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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 latitudinalcline 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-
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 Gpi100 allele than beaches inside Long Island Sound (site 4m) and Fishers Island Sound (site 1m). This suggested that the Gpi100 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 Gpi100 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 Alat100 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 Alat100 allele may be selected against in protected areas, which gen-

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**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.
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.

- Generally 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 sev-
eral loci, including Gpi, Alat, and Mpi, between the
estuarine Baltic Sea and the more marine Kattegat (Sieg-
ismund, 1985; Siegismund et al., 1985). *Traskorch-
estia traskiana* differs in Mpi and amylase allele fre-
quency between two exposed beaches and nearby
brackish ponds (Busath, 1980). *Megalorchestia cali-
iforniana* 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 (Vo-
gel, 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 un-
certain. 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 in-
fluence 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 diur-
nally, 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 sedi-
ments 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 se-
lection at enzyme loci.

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