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Review

PROTEIN POLYMORPHISMS AND GENETIC DIFFERENTIATION OF MARINE INVERTEBRATE POPULATIONS

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SUMMARY

Protein polymorphisms have been used to investigate the genetic structure of natural populations of a diversity of marine invertebrates. Studies reviewed here indicate that previously described relationships between length of planktonic larval life and the geographic boundaries of panmictic populations are not strongly supported. In particular, substantial differentiation has been observed in several species that appear to have high dispersal capabilities. While some of this differentiation may be the result of natural selection, other cases (where populations are found to have unique alleles in high frequency) seem to reflect restricted dispersal and gene flow among conspecific populations.

Key words: polymorphism, population structure, genetic differentiation, marine invertebrates.

INTRODUCTION

The application of biochemical genetic techniques to the study of geographically separated populations of marine and estuarine invertebrates over the past decade and a half has significantly enhanced our understanding of population differentiation in the marine environment (see Gooch, 1975; Battaglia and Beardmore, 1978; Levinton, 1980, 1982; Burton and Feldman, 1982a). When properly employed, these techniques (primarily gel electrophoresis of enzymes) can often provide substantial insight into the locus by locus genetic structure of conspecific populations of organisms which cannot readily be reared in the laboratory for formal genetic studies. Since laboratory rearing of marine species is frequently difficult and often impractical, enzyme electrophoresis is a powerful tool for studying population differentiation in the absence of morphological variation under simple genetic control. The purpose of this paper is not to exhaustively review electrophoretic studies of marine invertebrates, but rather to point out some of the major contributions such studies have made and to suggest areas where further work might be most profitable.

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Before beginning, a few comments should be made concerning the genetic interpretation of protein banding phenotypes on electrophoresis gels. First, it is now well known that a variety of factors other than genetic polymorphism can result in banding variation; developmental changes in isozyme expression (e.g. Gooch, 1977; Morgan et al., 1978), environmental effects (e.g. Oxford, 1975; Flowerdew and Crisp, 1976), and experimental artifacts can all generate banding variation which can potentially be confused with genetic polymorphism. Obviously, the preferred protocol for verifying the genetic control of banding variation is via formal pair crosses or parent–offspring analyses. However, when such studies are impractical, single-locus genetic polymorphism can generally be distinguished from other sources of band variation if care is taken in meeting the following two criteria: (1) banding patterns should be consistent with the suspected quaternary structure for the enzyme in question (i.e., two-banded heterozygotes for monomeric enzymes, three-banded heterozygotes with 1:2:1 staining intensity for dimers, etc.), and (2) genotype frequencies should approximate Hardy–Weinberg expected proportions at least to the extent that genotypes expected to be in high frequency are not totally absent. These criteria were suggested by Selander et al. (1970) and have achieved general acceptance among population geneticists as conservative standards where formal analysis is not possible.

CORRELATIONS BETWEEN THE ENVIRONMENT AND POPULATION DIFFERENTIATION

Correlations between allele frequencies at enzyme-coding gene loci and various parameters of the marine environment have been observed in a number of invertebrate systems. In their study of the marine ectoproct Schizoparella errata (Syn: S. unicornis), Schopf and Gooch (1971) found significant increases in the frequency of the Lap-3<sup>b</sup> allele in a series of population samples taken from the south shore of Cape Cod and the Elizabeth Islands, where a steep gradient in summer temperatures occurs. Later work (Schopf, 1974) showed that a second locus, Got, showed a similar clinal pattern in allele frequencies correlated to summer water temperatures. Since S. errata larvae cannot feed in the plankton and consequently must settle within a few hours, Schopf (1974) estimated that mean larval transport per generation is probably less than 1 km (the population samples covered a range of almost 100 km). Temporal stability of allele frequencies combined with the fact that each population was found to be in Hardy–Weinberg equilibrium at both loci led Schopf to conclude that local selection was the most likely explanation for the observed clines and their correlation with water temperature.

Climes in allele frequencies have also been observed on a larger geographic scale. Corbin (1977) found that while the distribution of phosphoglucone isomerase (PGI) alleles was not statistically heterogeneous among a series of 14 Emerita talpoida populations sampled between Massachusetts and Florida, two (of three) common alleles showed significant relationships with latitude. Noting that some of the allele
and genotype frequencies were correlated with mean low temperature and/or mean high salinity, Corbin concluded that local selection was responsible for the allele frequency–latitude relationship. A number of caveats should be expressed with respect to these conclusions. First, it was assumed that because *E. talpoida* has long-lived planktonic larvae, the range of populations sampled formed a single panmictic unit; hence, relationships between allele frequencies and latitude were assumed not to be attributable to population structure. As detailed below, assumptions of panmixis on this scale, though not uncommon in the literature, are not necessarily justified. Following the arguments of Christiansen and Frydenberg (1974), data on additional polymorphic loci might be used to clarify this issue in future investigations. A second problem involves the fact that while *E. talpoida* is an intertidal to shallow subtidal species, the environmental data used in the regression analyses were obtained from the records of off-shore lightships stationed in the general vicinity of Corbin’s collecting sites; the extent to which such data reflect relevant habitat parameters is unclear.

In interpreting observed clines in allele frequencies such as those discussed above (also see O’Gower and Nicol, 1968, and Hoffmann, 1981a), it should be noted that in the absence of evidence directly relating allelic function and environment via natural selection, correlations should not be taken as conclusive evidence for causation. As pointed out by Lewontin (1974), geographic patterns in allele frequencies at single gene loci can often be attributed to parameters of population structure and history rather than natural selection; such patterns might then show spurious correlations to environmental factors. For example, at the genotypic level Corbin (1977) noted consistent heterozygote deficiencies (compared to Hardy–Weinberg expected proportions) at PGI in *E. talpoida* which he attributes to selection against heterozygotes. An alternate explanation is that the heterozygote deficiencies are simply the result of mixing of locally differentiated populations (the Wahlund effect). Lassen and Turano (1978) discuss how the Wahlund effect may account for much of the heterozygote deficiency observed in *Mytilus edulis* populations in Long Island Sound.

**EVIDENCE FOR NATURAL SELECTION IN THE BIOCHEMICAL GENETIC DIFFERENTIATION OF INVERTEBRATE POPULATIONS**

The extent to which natural selection is responsible for population differentiation at the enzyme level remains a controversial issue. Attempts to understand the mechanisms by which allele frequencies are linked with the environment have, however, made significant progress in a number of marine invertebrate systems. One approach has been to investigate the functional properties of allelic enzymes (allozymes) with respect to relevant environmental parameters (e.g., are heat-sensitive allozymes found in reduced frequencies in the warmest regions of the species range?). Among marine invertebrates, the work of Koehn and collaborators
(detailed below) on a salinity-related aminopeptidase polymorphism in *Mytilus edulis*, and Hoffmann (1981b) on a temperature-related PGI polymorphism in the anemone *Metridium senile* are examples.

With respect to a mechanistic understanding of how marine invertebrate populations differentiate at the biochemical genetic level, the *Mytilus* studies are currently most complete (Koehn, 1978; Koehn et al., 1980; Hilbish et al., 1982). The scope of population sampling of allele frequencies in *Mytilus edulis* is apparent from Koehn et al. (1976); approximately 25,000 individuals from 150 samples were studied at six loci. Work has focused, however, on the sharp differentiation of populations along Long Island Sound at the aminopeptidase-I locus (or *Lap*). Allele frequencies at this locus are correlated with environmental salinity, as the frequency of the $Lap^{54}$ allele drops from approximately 0.55 in populations outside of the Sound to 0.15 at a distance of about 50 km into the Sound (Koehn, 1978). Boyer (1974) and Theisen (1978) have observed similar clines in *Lap* allele frequencies with salinity. While such clines might simply reflect restricted larval dispersal between genetically differentiated populations (oceanic versus estuarine), the alternate explanation of more or less free gene flow and differential selection along the cline is strongly supported by the work of Koehn et al. (1980). By monitoring size-dependent *Lap* allele frequencies in a series of five sites along the Long Island Sound cline, these authors observed an annual influx of oceanic recruits (marked with a high $Lap^{54}$ frequency) into the Sound, followed by selection against genotypes possessing $Lap^{54}$, as evidenced by decreases in allele frequency with shell size at sites within the Sound (having reduced salinity). Theisen (1978) also obtained evidence for selection at the *Lap* locus via a transplant experiment.

The mechanism by which environmental salinity selects for population differentiation at the *Lap* locus has been proposed by Koehn (1978) and Hilbish et al. (1982). In brief, allozymes at this locus have been observed to differ in specific activity due to a high $k_m$ for $Lap^{54}$ as compared to other allozymes. This higher activity, while potentially adaptive under conditions of hyperosmotic stress since it results in faster accumulation of free amino acids used to regulate cell volume, is apparently disadvantageous under reduced salinity conditions since excess free amino acids are produced by protein catabolism only to be excreted away in maintaining cell volume. Hence, under low salinity conditions, genotypes with the high activity $Lap^{54}$ are selected against because of excessive loss of nitrogen resources.

In another study of how salinity variation in the environment might result in selection for biochemical genetic differentiation of populations, Burton and Feldman (1983) have investigated the physiological consequences of a glutamate–pyruvate transaminase (GPT) polymorphism in the intertidal copepod * Tigriopus californicus*. This species inhabits high intertidal rock pools which experience extreme fluctuations in salinity depending on the proximity of the pools to wave splash and freshwater runoff. GPT was selected for study because rapid alanine synthesis has been observed to occur in *T. californicus* during hyperosmotic stress (Burton and
Feldman, 1982b), and GPT catalyzes the final step in alanine biosynthesis. Allozyme genotypes for GPT have been shown to differ in rates of alanine accumulation during hyperosmotic response; this difference appears to account for differential larval survivorship among genotypes when subjected to a hyperosmotic shock (Burton and Feldman, 1983). Interestingly, no correlation was found when allele frequencies of populations occupying pools of different salinity were determined (Burton, unpubl. data); however, since alanine levels are rapidly adjusted only in response to changes in salinity, a direct correlation with salinity would not necessarily be expected. In a pilot study of the relationship between allele frequency and variability of salinity (i.e., how often is adjustment of alanine concentration required?), higher frequencies of the more active allozyme have been found at sites with greater salinity variation than at neighboring locations with little variation (Burton, manuscript in prep.). These data suggest that population differentiation in T. californicus might result from local differences in the frequency and magnitude of salinity fluctuations rather than from relatively stable salinity differences between sites (as in the Long Island Sound Mytilus example discussed above).

The above examples indicate that natural selection can result in population differentiation at the biochemical genetic level, but that all correlations between allele frequencies and environmental parameters should not be accepted as proof for such selection. Furthermore, when it occurs, selection at the enzyme level can be sufficiently strong as to overcome high levels of gene flow (e.g. as in Mytilus) and maintain population differentiation. In this regard, biochemical genetic variation is clearly equal in its adaptive importance to more macroscopic genetic variation, such as shell morphology (see Struhsaker, 1968).

RELATIONSHIPS BETWEEN LARVAL DISPERSAL AND GENETIC DIFFERENTIATION OF MARINE INVERTEBRATE POPULATIONS

Like their terrestrial counterparts, marine organisms are not uniformly distributed in space; rather, they are typically restricted on a local scale to patches of favorable habitat. To the extent that such habitat patches are isolated from one another, populations of more or less sedentary adult marine invertebrates are geographically isolated from conspecific populations and may genetically differentiate in response to local selective pressures (as discussed above) and to random drift. The importance of genetic drift in population differentiation will depend on the effective sizes ($N_e$) of the populations, the level of gene flow between populations, and the length of time over which the process has taken place (see recent discussion in Hedgcock, 1982).

In the marine environment, gene flow among invertebrate populations is probably most frequently the result of interpopulational exchanges of planktonic larval stages. Depending on the species, larvae may be at liberty in the water column for a matter of minutes or for several months. Hence, potential for dispersal and gene
flow varies greatly among taxa. Using electrophoretically determined esterase polymorphisms as genetic markers, Berger (1973) assessed the importance of larval dispersal on population differentiation in three species of the genus *Littorina*: *L. obtusata* and *L. saxatilis*, both of which lack planktonic larval forms, and *L. littorea*, which has planktonic development lasting several weeks. Berger concluded that the former two species showed higher levels of population differentiation than the latter species, suggesting that the life history differences among the species result in corresponding differences in population structure. Further support for an inverse relationship between the capacity for larval dispersal and the extent of population genetic differentiation comes from the work of Gooch et al. (1972) and Snyder and Gooch (1973) which compared *L. saxatilis* (no planktonic larvae) to *Nassarius obsoletus* (with long-lived planktonic larvae) and found significantly greater differentiation in the former species.

From the sort of data discussed above, Crisp (1978) has concluded that, in general, one can predict from the length of larval life the order of magnitude of dispersal and the geographic boundaries of panmictic populations. With regard to species which lack planktonic larvae, it seems that Crisp’s conclusions are well borne out. Campbell (1978) found strong differentiation at several enzyme loci in a series of 12 *Thais lamellosa* on the west coast of North America, which is in accord with its life history, which lacks a planktonic stage. Bulnheim and Scholl (1981) observed significant differentiation among neighboring populations in the amphipod *Gammarus zaddachi*, but somewhat less differentiation among populations in the sibling species *G. salinus*. While both species lack planktonic larvae, the higher level of differentiation in *G. zaddachi* versus *G. salinus* may be attributable to the former’s restriction to low salinity habitats, thereby preventing gene flow in the adult stage which apparently occurs to some extent in the latter. Similarly, Parker et al. (1981) found evidence for differentiation among estuarine populations of the isopod *Cyathura polita*, which lacks a planktonic stage. Hence, as might be expected, species in which adults are relatively sedentary and which lack planktonic larvae almost always have been found to show significant differentiation of populations on a relatively small geographic scale.

With respect to species that have more or less long-lived planktonic larval stages, the data are not as conclusive. While the early work on gastropods (Gooch et al., 1972; Berger, 1973) supported the intuitively attractive notion that the longer the larvae are at liberty in the water column, the broader are the boundaries of panmictic populations, it is not at all clear that the majority of studies now available substantiate such a conclusion.

Scheltema (1975) has pointed out that dispersal not only reflects the length of planktonic larval life, but also local hydrographic conditions and larval behavior. These effects are clearly apparent in the data of Scheltema (1971) which show that there is little correlation between length of larval life of a given species and the extent to which the larvae of that species are found dispersed offshore (on the basis
of extensive plankton sampling). From observed larval distributions, Scheltema (1971) concluded that trans-oceanic dispersal is not infrequent in a number of gastropod species. While this conclusion gains some support from a qualitative correspondence between levels of morphological divergence between eastern and western Atlantic conspecific populations and the extent to which larvae were observed in the plankton, no genetic evidence was presented to support the hypothesis of trans-oceanic gene flow.

Existing evidence for extensive gene flow among geographically separated invertebrate populations is, in fact, limited. The early conclusions of Berger (1973) concerning Littorina littorea were essentially based on a single gene locus (Est 3) studied in small population samples (mean n = 14 individuals per site). It is also important to note that this locus was near fixation (on the same allele) in nearly all populations examined; just as monomorphic loci possess no information relevant to discerning population structure, weakly polymorphic loci give little insight (especially when sample sizes are small). The uniformity of alleles among Nassarius obsoletus populations presents a much stronger case for extensive gene flow (Gooch et al., 1972), but surprisingly few other invertebrate species have shown this pattern of genetic similarity over broad geographic ranges.

In extending his earlier studies of littorinids, Berger (1977) compared conspecific L. saxatilus, L. obtusata, and L. littorea populations from eastern (France) and western (U.S.) Atlantic coasts and found striking differentiation in all three species. Of particular interest in such comparisons are alleles which occur in high frequency on one side of the Atlantic but are not present on the other side. In L. saxatilus, for example, the Est-A allele ‘F’ accounts for 49% of the allelic composition in the French sample, but does not occur in the U.S. sample. In L. obtusata, 70% or more of the total alleles observed were unique to one of the populations at both the phosphoglucose isomerase and the protein-2 loci. The most extreme differentiation was between L. littorea populations; at nine loci (of 12) there is a nearly complete lack of overlap in alleles present. This observation is particularly striking in view of the fact that L. littorea has long-lived planktonic larvae and might have been considered a likely candidate for trans-oceanic dispersal; the biochemical genetic data are clearly incompatible with any trans-oceanic gene flow in L. littorea and probably the other littorinids as well.

Marcus (1977) determined allele frequencies at six polymorphic gene loci in four populations of the sea urchin Arbacia punctulata ranging from Massachusetts to North Carolina. While the overall genetic distances among the populations appeared unremarkable, differentiation on a locus by locus basis is quite striking for a species with a planktonic larval life of up to 8 wk. As noted by Marcus (1977), such differentiation could be the result of geographic isolation and/or strong selection. A closer look at the data, particularly the acid phosphatase (ACPH-2) locus, is instructive: the most common allele at the Willis site (Virginia) is the 0.79 allele which has a frequency of 62%. This allele was completely absent from all other
population samples (n > 75 for each Atlantic coast site). The high frequency of this unique allele in the middle of the sampled range of this species which would be expected to have extensive dispersal capacities seems to be striking evidence for highly restricted gene flow among populations in this species. The fact that some loci (e.g. amylase-1) show little differentiation among the same set of populations warns against basing conclusions about population structure on the results of a single or even a small number of loci. It should be apparent at this point that inferences of population structure from genetic information are asymmetrical - while we can safely infer restricted gene flow from the occurrence of unique alleles in high frequency at a single locus, essentially no data from a single locus can yield a strong conclusion for high gene flow.

Another example of restricted gene flow among conspecific populations is in the copepod *Tigriopus californicus*. This species inhabits ephemeral rock pools in the high intertidal zone, suggesting an important role for dispersal to colonize or recolonize freshly filled pools; the life history of the species includes both free-swimming adults and larval forms that appear capable of such dispersal. However, genetic data presented by Burton et al. (1979) and Burton and Feldman (1981, 1982a) give strong evidence for genetic isolation of populations on both a regional and local scale. The occurrence of unique alleles at one or more loci is shown in Table I for a series of geographically separated populations. Despite the strong differentiation of populations, inter-site crosses are easily achieved in the laboratory and result in fertile F<sub>1</sub> and F<sub>2</sub> progeny (Burton et al., 1981).

On a smaller geographic scale, population differentiation in *T. californicus* is equally striking. While populations inhabiting pools located on the same rock outcrop tend to be genetically homogeneous, those on different outcrops separated by as little as 500 m have been found to show strong, stable differentiation at one or more enzyme loci (Burton and Feldman, 1981). Hence, the geographic boundaries of more or less panmictic population units in *T. californicus* are apparently defined by the size of the rock outcrop on which a set of pools are located. High levels of gene flow within such a unit are anticipated since pools are periodically (but infre-

<table>
<thead>
<tr>
<th>Population</th>
<th>GOT 1</th>
<th>GOT 2</th>
<th>ME</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>100</td>
<td>96</td>
<td>90</td>
</tr>
<tr>
<td>Palos Verdes</td>
<td>0.02</td>
<td>0.98</td>
<td>1.0</td>
</tr>
<tr>
<td>Laguna Beach</td>
<td>0.73</td>
<td>0.07</td>
<td>0.25</td>
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<tr>
<td>Aliso Beach</td>
<td>0.98</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>La Jolla</td>
<td>0.65</td>
<td>0.02</td>
<td>1.0</td>
</tr>
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GOT 1: glutamate–oxaloacetate transaminase (cytosolic); GOT 2 (mitochondrial); ME: malic enzyme.
quently) totally submerged during storms; adults can be found swimming between pools on the wet rock following such a period of high wave activity. The degree of isolation between outcrops might be due to both behavioral mechanisms and/or heavy predation on *T. californicus* in lower intertidal habitats (Burton and Feldman, 1981).

To experimentally address the problem of gene flow in *T. californicus*, Swisher (pers. comm.) has attempted to transplant a unique allele into a site where that allele did not previously occur. Preliminary results indicate that the allele (*PGI*<sup>1.05</sup>) has been successfully established at the new site (less than 1 km from the source population) though at a lower frequency than in the source population. While this change in frequency might be due to selective differences in the environment, available evidence suggests that the drop in *PGI*<sup>1.05</sup> frequency resulted from a swamping effect of the transplanted population by local residents (despite efforts to remove residents from the transplant site). With respect to gene flow, Swisher has found the *PGI*<sup>1.05</sup> allele in all pools on the outcrop used as the transplant site. In a recent sample, approximately 1 yr after the transplant, this allele was still not found at a neighboring outcrop less than 50 m distant. These experimental observations are in accordance with expectations based on the original allozyme survey work discussed above (Burton et al., 1979; Burton and Feldman, 1981); further experimental transplants, monitoring several loci at once, could provide valuable insight into the mechanistic basis of population differentiation since multiple loci could serve as internal controls to discriminate between natural selection and drift–migration hypotheses.

Data on other invertebrates also indicate strong differentiation among populations in species with substantial dispersal capacity. Tracey et al. (1975) have shown that malic enzyme (ME) allele frequencies in the lobster *Homarus americanus* are sharply differentiated between inshore versus offshore populations (Woods Hole and Martha’s Vineyard compared to Georges Bank). Though not discussed by Tracey et al. a number of other comparisons of allele frequencies suggest significant differentiation among neighboring populations (Burton and Feldman, 1982). For example, among the inshore samples, Woods Hole had the *MDH-2*<sup>1.05</sup> allele at a frequency of 0.20, while the nearby Martha’s Vineyard sample of 45 individuals completely lacked this allele. The same allele had a frequency of 0.11 at the offshore LSA site but was absent from the LSB site (n = 60) located 30 km away. While further evidence is clearly needed, available data indicate substantial differentiation among local *H. americanus* populations despite this species’ planktonic larval stages.

Further evidence for genetic differentiation among populations of marine invertebrates with planktonic larvae is presented in Burton and Feldman (1982a). While some of the inferences made are inherently weak because they require comparing results from different laboratories using slightly different techniques, the data certainly suggest that differentiation is not a rare event among such species.
Furthermore, the occurrence of unique alleles in high frequency in populations of species with high dispersal capacity indicates that we must be cautious in inferring gene flow from dispersal capacity alone.

DISCUSSION

The use of biochemical genetic techniques in the study of genetic differentiation of marine populations has resulted in some surprising insights into the ecology and evolution of invertebrate populations. Perhaps the single most important contribution of this work has been to elucidate the geographic scale of population differentiation. While available data represent few species, they suggest that local differentiation is more widespread than might be anticipated from the life histories of those species. It is particularly important to note that the genetic structure of natural populations of marine invertebrates cannot be reliably inferred from their apparent dispersal capacities. This conclusion contrasts with those of Gooch (1975) and Crisp (1978), but seems justified by the data reviewed above and in Burton and Feldman (1982a). Despite what appears to be a recent decline in interest in large scale electrophoretic surveys of animal populations, it is clear that such efforts are still needed to address population structure in the marine environment. Such efforts might best be focused on species that have planktonic development stages since work on species lacking such dispersal stages has consistently shown sharp differentiation.

While it has been long appreciated that natural selection can result in population differentiation even where significant gene flow occurs (e.g., Struhsaker, 1968; Antonovics and Bradshaw, 1970), the fact that dispersal capacities may not accurately reflect levels of gene flow among populations has received relatively little attention. Restriction of gene flow in species with what appears to be high dispersal capacity can be the result of two quite different processes: (1) apparent dispersal capabilities are not realized because of some biotic or abiotic barrier, or (2) while dispersal successfully introduces immigrants to a new population, those immigrants fail to reproduce at the new site. Evidence cited above indicates that both processes are probably important. In the case of Mytilus edulis, for example, oceanic immigrants apparently succeed in dispersing to Long Island Sound populations but are subsequently selected against contributing to the gene pool within the Sound. In contrast, the sharp differentiation of Tigrionus californicus populations seems to indicate that the dispersal capacities of this species are, in fact, not realized.

A variety of factors might prevent dispersal capacities from being realized. Among abiotic factors, currents could have obvious effects on the passive dispersal of planktonic larvae. The unfavorable temperatures and/or salinities present in river flumes may be effective barriers to dispersal along coastlines as larvae entering such water masses may experience 100% mortality. At a more subtle level, Fish (1972) noted that while Littorina littorea egg capsules are only slightly denser than sea water, this difference is exaggerated in less saline environments; hence, egg capsules
might tend to settle out of the water column when released by estuarine populations. In his study of British estuarine populations of *L. littorea*, Fish (1972) concluded that a considerable proportion of egg capsules undergo their full development in the benthic detritus of the estuary rather than in the oceanic water column.

Biotic factors that may influence dispersal include food supply, predation and behavior. Burton and Feldman (1981) suggested that the latter two might be responsible for restricted gene flow in *Tigriopus*. Predation plays a major role in limiting the ecological distribution of this species to the highest intertidal rock pools (Dethier, 1980); potential dispersers between populations are likely to experience high predation. Behavior of individual copepods might also restrict gene flow as Bozic (1975) has shown that they have a strong, chemically oriented tendency to swim toward pools occupied by conspecifics. This suggests that individuals washed from pools during periods of high wave activity would show an oriented return to those same pools.

Despite extensive documentation of the behavioral responses of marine invertebrate larvae to light, salinity, temperature, and hydrostatic pressure, the extent to which these responses influence the dispersal of larvae remains controversial. On the basis of limited genetic data, Burton and Feldman (1982a) concluded that species with strong-swimming pelagic larvae tended to show more population differentiation than species that release eggs or weak-swimming larvae into the water column. If such differences in population structure exist, they may reflect the differences in larval capacity to actively influence their position in the water column. In the simplest scenario, larvae transported just offshore from the parental habitat might actively retain their geographic position by attaching to benthic algae or substrate until metamorphosis to the adult form when they might simply return to the adult habitat. While not favoring this hypothesis, Efford (1970) does pose it as a possible recruitment pattern in *Emerita analoga* (‘the nursery area hypothesis’). Efford points out that such a recruitment mechanism would lead to population differentiation, while the coastal ‘counter current hypothesis’ he favors for *Emerita* would result in extensive population mixing. Unfortunately, genetic studies that might distinguish between these hypotheses have yet to be attempted; such studies may provide important insight into both the recruitment process itself and the geographic scale at which we might expect evolutionary adaptation of populations to their environment.

With regard to the latter issue, it should be pointed out that of all the population differentiation documented via electrophoretic studies, the adaptative significance of the differentiation has been addressed in only a few cases and is even partially understood in fewer still. The extent to which protein polymorphisms result in adaptative physiological variation remains poorly understood, though the work of Hilbish et al. (1982) and Burton and Feldman (1983) presents one approach to understanding this problem. Such a mechanistic approach is attractive in that it can provide a predictive framework for the study of adaptive differentiation of populations; it
does, however, suffer from the obvious limitations of dealing with adaptation on a locus-by-locus basis. Most adaptation at a biochemical level is likely to involve many loci and their interactions and it is not clear whether or not a locus by locus approach can successfully deal with complex physiological adaptations to the environment.

While this review has focused exclusively on studies of protein polymorphisms, the data obtained from such studies have their greatest value when viewed in the light of alternate approaches to the study of genetic differentiation of populations. As we have come to see that population differentiation is widespread in the marine environment (due largely to the success of biochemical genetic techniques), interest in local physiological adaptation has grown (see Levinton, 1982). Whether such adaptation is the result of allozyme variation itself (target loci, in the terms of Levinton, 1980), or the result of ecotypic race formation which is coincidentally ‘marked’ by allozyme variation, it is clear that studies of protein polymorphisms will continue to contribute to our understanding of the genetic differentiation of marine invertebrate populations.

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