Genetic Variation in a Gradient of Environmental Variability: Marine Bivalvia (Mollusca)

Abstract. *Six bivalve mollusk species were sampled for genetic variability at two enzyme synthesizing loci. The effective number of alleles and absolute number of alleles decreased with depth of burial within the sediment, intertidally, and with depth of water, subtidally. It is proposed that environmental variability regulates genetic variability at these two loci.*

Since the discovery of an unexpectedly high proportion of polymorphic loci in bisexual outbreeding species (*1*), many attempts have been made to describe this phenomenon in some detail and elucidate its cause. The observed polymorphisms could involve selectively neutral alleles (*2*), but the many studies showing correlation of allele frequencies with environmentally significant parameters would suggest an adaptive significance (*3*). Kinetic properties of proteins at varying temperatures can also be shown to correlate with distributions of alleles in natural populations (*4*).

One prediction following from the selectionist point of view is that varying and heterogeneous environments should support species that are relatively more polymorphic, while monotonous environments that are geologically permanent should support monomorphic species (*5*). Lewontin (*6*) demonstrated a loss of chromosomal polymorphism in *Drosophila pseudoobscura* in a population raised for many generations in a constant environment. Similiarly, a heterogeneous environment was found to retard the loss of genetic polymorphism in experimental studies of *Drosophila* (*7*). In a consideration of theoretical possibilities, it was predicted that the constancy of the deep sea bottom should result in species with a high percentage of monomorphic loci (*5*). However, recent evidence tends to contradict this prediction (*8*).

The present study relates genetic variability to environmental variability in six species of shallow-water marine Bivalvia (Mollusca). Phosphohexose isomerase (PHI) (D-glucose-6-phosphate keto-isomerase; E.C. 5.3.1.9) and leucine aminopeptidase (LAP) (L-leucyl-peptide hydrolase; E.C. 3.4.1.1) were examined by the electrophoretic methods of Koehn and Mitton (*9*). Phosphohexose isomerase was detected by the methods of Brewer (*10*).

The species studied (Table 1) were selected because they live in an inferred gradient of environmental variability. Sanders (*11*) discussed the role of water depth in damping variability in water temperature. There is a range of 27°C in the seawater temperature at the surface near Gay Head, Massachusetts; but a variation of only 1°C at a depth of approximately 500 m. Variability in parameters such as temperature and salinity is similarly reduced with depth into the sediment. At a depth of 5 cm within the sediment, a surface variation of 26 per mil in salinity is reduced to less than 1 per mil in a boreal estuary (*12*). Because of this, organisms living in soft bottom intertidal environments tend to be deep burrowing (*13*). Epifaunal species such as barnacles typically have mechanisms to tightly seal off soft body parts. Thus, Table 1 includes species ranging from the epifaunal, byssally attached bivalve *Mytilus edulis*, to a shallow-burrowing species, *Nucula annulata*, living at a water depth of approximately 20 m. Populations of all species were collected within the Long Island Sound region (*14*). Some species, collected intertidally in this study, are also found in significant numbers in shallow subtidal environments (see Table 1). Considerations of parameters such as height in the intertidal zone might lead other workers to a somewhat different a priori ordering of the six species along an environmental variability gradient. Therefore, it is necessary to state that the specific hypothesis to be tested is that species which burrow more deeply have less genetic variability at these two loci. The deeper water *N. annulata* is predicted to have the least variability.

For LAP activity, two loci seem to be present in five of the six species (LAP could be detected, but electrophoresis bands could not be identified in *N. annulata*). However, bands corresponding to the slower migrating locus could only be identified in *Macoma balthica* and *Mya arenaria*. For PHI, there seems to be one zone of polymorphic activity that can be ascribed to a single segregating locus in all six species. In LAP, homozygotes were inferred from single strong bands, whereas heterozygotes were inferred from double bands that were weaker in color. In PHI, homozygotes were inferred from single bands, and heterozygotes from triple bands, indicating the dimeric structure of the molecule.

Two estimates of polymorphism are reported. One is the absolute number of alleles (A) found in the population. If environmental heterogeneity increases polymorphism, the number of alleles might crudely correlate with the number of alternative environmental states (or the range of variation of a continuous parameter such as temperature) that the population confronts.

Second, the effective number of alleles (n_e) was estimated, as proposed by Kimura and Crow (*15*); n_e is calculated as the reciprocal of the sum of the squares of the allele frequencies, thus diminishing the contribution of rare alleles. If all of the alleles are equal in abundance, then n_e equals A, and the frequency of heterozygotes obtainable with Hardy-Weinberg equilibrium is maximized. Thus, n_e may be a measure of (i) heterozygosity, or (ii) the relative availability of a series of environmental states, as discussed above.

Both estimates of genetic variability are problematical in their application to samples from natural populations. The number of alleles observed at a locus is dependent on sample size. Thus, sample size has been kept as uniform as possible in this study, and many samples have been collected to determine the variance of A in some

Table 1. Genetic variation of bivalves at the PHI locus. Species 1, 3, 4, and 5 are also found in shallow subtidal bottoms. The abbreviations are A, absolute number of alleles; n_e, effective number of alleles; N, number of samples.

Species	Life habit	A	n_e	N
1) *Mytilus edulis*	Epifaunal, intertidal	7	3.9	70
2) *Modiolus demissus*	Semi-infaunal, intertidal	6	2.6	62
3) *Mercenaria mercenaria**	Shallow-infaunal, intertidal	6	2.5	55
4) *Macoma balthica*	Medium infaunal (10 cm), intertidal	3	2.1	85
5) *Mya arenaria*	Deep infaunal (15 to 30 cm), intertidal	3	1.7	58
6) *Nucula annulata*	Infaunal, subtidal (depth, 20 m)	2	1.2	71

* This species showed a significant deviation from Hardy-Weinberg frequencies (chi-square goodness of fit, $P < .05$).

of the species discussed in this report (*16*). Furthermore, if allele frequencies change as a function of geography (clinal variation), n_e will change accordingly. Evidence presently available indicates that PHI allele frequencies are uniform in *Mytilus edulis* from Virginia to Cape Cod. However, at the LAP locus, over this range there are significant departures from otherwise uniform allele frequencies, north of Cape Cod and within Long Island Sound (*14*).

Tables 1 and 2 show the values of these parameters for the species studied. In PHI, there is a steady diminution in both the absolute and effective number of alleles down the burrowing depth gradient discussed above. A high correlation exists between n_e and the number of alleles observed ($r_s = .96$, $N = 6$, $P < .05$). In LAP, this same phenomenon is observed for n_e and for A with a significant correlation between these two parameters ($r_s = .92$, $N = 5$, $P < .05$).

Despite the problems of the measures employed, it seems clear that species supposed to be experiencing more environmental variability are more polymorphic than those living in more constant environments. Two hypotheses, one involving selection for heterozygotes in a varying environment, the other involving diversifying selection in a heterogeneous environment with extensive gene flow, may explain the above data. Some data obtained by Koehn *et al.* (*17*) suggest that selection for heterozygotes in *Modiolus demissus* does occur in the high intertidal zone, relative to the low intertidal zone. However, environmental heterogeneity should not be dismissed as an important factor. *Mytilus edulis* occurs in a wide variety of substrata in Long Island Sound, including rock, gravel, sand, and wood pilings. With a planktonic larva, gene flow could extensively mix all of these populations. By contrast, *Nucula annulata* is collected in only subtidal muddy substrata (*18*).

It is significant that there is a correlation in n_e between the two different loci, over the species studied ($r_s = .95$, $N = 5$, $P < .05$). This suggests that epistatic interactions, environmental heterogeneity selecting for genetic diversity, or selection for heterozygosity is controlling genetic polymorphisms at these two loci. The latter two alternatives, taken singly or in combination, are consistent with the data presented above. Thus, environmental variability selects for increased A and n_e, independently at both loci. More work is needed to determine whether heterozygosity is selected in a fluctuating environment, thus increasing polymorphism, or whether diversifying selection increases polymorphism in a spatially heterogeneous habitat. Both processes are probably important in shallow-water marine bottoms. It is also of great importance to critically evaluate objective measures of environmental variability. It is here proposed that living depth within the sediment is an imperfect, but adequate, indicator.

Table 2. Genetic variation of bivalves at the LAP locus (most anodally migrating locus).

Species	A	n_e	N
1) *Mytilus edulis*	5	3.0	52
2) *Modiolus demissus*	4	2.5	66
3) *Mercenaria mercenaria*	4	2.6	60
4) *Macoma balthica*	4	2.2	50
5) *Mya arenaria*	3	1.1	64

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References and Notes

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Two Morphologically Distinct Blood-Brain Barriers Preventing Entry of Cytochrome c into Cerebrospinal Fluid

Abstract. *After intravenous injection, cytochrome c does not enter the cerebrospinal fluid. In most areas of the brain, the marker is prevented from leaving cerebral vessels by the capillary endothelium. In the choroid plexus, the marker passes freely out of capillaries into the extracellular space. However, it does not traverse tight junctions between epithelial cells and is rapidly incorporated into membrane-bound vesicles within the cell cytoplasm. Thereafter, cytochrome c is apparently removed by lysosomal degradation. These data suggest that there are at least two morphologically distinct blood-brain barriers to cytochrome c and that pinocytosis may be a mechanism for intracellular degradation rather than transcellular transport.*

Although it is firmly established that a major fraction of the cerebrospinal fluid (CSF) is formed constantly in the cerebral ventricles (*1*), the specific sites and mechanisms of CSF formation remain unclear. Among the many obstacles that confront investigators in this field is the lack of an electron-dense marker that can be injected into the blood and recovered in the CSF. With respect to the commonly employed intracerebral markers, ferritin and horseradish peroxidase, there is some question about tissue toxicity (*2*) and no proof that the markers enter the CSF.

In the continuing search for a suitable blood-CSF marker that can be used for examining mechanisms of CSF formation, we were recently led to a study of cytochrome c. This hemo-