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Genetics

Maintenance of an aminopeptidase allele frequency cline by natural selection

(protein polymorphism/gene flow/lysosome function/osmoregulation/*Mytilus edulis*)

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ABSTRACT **The product of the *Lap* locus in the marine bivalve *Mytilus edulis* is a neutral, membrane-associated aminopeptidase that is primarily localized on intestinal microvilli and in digestive cell lysosomes. Natural populations are genetically differentiated at the *Lap* locus between areas of differing salinity. A steep (0.55–0.15) allele frequency cline connects differentiated populations between the Atlantic Ocean and Long Island Sound. We demonstrate an annual gene flow/mortality cycle in cline populations whereby gene frequencies after mortality are correlated with salinity and enzyme activity. The cline is spatially and temporally unstable in immigrants, but stable in residents after mortality. Mortality is nonrandom with regard to the *Lap* locus; genotype-dependent properties of the aminopeptidase enzyme apparently lead to a differential rate of the utilization of nutrient reserves because selected genotypes exhibited an increased rate of tissue weight loss. Aminopeptidase genotypes are differentially adapted to different temperatures and salinities, which provides a mechanism for the relationship among biochemical, physiological, and population phenotypes.**

The allele frequencies of polymorphic genes can often be statistically correlated with spatial or temporal variation of environmental factors (1–9). However, the causal basis for a correlation between the population frequency of an enzyme allele and some environmental components cannot usually be demonstrated. It is not clear how biochemical phenotypes of allozymes are translated into physiological and fitness differences (10–13), but recent studies have begun to elucidate relationships between biochemical differences of allozymes and physiological and behavioral phenotypes (14–18).

In the marine bivalve mussel *Mytilus edulis* there are significant allele frequency differences at the *Lap* locus among some populations on the eastern coast of North America (19). The product of this gene is an aminopeptidase (aminopeptidase-I; EC 3.4.11.-), a dimer of size 68,000 daltons (20), which has highest affinity for neutral and aromatic NH_2-terminal residues of oligopeptides (20). Moore *et al.* (21) immunocytochemically localized the enzyme to both the intestinal brush border and lysosomes of digestive tubule cells of the digestive gland, sites of active protein catabolism (21), where it may act in peptide transport, as has been postulated for other membrane-associated aminopeptidases (22–29). Digestive tubule cells are rich in lysosomes (30, 31), and aminopeptidase-I has been demonstrated in cell-free lysosome preparations.

Cell volume regulation in *M. edulis*, as in many other marine invertebrates, occurs by changes in the concentration of the cellular free amino acid pool in response to environmental salinity variation (32). Catabolism of protein is one possible mechanism for provisioning this pool (33), and it is therefore of special interest that total aminopeptidase-I activity can be experimentally acclimated to different levels at different salinities (21, 34).

Several studies have focused on the evolutionary mechanisms that can explain a steep cline connecting Atlantic Ocean populations, where the frequency of the Lap^{94} allele is about 0.55, with populations in Long Island Sound, where the frequency of Lap^{94} equals 0.15 (19, 35–38). Lassen and Turano (36) dismissed natural selection in favor of hydrographical isolation for maintenance of the Lap^{94} cline and attributed deficiencies of heterozygotes in the youngest mussels to population mixing, or the Wahlund effect (39), in a narrow zone of larval mixing. This is difficult to envisage because of the prolonged larval dispersal of 3–7 weeks (40) in this species.

Laboratory shock experiments, using different combinations of salinities and temperatures, were used by Levinton and Lassen (37, 38) in an attempt to shift *Lap* allele frequencies by differential mortality. Their results were largely uninformative because intense laboratory shock treatments are very artificial and their experimental controls were unusual (p. 246 of ref. 38); the allele frequencies in controls differed more from starting frequencies than did treatments.

We will demonstrate that there is an annual cycle of gene flow into cline populations, followed by genotype-dependent mortality of immigrants. This cycle produces temporal variation in the spatial location of the preselection cline in young mussels, but the postselection cline in adults is both temporally and spatially stable. The spatial position of the postselection adult cline corresponds with a gradient in the environment (salinity), which has a demonstrable effect on the enzyme product of the *Lap* locus (34). Selection is apparently a consequence of differences in reaction rate among *Lap* allozymes that lead to differing degrees of physiological stress and, thus, to differential mortality.

METHODS

Large samples of *M. edulis* were randomly collected by hand during July (1976–1978) in the intertidal zone at six sampling sites (Fig. 1) and shell length was measured to the nearest millimeter. Allele frequency data were collected from the electrophoresis of homogenates of digestive gland (19). Data are presented on recruits (≤15 mm shell length) and resident adults (>15 mm shell length) because the annual settling cohort can be easily recognized in July as a group with shell size <15 mm (35). Settlement of pelagic larvae occurs in June–July. The frequencies of *Lap* alleles in the annually recruited cohort were identical to those in oceanic populations (19); therefore, individuals of the cohort are termed "immigrants" to denote their origin from oceanic populations. The waters of Long Island Sound are of low salinity (26 parts per thousand). There is a typical estuarine diminution in salinity at the eastern entrance of Long Island Sound. Salinity and aminopeptidase-I enzyme activity were determined at each sampling site. Enzyme ac-

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tivity was measured in adults with L-leucyl-β-naphthylamide as substrate (20).

The dry tissue weight of molluscs, expressed in relation to some shell parameter (e.g., weight, volume, or size), has frequently been used as a physiological condition index (41–45). In *M. edulis*, this index varies seasonally (46); both the weight of germinal tissue varies, due to the buildup and liberation of gametes, and the weight of somatic tissue varies as nutrient reserves are stored and used (47, 48). We have used total dry tissue weight in relation to shell length as the index of physiological condition that would reflect any genotype differences in energetic stress. The relationships between dry tissue weight and shell length in two groups of genotypes with and without the Lap^{94} allele were determined for 200 animals in each group. Collections were made in both July and October, 1979, from site 3. Animals ranged in shell length from 5 to 55 mm. The frequency of the Lap^{94} allele was 0.55 in recruits, thus insuring that measurements included an immigrant cohort. Individual animals, measured to the nearest 0.1 mm, were opened and the digestive gland was slightly macerated with a probe. A paper wick (Whatman no. 3) of standard size was used to obtain a constant volume of body fluid and was then introduced directly into starch gels for electrophoresis. For animals <20 mm, the shell and tissue were dried to constant weight at 95°C. After the dried animal was weighed, it was soaked in distilled water, the tissue was scraped from the shell, and the shell was redried and weighed. For animals >20 mm, body tissues were excised from the shell onto tared aluminum foil and dried to constant weight at 95°C.

The weight/length data were transformed to $\log_{10}$ and a best-fit line was determined by least-squares regression separately for two groups of genotypes, those with and those without the Lap^{94} allele. The significance of differences between the regression parameters was estimated by an analysis of covariance (49).

RESULTS

Migration. Study sites that encompass the cline in frequency of the Lap^{94} allele at the eastern entrance of Long Island Sound from the Atlantic Ocean are shown in Fig. 1.

The frequency of Lap^{94} in oceanic populations south of Cape Cod varies closely around a value of 0.55, and within Long Island Sound its frequency is approximately 0.15 (19). The decline in frequency at the entrance to Long Island Sound occurs in less than 20 miles (Fig. 2). The cline in immigrants was observed as far as 12 miles into Long Island Sound (locality 4), but the decrease in the frequency of Lap^{94} in resident adults occurred between miles 0 and 12 (Fig. 2). The immigrant frequency of Lap^{94} was significantly higher at most localities in 1977, but we did not observe a large difference in the position of the allele frequency cline between immigrants and adults. However, in animals with a shell length < 10 mm, the frequency of Lap^{94} was 0.40–0.55 at sites 1–4 (this size range was absent at sites 5 and 6), suggesting that the 1977 sample was later in the settlement period than the 1976 and 1978 samples. The oceanic frequency of Lap^{94} in immigrants in 1978 was observed as far as 17 miles (locality 5) into Long Island Sound, producing a steep, abrupt change in frequency between miles 17 and 22. In adults, the cline in 1978 was spatially coincident with adult clines in previous years (Fig. 2; see below).

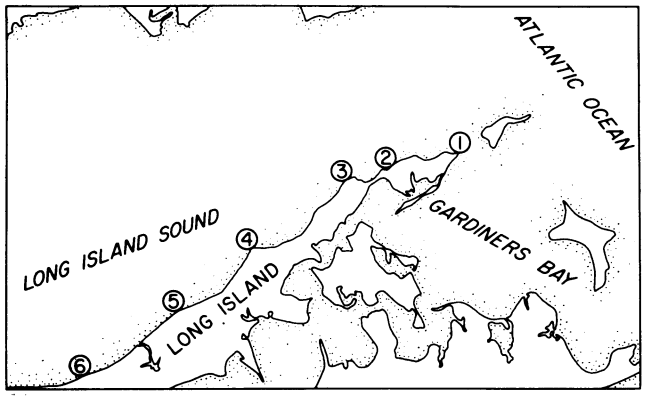

FIG. 1. Geographic location of sample sites at the eastern entrance to Long Island Sound from the Atlantic Ocean. Numbered localities correspond to those in Figs. 2 and 3 and in the text.

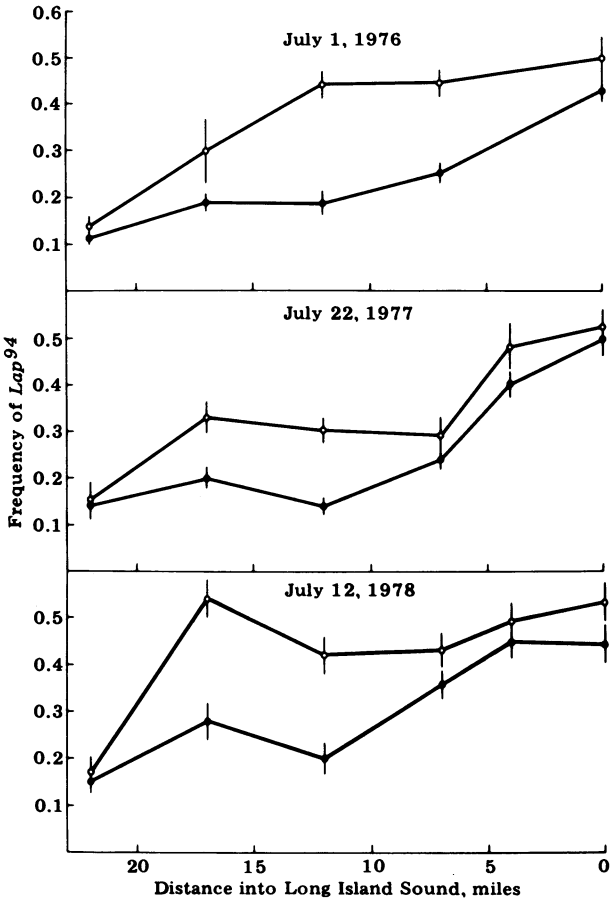

FIG. 2. Clinal decrease in the frequency of Lap^{94} at the entrance to Long Island Sound (see Fig. 1) for immigrants (○) and adults (●) in 1976–1978. Data for 1976 are from Lassen and Turano (36). Sample size for each point averaged 119 individuals. Locality 1 is at mile 0.

Mortality of Immigrants. The annual immigration of oceanic larvae, carrying a high frequency of the Lap^{94} allele (Fig. 2), coupled with a low frequency of the Lap^{94} allele at these same sites in resident adults, illustrates that annual immigration is followed by mortality. When mussels at localities 1–5 were examined by 5-mm size classes, there was a decreased frequency of the Lap^{94} allele with increasing shell size (Fig. 3). The pattern of decrease in the frequency of Lap^{94} was constant at each site in each year but differed among sites. At localities 1 and 2 there was only a slight decrease in the frequency of Lap^{94} with size, but a large and gradual decrease in the frequency of Lap^{94} with shell size at localities 4 and 5 (Fig. 3). At East Marion (locality 3), the decrease of Lap^{94} with increasing shell size was most rapid in both 1977 and 1978.

In each year, pairwise product–moment correlations were computed among allele frequency, enzyme activity, and environmental salinity separately for immigrants and adults.

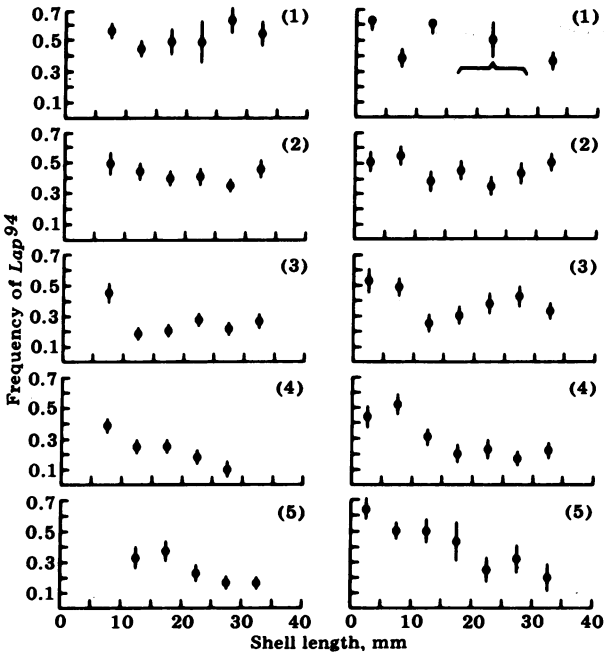

FIG. 3. Size-dependent decrease in the frequency of *Lap*^{94} with increasing shell size at localities 1–5 (Fig. 1) in 1977 (*Left*) and in 1978 (*Right*). 1, Orient Point; 2, Orient Town; 3, East Marion; 4, Horton's Point; and 5, Depot Road.

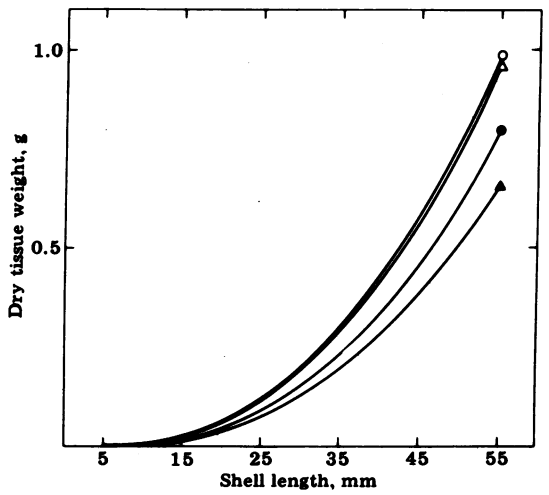

FIG. 4. Relationship between dry tissue weight and shell length in July, 1979 (○ and △) and October, 1979 (● and ▲) for individuals with (△ and ▲) and without (○ and ●) the *Lap*^{94} allele. Although statistical analyses were performed on $\log_{10}$-transformed variables, the illustrated curves are the computed regression lines, which were detransformed. Each regression involved >200 data points.

Correlations were computed between allele frequencies in different years. Several important results emerged from this analysis (Table 1). Because total aminopeptidase-I enzyme activity rapidly acclimates to environmental salinity (21, 34), it was not surprising that there was a significant correlation between measured enzyme activity and salinity among localities 1–5. Second, the frequency of *Lap*^{94} in immigrants was not significantly correlated with either environmental salinity or total enzyme activity (measured in adults). There were no significant among-year correlations in the frequency of *Lap*^{94} in immigrants, illustrating the annual variation of immigration distance by oceanic larvae. In contrast, nearly all correlations among allele frequency, salinity, and enzyme activity in resident adults were statistically significant (Table 1). Also, the frequency of the alleles in resident adults was highly correlated among years, illustrating the *temporal stability* in the position of the allele frequency cline in adults.

Tests of physiological condition compared two groups of genotypes: individuals with and without the *Lap*^{94} allele. In July, a time of high food availability, there were no significant differences between groups in exponents or intercepts relating dry tissue weight to shell length (Fig. 4). In October, a period when nutrient levels are declining, there was no significant heterogeneity between groups in exponents ($F_{1,401} = 2.625$; not significant), but intercepts differed significantly between groups ($F_{1,402} = 40.465$; $P < 0.001$). This result demonstrates that animals possessing the *Lap*^{94} allele, in either heterozygous or homozygous condition, had, on the average, a significantly larger amount of tissue loss than animals without the *Lap*^{94} allele during the period July–October, when differential mortality occurred (Table 2). Because intercepts (but not exponents) differed, there was a proportional weight loss in animals of each size.

DISCUSSION

Lassen and Turano (36) have claimed that this cline was due to population mixing of pelagic larvae from Atlantic Ocean and Long Island Sound populations, a conclusion based partly on two cases of significant heterozygote deficiency at localities 4 and 5 (Fig. 2) in immigrants. We can find no evidence to sup-

Table 1. Correlations between allele frequency (*Lap*^{94} and *Lap*^{96}) in different years, salinity, and aminopeptidase-I activity (arbitrary units/ml, under standard conditions) among localities (Fig. 1)

	Immigrants			Adults		
	1976	1977	1978	1976	1977	1978
Lap^{94}						
Salinity	0.873*	0.750	0.049	0.919*†	0.850	0.849
Enzyme activity	0.751	0.800	0.172	0.918‡	0.884†	0.949†
Lap^{94}: 1976	—	0.416	−0.351	—	0.990‡	0.900†
1977	—	—	0.662	—	—	0.909†
Lap^{96}						
Salinity	−0.892*	−0.663	−0.725	−0.715*	−0.911†	−0.894
Enzyme activity	−0.793	−0.762	−0.852	−0.867	−0.965‡	−0.913
Lap^{96}: 1976	—	0.277	0.601	—	0.956‡	0.932†
1977	—	—	0.538	—	—	0.945†

Correlation between salinity and enzyme activity was nearly unity.
* Degrees of freedom (df) = 2; all others, df = 3.
† $P < 0.05$.
‡ $P < 0.01$.

Table 2. Frequencies of Lap^{94} (±SE) in samples of immigrants and adults in July and October 1979 at East Marion (locality 3) from which the condition index (Fig. 4) was determined

Month	Immigrants	Adults
July	0.50 ± 0.024	0.44 ± 0.027
October	0.21 ± 0.028	0.41 ± 0.020

port this claim, and several observations are inconsistent with their interpretation. These points are elaborated elsewhere (50). Briefly, the evidence against population mixing includes differences at each site in both the magnitude and sign of Wright's fixation index (51), F, from a Wahlund expectation; significant values of F only rarely occur at localities with intermediate (i.e., "mixed" allele frequencies; and the spatial distribution of F values in the immigrant and resident clines are different from one another.

Larvae of *M. edulis* are pelagic between 3 and 7 weeks (40). Our results do not suggest what forces limit larval dispersal into Long Island Sound to less than 20 miles, but the mortality of the immigrant groups would indicate that oceanic larvae are not adapted to the Long Island Sound environments. Growth rates of larvae from oceanic environments are reduced in low salinity (40).

Although the position of the allele frequency cline in immigrants varies from year to year, the cline in resident adults after mortality is spatially stable (Table 1) and the rate of mortality is site specific. Decrease in Lap^{94} frequency in immigrants could be due to mortality of physiologically differentiated individuals from oceanic populations that are coincidentally marked by a high frequency of Lap^{94}, as proposed by Levinton and Lassen (37, 38). This explanation does not require selection at the *Lap* locus. Alternatively, significantly different fitnesses among individuals of different *Lap* genotypes are required, which lead to a population after selection characterized by a low frequency of Lap^{94} genotypes specifically because of differences in aminopeptidase-I allozymes.

A clear distinction between these alternative explanations requires some direct evidence of differential mortality among *Lap* genotypes. Ideally, such evidence should be related to aminopeptidase-I function. The change in the relationships of tissue weight and shell size between July and October demonstrates that catabolism of nutrient reserves occurs in the same time period as the mortality of a large proportion of the immigrants possessing a Lap^{94} allele. The significantly different tissue weights between postselected (Fig. 4) individuals with and without the Lap^{94} allele is indicative of greater stress in Lap^{94} individuals. The postselected population apparently regains tissue reserves by the following summer.

Any hypothesis that attempts to explain differentiation of *Lap* allele frequencies among different areas must take account of the effect of both salinity and temperature upon both digestive and osmoregulatory physiologies. The frequency of Lap^{94} declines in estuaries south of Cape Cod but it also declines in a northward direction at Cape Cod, where there is no change in environmental salinity but a decrease in water temperature (19).

High temperatures elicit increased enzyme concentration in lysosomal enzymes (52). High salinity induces high aminopeptidase-I activity as an apparent adaptive response to effective cell volume regulation. We would therefore expect the evolution of a hydrolytic enzyme (or allozyme) with high catalytic rate in an environment with high temperatures and oceanic salinities. At warm temperatures and high oceanic salinities, increased reaction rate is adaptive because effective osmoregulation requires relatively higher enzyme activity and elevated activity is thermally compensatory. Lap^{94} is found in high frequency only where these two environmental conditions occur together (19). The Lap^{94} phenotypes seem to effectively extend the thermal compensatory capacity of the aminopeptidase-I enzyme because genotypes with the Lap^{94} allele exhibit higher specific activity relative to total protein (34) and per unit enzyme concentration (53).

Low salinity modulates aminopeptidase-I activity to lower levels. What might be the disadvantage of the Lap^{94} allele at low salinities and warm temperatures, such as occur in Long Island Sound? This appears to be a function of the potential "cost" of increase V_{max} in digestive physiology. Permitting the absorption of the products of protein digestion to proceed too rapidly would be disadvantageous (54). We might then suggest that at low salinities, where overall aminopeptidase-I activity is low, the Lap^{94} phenotypes will have a decreased efficiency of nitrogen assimilation because of the imbalance between uptake rate of amino acids from the gut and the rate of amino acid utilization. At times when environmental nitrogen is limiting, this would lead to an increased rate in the utilization of protein reserves and, thereby, to reduced tissue weight (32), such as we have observed.

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