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Enzyme Polymorphism Associated with Habitat Choice in the Intertidal Snail *Tegula funebralis*

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The black turban snail, Tegula funebralis, *is found in the intertidal zone of the North American Pacific coast, a region of extreme spatial heterogeneity. Associations between genetic polymorphism and habitat choice are predicted by theoretical models of the maintenance of genetic polymorphism in spatially heterogeneous environments, such as that occupied by* Tegula funebralis. *Habitat choice was studied in individually marked snails at two locations. Genotypes of marked snails were then determined at two highly polymorphic enzyme loci, leucine aminopeptidase (LAP) and phosphoglucose isomerase (PGI), using starch gel electrophoresis. Multiple regression analysis showed that a snail's size, sex, and enzyme genotype were associated with its habitat selection behavior.*

KEY WORDS: habitat choice; enzyme polymorphism; intertidal snail; *Tegula funebralis*; individual-marking technique.

INTRODUCTION

Theoretical models predict an association between habitat choice and genetic polymorphism (Levene, 1953; Hedrick *et al.*, 1976; Powell and Taylor, 1979). If individuals choose habitats in which their fitness is highest, the severity of natural selection required to maintain a balanced polymorphism in a heterogeneous environment is expected to be reduced. Conversely, if a polymorphism is being maintained by selection in a spa-

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tially heterogeneous environment, such selection might be a force which would promote the evolution of habitat choice (Maynard Smith, 1966; Silcock and Parsons, 1973; Taylor, 1975; Powell and Taylor, 1979). Such theoretically predicted associations between habitat choice and genetic polymorphism have been demonstrated in many animal species, including insects (Kettlewell, 1955; Sargent, 1966; De Souza *et al.*, 1970; Trpis and Hausermann, 1975), mollusks (Sedlmair, 1956; Giesel, 1970), and mammals (Wecker, 1963; Silcock and Parsons, 1973). Associations between habitat choice and allozyme polymorphism have also been reported (Taylor and Powell, 1978; Cavener, 1979).

Habitat choice has been observed in the intertidal snail *Tegula funebralis* (Byers and Mitton, 1981). In studies using a mass-marking technique, most snails found either above or in permanent tidepools at low tide returned to their original habitats within a few days after experimental habitat reversal. Experiments with individually marked snails showed that they prefer specific intertidal levels, and not merely above-pool or in-pool habitats.

These habitat-choice studies using individually marked *Tegula funebralis* differ in two ways from other studies of habitat choice which have been reported. First, whereas previous studies used qualitative measures of habitat preference, a quantitative measure, the height above or below the surface of the study pool, was used in these studies. Second, individual marking allows repeated measurement of the height preference of each snail, so the variance of this measure can be calculated. Since habitat choice occurs in this species (Byers and Mitton, 1981), variance in the height chosen could be interpreted as a measure of the strength of habitat preference. Quantification of this aspect of habitat choice has not been reported previously.

This report gives evidence for associations between enzyme polymorphism and habitat selection behavior. Starch gel electrophoresis was used to determine the enzyme genotypes of snails whose intertidal height preferences had been measured in the field. Stepwise multiple regression analysis showed some associations between the size and enzyme genotype of snails and their mean intertidal height, and between their size, sex, and enzyme genotype and the standard deviation of their height.

MATERIALS AND METHODS

Measurement of Habitat Preference

Habitat choice was studied in the field at two sites on the northern U.S. Pacific coast, Cape Arago, Oregon, and Mukkaw Bay, Washington, using an individual-marking technique (Byers and Mitton, 1981). In these experiments, the height of equal numbers of snails originally found above or below the surface of a permanent tidepool was first measured to the nearest centimeter. Snails were then marked by gluing numbered plastic tags to their shells. Marked snails were released at the surface of the study pool, their height was measured 48 h later, and they were again moved to the waterline of the pool. Positions of marked snails which were not found 48 h later were measured on the first subsequent day they could be found, and they were then restarted at the waterline of the pool. During an experimental series, the intertidal height of most marked snails was measured at least twice, and in some cases four or five times. Since snails were placed at the waterline of the pool after each position measurement, a number of independent measures of intertidal height preference were obtained for each individual. Using data from these experiments, the mean and standard deviation of intertidal height chosen by each snail were calculated.

Sample Preparation

After habitat-choice measurements were completed, snails were shipped alive to the laboratory in Boulder, Colorado. They were then frozen immediately and held at −60°C for processing. Shell diameter, taken as the maximum width across the umbilicus from the upper margin of the shell lip to the opposite side of the body whorl (Frank, 1975), was measured to the nearest 0.1 mm using vernier calipers. Shells were then carefully cracked and the sex of the individual was determined from the color of the gonads—these are dark green in females and cream-colored in males. Entire snails then were cut into small pieces with dissecting scissors while still mostly frozen and ground with approximately 1 ml of deionized water using a mortar and pestle. This material was transferred to small test tubes and refrozen at −60°C until gels were run. Some samples were stored for 2 years in the freezer with only a slight decrease in the activity of most enzymes.

Electrophoretic Systems

Eleven enzyme loci could be reliably resolved using horizontal starch gel electrophoresis. Five of these were polymorphic, and six appeared to be monomorphic (Byers, 1980). Of the five polymorphic loci, two were highly polymorphic, and only these are discussed in this report.

Breeding studies needed to demonstrate the Mendelian inheritence of enzyme phenotypes would be difficult and time-consuming, if not impossible, in *Tegula funebralis*, since this species probably has a long planktonic larval stage (Frank, 1975) and does not become sexually mature until several years old. However, four observations support the interpretation that the variation in enzyme phenotypes is the result of allelic variation at structural gene loci. (1) Enzyme phenotypes fit a pattern of diploid Mendelian inheritence. No individual has more than two enzyme forms, or three in the case of the dimeric enzyme PGI, where homo- and heterodimers are found. All possible phenotypes were observed, except for a few homozygous combinations of the rarest alleles, which were not expected in samples of the size analyzed. (2) Enzyme phenotypes do not change qualitatively with age; the same patterns are seen in newly settled snails, juveniles, and 25-year-old adults. (3) Different tissues (foot muscle, gut, and gonads) from an individual all show the same enzyme phenotype. (4) Observed phenotypic distributions fit Hardy–Weinberg expectations for both loci in all populations sampled. The following analysis assumes that the variation revealed by electrophoresis is allozyme variation.

Leucine aminopeptidase (LAP) had five electromorphs; the most common of these had a frequency of about 0.50 in the populations of *Tegula funebralis* surveyed. Phosphoglucose isomerase (PGI) had six electromorphs; the frequency of the most common of these was about 0.55.

Both of these enzymes were resolved using the discontinuous buffer system of Mitton *et al.* (1977) and 12% starch gels (Sigma starch). The staining solution for LAP contained 10 ml of 0.16 *M* potassium phosphate buffer (pH 7.0), 20 mg of fast garnet GBC salt, 10 mg of L-leucyl β-naphthylamide dissolved in 5 ml of *N,N*-dimethylformamide, and 90 ml of distilled water. Phosphoglucose isomerase was stained using 10 ml of Tris–HCl buffer (pH 8.0), 10 mg of NAD, 10 mg of fructose 6-phosphate, 5 mg of MTT, 3 mg of PMS, and 10 U of glucose 6-phosphate dehydrogenase, applied in an agar solution.

Statistical Analysis

For the purposes of statistical analysis, enzyme genotypes at each locus were scored according to the presence or absence of each of the five or six alleles at that locus. Each individual received a score of 0 or 1 for each of five or six independent variables corresponding to each of the five LAP or six PGI alleles. For example, a PGI^{23} heterozygote would score 1 at both PGI^{2} and PGI^{3} measures and 0 at PGI^{1}, PGI^{4}, PGI^{5}, and PGI^{6}. A PGI^{22} homozygote would also score 1 at PGI^{2} but 0 at PGI^{1}, PGI^{3}, PGI^{4}, PGI^{5}, and PGI^{6}.

A stepwise multiple regression program (Nie *et al.*, 1975) was used to analyze the data; size, sex, and presence or absence of each of the alleles at each locus were the independent variables, and either mean intertidal height or standard deviation of height was the dependent variable. To simplify presentation and interpretation of the results, groups possessing or lacking a particular allele were compared using *t* tests if the multiple regression procedure showed a significant association of that allele and habitat choice measures.

Correlations between mean height and standard deviation of height were calculated; a partial correlation procedure (Nie *et al.*, 1975) was used to control for the possible influence of size on this correlation.

RESULTS

Mean Intertidal Height

Stepwise multiple regression showed that both size and LAP genotype were significantly associated with mean intertidal height at Cape Arago, Oregon (Table I). The negative sign of the regression coefficient, *b*, of mean height on size indicates that, as snails get larger, they tend to choose lower positions in the intertidal. The presence or absence of the LAP^2 allele in a snail's genotype made a significant contribution to the regression solution. The positive regression coefficient, *b*, for this independent variable indicates that snails with the LAP^2 allele in their genotypes chose higher mean heights than snails with genotypes lacking this allele. Snails with LAP^2 had a mean height ($\pm$SD) of 15.5 ($\pm$18.1) cm, while those lacking the LAP^2 allele had a mean height of 8.2 ($\pm$14.6) cm, a statistically significant difference ($t = 2.17$, df $= 162$, $P < 0.05$). Analysis of the Mukkaw Bay data also showed that size was associated with mean height but did not show an association of LAP genotype and mean height (Table I).

Regression analysis showed no association between presence or absence of any PGI alleles and mean intertidal height at Cape Arago (Table I). At the Mukkaw Bay site, the analysis showed that, in addition to size,

Table I. Summary of Stepwise Multiple Regression of Mean Intertidal Height on Size, Sex, and Genotypic Score

Site	Variable entered	*F* to enter	Statistical significance	*b*
Cape Arago[a]	Size	28.59	$P < 0.001$	−5.86
	LAP^2	3.95	$P < 0.05$	8.73
Mukkaw Bay[b]	Size	16.91	$P < 0.001$	−3.69
	PGI^5	3.79	$P \cong 0.05$	−8.28

[a] $N = 164$.
[b] $N = 220$.

the presence or absence of the PGI^5 allele in a snail's genotype made a significant contribution to the regression solution. The mean height of snails with the PGI^5 allele was −15.2 (±12.6) cm (15.2 cm below the pool surface) at this site; that of snails lacking the PGI^5 allele was −5.2 (±17.7) cm. This difference is significant (t = 2.01, df = 218, $P < 0.05$). The PGI^5 allele is relatively rare and is found in only 13 individuals in the sample from this site.

Standard Deviation of Height

Multiple regression analysis showed that both sex and genotype at the PGI locus were associated with standard deviation of height in the Cape Arago population (Table II). The presence or absence of the PGI^5 allele in the genotype is the first independent variable to enter the regression solution. The negative sign of the regression coefficient indicates that snails with the PGI^5 allele have smaller standard deviations of height than those lacking this allele. The mean standard deviation of height of individuals with PGI^5 is 7.6 (±8.7) cm, while that of snails lacking PGI^5 is 21.5 (±13.1) cm, a statistically significant difference (t = −2.58, df = 162, $P < 0.01$). As at Mukkaw Bay, PGI^5 is a rare allele; only six individuals with this allele were found in the sample from Cape Arago. The second independent variable to enter the regression solution is sex. Since males were assigned a score of 1 and females a 2, the positive sign of the regression coefficient indicates that females have greater standard deviations of position than males. The analysis suggested that the contributions made by the presence or absence of both the PGI^2 and the PGI^1 alleles are close to significance, at $P \cong 0.06$ (Table II). LAP genotype is not significantly associated with standard deviation of height at Cape Arago (Table II).

Multiple regression analysis showed an association between size and

Table II. Summary of Stepwise Multiple Regression of Standard Deviation of Height on Size, Sex, and Genotypic Score

Site	Variable entered	F to enter	Statistical significance	b
Cape Arago[a]	PGI^5	6.65	$P \cong 0.01$	−11.36
	Sex	5.75	$P < 0.05$	5.07
	PGI^2	3.55	$P \cong 0.06$	4.32
	PGI^1	3.75	$P \cong 0.06$	8.39
Mukkaw Bay[b]	Size	5.97	$P < 0.05$	−1.80

[a] N = 164.
[b] N = 220.

marked snails were included in the calculations. Because the tidepools at both sites were only about 0.5 m deep, while rocks above the pools were several meters high, these correlations could be the result of a "floor effect," caused by snails with low mean heights congregating at the bottom of the pools and, therefore, having lower standard deviations of height than snails found higher. However, significant correlations between mean height and standard deviation of height were found even when all snails with mean heights below the pool surface were excluded from the calculations. This indicates that a floor effect is not the main cause of these correlations. A possible explanation for the correlations may be that snails found higher in the intertidal are forced to move more often than snails found lower due to intolerable environmental extremes, such as high temperature. These correlations appear to strengthen the conclusion that the presence or absence of the PGI^5 allele has an influence on the habitat choice. The presence or absence of PGI^5 was associated with mean height at Mukkaw Bay and with standard deviation of height at Cape Arago; since mean height and standard deviation of height are correlated at both sites, an association between this allele and habitat choice is suggested. The strength of the evidence for this association between PGI^5 and habitat choice should perhaps be tempered by noting that it is a relatively rare allele in these populations.

The independent variables associated with measures of habitat choice differ at the two study sites. An explanation of these differences requires further research. However, the Mukkaw Bay study pool was estimated to be 0.25 to 0.5 m higher in the intertidal zone than the study pool at Cape Arago, and this ecological difference between the two sites may influence the specific associations observed (Byers, 1980). By studying several pools at different heights at each site, the generality of specific associations between the genotype, sex, and size of snails and the measures of habitat choice could be tested.

The inverse relationship found in this study between size and intertidal height in *Tegula funebralis* has been reported previously (Paine, 1969; Frank, 1975). Studies by Fawcett (1979) suggest that this inverse relationship occurs only at sites where predators of black turban snails are not abundant.

Genetic polymorphism at the LAP locus in the blue mussel, *Mytilus edulis,* has been the subject of several studies (Koehn *et al.*, 1976; Koehn, 1978; Young *et al.*, 1979), and these suggest testable hypotheses about its function in *Tegula funebralis*. Black turban snails found higher in the intertidal zone are subjected to higher temperatures, greater temperature variation, and reduced salinity during periods of rain at low tide. These factors could contribute to selection acting on LAP phenotypes.

The relationship between environmental temperature and PGI postandard deviation of height at Mukkaw Bay (Table II). The negative sign of the regression coefficient indicates that larger snails have a smaller standard deviation of height than smaller snails. No significant association of genotypic measures and standard deviation of height was found at this site.

Correlation of Mean Height and Standard Deviation of Height

The partial correlation between mean height and standard deviation of height for all marked snails at Cape Arago is 0.29 (df = 161, $P < 0.001$), and that at Mukkaw Bay is 0.48 (df = 217, $P < 0.001$). When only snails whose mean height was above the pool surface were used in the calculations in order to reduce possible "floor effects," these partial correlations were 0.27 (df = 128, $P < 0.001$) at Cape Arago and 0.28 (df = 64, $P < 0.01$) at Mukkaw Bay.

DISCUSSION

Size, sex, and the presence or absence of some alleles at two highly polymorphic enzyme loci are associated with measures of habitat choice in *Tegula funebralis*. At Cape Arago, a snail's size and the presence or absence of the LAP^2 allele in its genotype were significant predictors of its mean intertidal height. In the Mukkaw Bay population, size and the presence or absence of the PGI^5 allele were associated with mean height. The sex of a snail and the presence or absence of the PGI^5 allele in its genotype were associated with the standard deviation of its height at Cape Arago. Only size was associated with this measure at Mukkaw Bay.

The statistically significant associations between genotypes at two polymorphic enzyme loci and measures of habitat preference in *Tegula funebralis* fit the prediction of the theoretical models discussed in the Introduction, if it is assumed that some of the genetic variation at these loci, or at closely linked loci, is related to fitness in the heterogeneous intertidal environment.

Associations between measures of habitat choice and enzyme polymorphism were not universally found. No associations were found between any PGI alleles and mean height at Cape Arago, or any LAP alleles and mean height at Mukkaw Bay. Neither the PGI nor the LAP genotype was associated with the standard deviation of the height at Mukkaw Bay, and the LAP genotype was not associated with this measure at Cape Arago.

Highly significant positive correlations between mean height and standard deviation of height were found at both sites when data from all lymorphism has been studied in the anemone *Metridium senile* (Hoffmann, 1981a,b), *Colias* butterflies (Watt, 1977), and several species of marine mollusks (Wilkins, 1977; Wilkins *et al.*, 1978). Based on these studies, it seems useful to speculate that PGI variation in *Tegula funebralis,* and perhaps its behavioral correlates also, is somehow related to thermal adaptation. Some evidence supporting such speculation exists. The distribution of *T. funebralis* populations in the intertidal zone is influenced by temperature contrasts (Byers, 1980). Following warm, sunny days, when temperature differences between in-pool and above-pool habitats are great, snail populations move lower in the intertidal zone and constrict their range compared to that on days following cool, overcast weather. The population distribution is determined by the movement of the individual snails comprising it, so it is logical to assume that temperature contrasts influence the height preferences of individuals. As described in the present report, the mean height of individual snails was associated with the PGI genotype at Mukkaw Bay, and the standard deviation of the height and the PGI genotype were associated at Cape Arago. Because both temperature contrasts and the PGI genotype are associated with the intertidal height preferences of snails, these two factors may somehow be functionally related, perhaps through an effect of the PGI phenotype on thermal physiology.

One of the most interesting results of this study is the association, found at Cape Arago but not Mukkaw Bay, between the PGI polymorphism and the standard deviation of the height of individual snails. Because habitat choice occurs in this species (Byers and Mitton, 1981), the standard deviation of the height of individuals could be interpreted as a measure of the strength of their habitat preference. Many examples of associations between genetic polymorphism and absolute habitat preferences have been reported in the literature (Kettlewell, 1955; Sedlmair, 1956; Wecker, 1963; Sargent, 1966; De Souza *et al.*, 1970; Giesel, 1970; Silcock and Parsons, 1973; Trpis and Hausermann, 1975; Taylor and Powell, 1978; Cavener, 1979). However, no example of an association between genetic polymorphism and strength of habitat preference has been reported previously. Studies of marked individuals whose habitat preferences can be measured repeatedly are needed to demonstrate such associations. Since previous studies have used mass-marking rather than individual-marking techniques, data needed to calculate the variance of repeated habitat choices of individuals have not been available.

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REFERENCES

Byers, B. A. (1980). *The Ecological Behavioral Genetics of Habitat Selection in an Intertidal Snail,* Tegula funebralis, Ph.D. thesis, University of Colorado, Boulder.

Byers, B. A., and Mitton, J. B. (1981). Habitat choice in the intertidal snail *Tegula funebralis*. *Mar. Biol.* **65**:149–154.

Cavener, D. (1979). Preference for ethanol in *Drosophila melanogaster* associated with the alcohol dehydrogenase polymorphism. *Behav. Genet.* **9**:359–365.

De Souza, H. M. L., Da Cunha, A. B., and Dos Santos, E. P. (1970). Adaptive polymorphism of behavior evolved in laboratory populations of *Drosophila willistoni*. *Am. Nat.* **104**:175–189.

Fawcett, M. H. (1979). *The Consequences of Latitudinal Variation in Predation for Some Marine Intertidal Herbivores,* Ph.D. thesis. University of California, Santa Barbara.

Frank, P. W. (1975). Latitudinal variation in the life history features of the black turban snail *Tegula funebralis* (Prosobranchia: Trochidae). *Mar. Biol.* **31**:181–192.

Giesel, J. T. (1970). On the maintenance of a shall pattern and behavior polymorphism in *Acmaea digitalis,* a limpet. *Evolution* **24**:98–119.

Hedrick, P. W., Ginevan, M. E., and Ewing, E. P. (1976). Genetic polymorphism in heterogeneous environments. *Ann. Rev. Ecol. Syst.* **7**:1–32.

Hoffmann, R. J. (1981a). Evolutionary genetics of *Metridium senile*. I. Kinetic differences in phophoglucose isomerase allozymes. *Biochem. Genet.* **19**:129–144.

Hoffmann, R. J. (1981b). Evolutionary genetics of *Metridium senile*. II. Geographic patterns of allozyme variation. *Biochem. Genet.* **19**:145–154.

Kettelwell, H. B. D. (1955). Recognition of appropriate backgrounds by the pale and black phases of Lepidoptera. *Nature* **175**:943–944.

Koehn, R. K. (1978). Biochemical aspects of genetic variation at the LAP locus in *Mytilus edulis*. In Battaglia, B., and Beardmore, J. A. (eds.), *Marine Organisms,* Plenum Press, New York and London.

Koehn, R. K., Milkman, R., and Mitton, J. B. (1976). Population genetics of marine pelecypods. IV. Selection, migration, and genetic differentiation in the blue mussel *Mytilus edulis*. *Evolution* **30**:2–32.

Levene, H. (1953). Genetic equilibrium when more than one niche is available. *Am. Nat.* **87**:331–333.

Maynard Smith, J. (1966). Sympatric speciation. *Am. Nat.* **100**:637–650.

Mitton, J. B., Linhart, Y. B., Hamrick, J. L., and Beckman, J. S. (1977). Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. *Theor. Appl. Genet.* **51**:5–13.

Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrenner, K., and Bent, D. H. (1975). *Statistical Package for the Social Sciences,* 2nd ed., McGraw-Hill, New York.

Paine, R. T. (1969). The *Pisaster-Tegula* interaction: Prey patches, predator food preference, and intertidal community structure. *Ecology* **50**:950–961.

Powell, J. R., and Taylor, C. E. (1979). Genetic variation in ecologically diverse environments. *Am. Sci.* **67**:590–596.

Sargent, T. D. (1966). Background selections of geometrid and noctuid moths. *Science* **154**:1674–1675.

Sedlmair, H. (1956). Verhaltens-, Resistenz-, und Gehäuseunterschiede bei den polymorphen Bänderschnecken *Cepaea hortensis* (Müll.) und *Cepaea nemoralis* (L.). *Biol. Zentr.* **75**:281–313.

Silcock, M., and Parsons, P. A. (1973). Temperature preference differences between strians of *Mus Musculus,* associated variables and ecological implications. *Oecologia* **13**:147–160.

Taylor, C. E. (1975). Genetic loads in heterogeneous environments. *Genetics* **80**:621–635.

Taylor, C. E., and Powell, J. R. (1978). Habitat choice in natural populations of *Drosophila*. *Oecologia* **37**:69–75.

Trpis, M., and Hausermann, W. (1975). Demonstration of differential domesticity of *Aedes aegypti* (L.) (Diptera, Culicidae) in Africa by mark-release-recapture. *Bull. Entomol. Res.* **65**:199–208.

Watt, W. B. (1977). Adaptation at specific loci. I. Natural selection on phosphoglucose isomerase of *Colias* butterflies: Biochemical and population aspects. *Genetics* **87**:177–194.

Wecker, S. C. (1963). The role of early experience in habitat selection by the prairie deer mouse, *P. Maniculatus bairdii*. *Ecol. Monogr.* **33**:307–325.

Wilkins, N. P. (1977). Genetic variability in littoral gastropods: Phosphoglucose isomerase and phosphoglucomutase in *Patella vulgata* and *P. aspera*. *Mar. Biol.* **40**:151–155.

Wilkins, N. P., O'Regan, D., and Moynihan, E. (1978). Electrophoretic variability and temperature sensitivity of phosphoglucose isomerase and phosphoglucomutase in Littorinids and other marine molluscs. In Battaglia, B., and Beardmore, J. A. (eds.), *Marine Organisms*, Plenum Press, New York and London.

Young, J. P. W., Koehn, R. K., and Arnheim, N. (1979). Biochemical characterization of "LAP," a polymorphic aminopeptidase from the blue mussel, *Mytilus edulis*. *Biochem. Genet.* **17**:305–323.

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