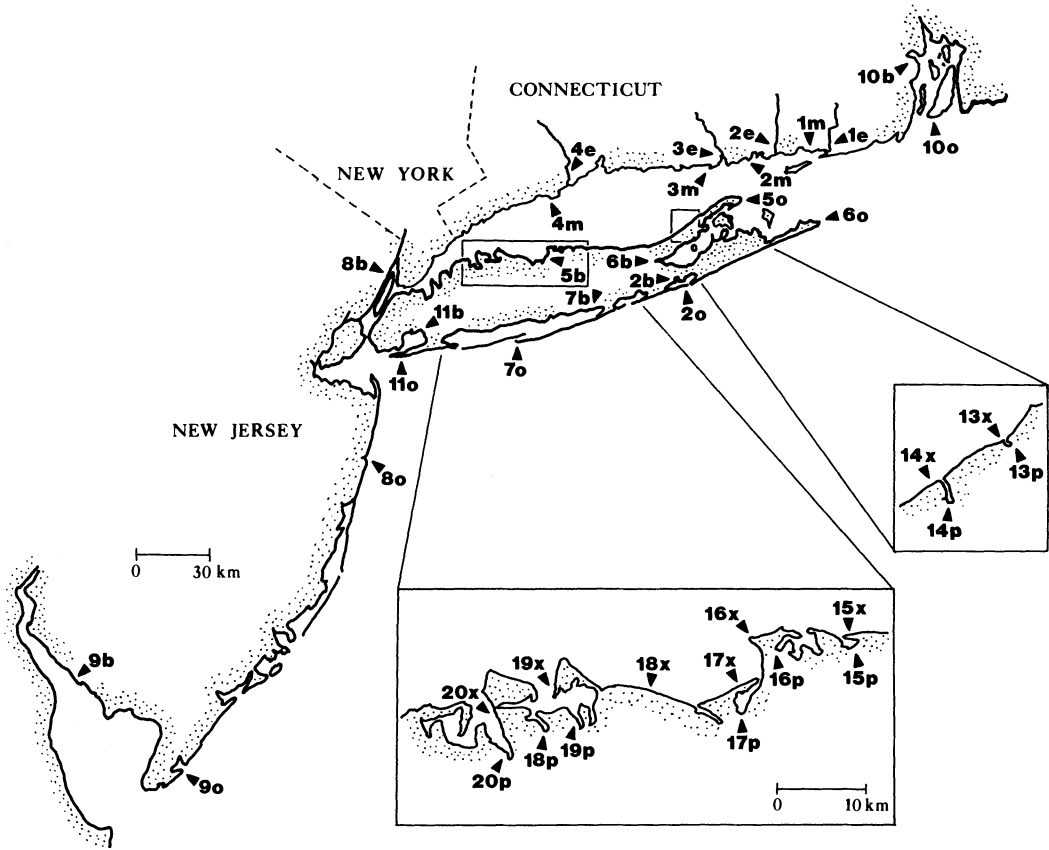


FIG. 1. Locations of *P. platensis* samples. Both insets use the same scale. Letters indicate the type of beach: e, estuarine; m, marine; b, bay; o, ocean; p, protected; x, exposed.

type. A hypothesis was formulated to explain the results of this initial survey, and this new hypothesis was then tested with a new set of pairs of locations. A sign test (Sokal and Rohlf, 1981 pp. 449–450) was used because the null hypothesis is that any one pair of locations might differ in allele frequency due to random drift, but the direction of difference would be random. A one-tailed test was used because a specific alternative hypothesis, predicting which allele would be associated with each habitat, was formulated before the second set of samples was collected. Because subjective, qualitative habitat classifications were used, it is important to emphasize that the pairs of locations were chosen and their habitat types were determined before the allozyme data were collected.

The initial survey of variation at the *Gpi* locus consisted of four estuarine-marine pairs (locations 1–4, Fig. 1). At each pair, an estuarine sample was collected at a beach on the estuarine portion of a river, and a marine sample was collected on a beach outside the mouth of the river. A consistent geographic pattern of *Gpi* allele frequencies was not evident within these pairs of locations (Fig. 2a). However, the beach most exposed to the moderating climatic influence of the Atlantic Ocean, site 2m, had a higher frequency of the *Gpi*¹⁰⁰ allele than beaches inside Long Island Sound (site 4m) and Fishers Island Sound (site 1m). This sug-

gested that the *Gpi*¹⁰⁰ allele might be selected against on the shores of sheltered bodies of water, where the climate is warmer in summer and cooler in winter than on ocean beaches. To test this hypothesis, eight bay-ocean pairs were surveyed (locations 5–12). Bay samples were collected on the shores of bays and large estuaries, as far as possible from the ocean, while ocean samples were collected on or near Atlantic Ocean beaches. At seven of these eight pairs of beaches, the *Gpi*¹⁰⁰ allele was indeed less common at the bay site (Fig. 2b), which is significantly different from the null expectation (one-tailed sign test, $P = 0.035$).

At the *Alat* locus, the initial survey consisted of the four estuarine-marine pairs and the first five bay-ocean pairs. At that point the *Alat*¹⁰⁰ allele was less common at the estuarine and bay beaches in seven of the nine pairs (Fig. 2c). The most striking exception was Delaware Bay (sites 9o and 9b). The beach within Delaware Bay, like other bay beaches, is probably warmer in summer, cooler in winter, and lower in salinity than the nearby ocean beach. However, unlike other bay beaches, where the *P. platensis* habitat is generally mud and fine sand, the Delaware Bay beach consists of coarse sand, gravel, and large rocks. In this respect the Delaware Bay beach is similar to *P. platensis* habitat on ocean beaches. This suggested that the *Alat*¹⁰⁰ allele may be selected against in protected areas, which gen-

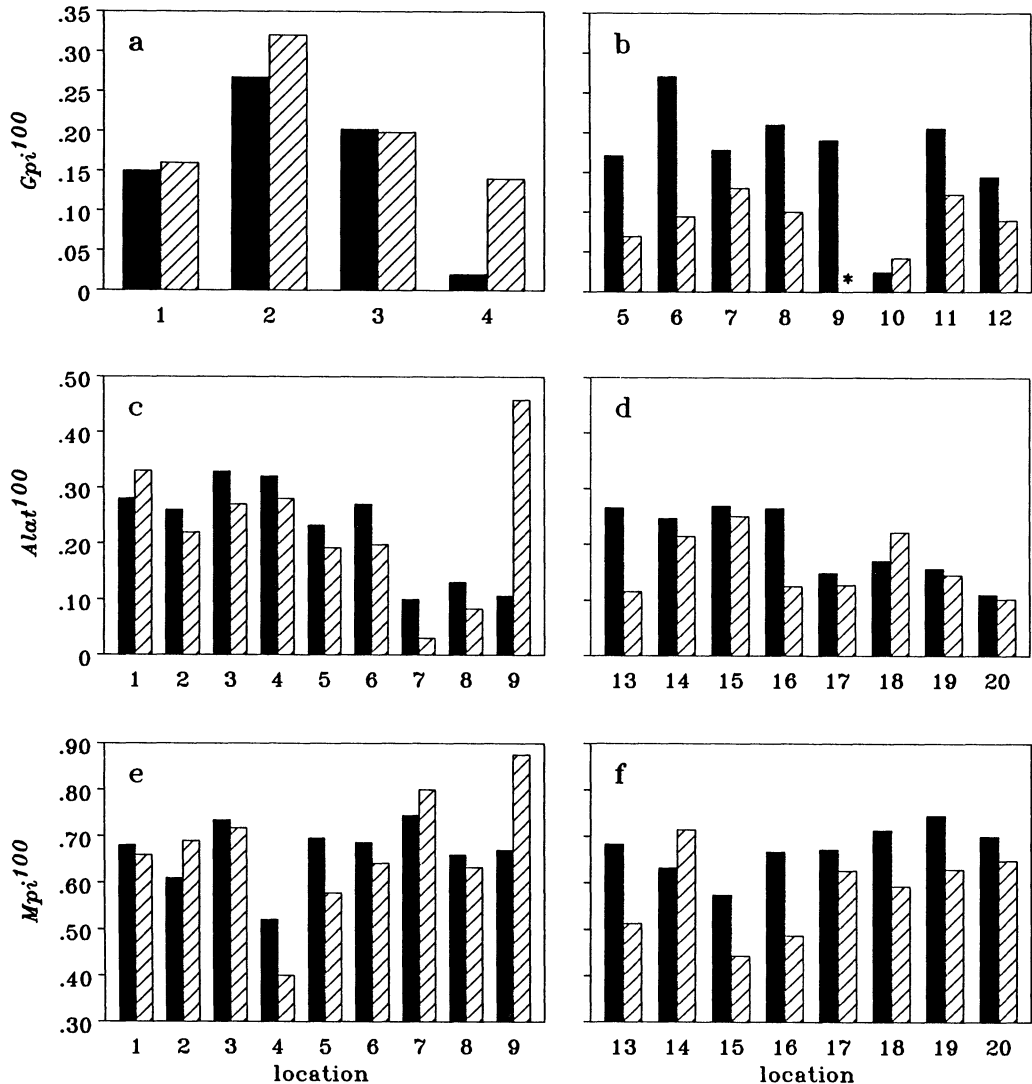


FIG. 2. Allele frequencies at the locations shown in Figure 1. For each pair of locations, the solid bar represents the marine, ocean, or exposed beach; and the striped bar represents the estuarine, bay, or protected beach. a) *Gpi*, initial survey. b) *Gpi*, bay-ocean pairs. * Indicates that the frequency of *Gpi*¹⁰⁰ is 0. c) *Alat*, initial survey. d) *Alat*, protected-exposed pairs. e) *Mpi*, initial survey. f) *Mpi*, protected-exposed pairs.

erally have muddier sediment than beaches exposed to more wave action. To test this hypothesis, eight protected-exposed pairs on the north shore of Long Island were surveyed (locations 13–20, Fig. 1). Exposed beaches on Long Island Sound consist of coarse sand and gravel, while protected beaches on the small bays are much muddier. At seven of the eight pairs of locations, the *Alat*¹⁰⁰ allele was less common in the protected site (Fig. 2d), a significant difference (one-tailed sign test, $P = 0.035$).

The pattern at the *Mpi* locus was similar to that at *Alat*. At six of the first nine pairs of locations, the *Mpi*¹⁰⁰ allele was less common at the estuarine and bay sites, again with the striking exception of Delaware Bay (Fig. 2e). The hypothesis that the *Mpi*¹⁰⁰ allele is selected

against on protected beaches was tested at the eight small bays on Long Island Sound, and at seven of these the *Mpi*¹⁰⁰ allele was less common on the protected beach (Fig. 2f; $P = 0.035$, one-tailed sign test).

One explanation for the unusual *Alat* and *Mpi* allele frequencies at the site inside Delaware Bay could be inadvertent sampling of a second species. The amphipods from sites 9e and 9p were run for an additional five enzyme systems (aspartate aminotransferase, malate dehydrogenase, peptidase, phosphoglucomutase, and phosphogluconate dehydrogenase), yielding eight isozymes. The two populations were monomorphic for the same allele at all eight loci; because cryptic species in other genera of amphipods have been easily distinguished using allozymes (Bulnheim and Scholl,

1981; Siegismund et al., 1985; McDonald, unpubl.), it is unlikely that more than one species was present.

Small-scale geographic variation in allele frequencies has been found in several amphipod species. Four species of *Gammarus* differ in allele frequency at several loci, including *Gpi*, *Alat*, and *Mpi*, between the estuarine Baltic Sea and the more marine Kattegat (Siegismund, 1985; Siegismund et al., 1985). *Traskorchestia traskiana* differs in *Mpi* and amylase allele frequency between two exposed beaches and nearby brackish ponds (Busath, 1980). *Megalorchestia californiana* differs in *Gpi* allele frequency at one of two small estuaries (McDonald, 1985). Amphipods carry their young in a brood pouch, unlike most marine organisms, which have planktonic larvae. Because of the resulting limited dispersal abilities of amphipods and the patchy distribution of each species' habitat, all of these examples of differentiation could be the result of random drift. Both the young and adults of *P. platensis* are poor swimmers, like other talitrid amphipods (Vogel, 1985), further increasing the likelihood of random drift in isolated populations. However, the consistently repeated differences in allele frequency found at the *Alat*, *Gpi*, and *Mpi* loci in *P. platensis* suggest that differential selection is affecting each of these loci in this species, either directly or through a locus in linkage disequilibrium.

At this point, the environmental factors which may be responsible for the selection at each locus are uncertain. Because many of the environmental factors which vary among beaches are correlated with each other, the subjective criteria used to classify beaches in this study need not be of selective significance. When compared with a beach exposed to the moderating influence of the ocean or Long Island Sound, a bay or protected beach is likely to be warmer in summer, colder in winter, more variable in temperature diurnally, and to have lower and more variable salinity. The coarse sand and gravel of exposed beaches may provide less protection from fluctuating temperature and salinity than the finer sediments of protected beaches; however, amphipods burrowed in fine sediments may be at increased risk of low oxygen stress. *P. platensis* eat drift algae and grasses, which may differ in relative abundance between habitats. There are probably other, less obvious environmental factors which also differ among beaches and might cause selection at enzyme loci.

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