

## ADAPTIVE MAINTENANCE OF GENETIC POLYMORPHISM IN AN INTERTIDAL BARNACLE: HABITAT- AND LIFE-STAGE-SPECIFIC SURVIVORSHIP OF *Mpi* GENOTYPES

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**Abstract.**—In the northern acorn barnacle, *Semibalanus balanoides*, genotype frequencies of three genetic markers were tracked over time in four types of intertidal habitats. These habitats were selected to represent natural variation in several environmental parameters, specifically the degree of physical stress experienced by barnacles. Frequencies for one allozyme locus (*Gpi*) and a presumably neutral mtDNA marker were homogeneous among habitats in each temporal sample. Similarly, no temporal stratification in genotype frequencies was evident across the five sampling intervals: from planktonic larvae sampled in March to juveniles collected at the end of June. In contrast to the *Gpi* and mtDNA loci, *Mpi* genotypes significantly changed in frequency in two habitats in the high intertidal zone. On exposed substrate, the *Mpi*-FF homozygote increased in frequency, whereas the alternative homozygote, *Mpi*-SS, significantly decreased in frequency. Barnacles that were protected from environmental stress at high intertidal heights by the *Ascophyllum nodosum* algal canopy demonstrated the opposite pattern. In both habitats, the change in frequency of the heterozygote was intermediate to that of the homozygous genotypes. Furthermore, these patterns of genotype-by-environment association reflected a pulse of genotype-specific mortality that occurred over a two-week interval subsequent to metamorphosis from the larval to the adult form. These data indicate that each *Mpi* homozygote is the highest fitness genotype in some portion of the intertidal environment. Using the Levene (1953) model to evaluate the spatial variation in genotypic fitness, the stable maintenance of the *Mpi* polymorphism is predicted under certain subsets of conditions. Environmental heterogeneity in the intertidal zone translates to spatial variation in selection pressures, which may result in the active maintenance of the *Mpi* polymorphism in this species.

**Key words.**—Balancing selection, *Mpi*, *Semibalanus balanoides*.

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How organisms adapt to environmental heterogeneity remains a fundamental issue in evolutionary biology. The mechanisms by which a species responds to selection regimes that vary in space or time can be dependent on the correlation between the environments experienced by parent and offspring (e.g., Levins 1968). In turn, this relationship may be greatly affected by the spatial scale of environmental variation as well as life-history attributes of a given taxon. In marine systems, larval dispersal ability has often been hypothesized as the primary determinant of population genetic structure (Burton 1983; Hedgecock 1986; Palumbi 1995). A general association exists between mode of dispersal and the degree of genetic differentiation among populations (Bohonak 1999). Species characterized by limited dispersal potential may exhibit significant genetic structure at relatively small spatial scales (Ayre and Dufty 1994; Hellberg 1994); such evolutionary independence of distinct populations could allow local adaptation to and specialization in different environments (Holt and Gaines 1992; Ayre 1995). In contrast, species with planktonic larval or gametic dispersal often demonstrate no detectable population structure over thousands of kilometers (Hellberg 1996; McFadden et al. 1997; Lessios et al. 1998). High levels of gene flow among populations may result in open rather than closed population dynamics (Menge and Olson 1990), which may preclude local adaptation.

The maintenance of genetic polymorphism in natural populations can also reflect the process of adaptation to environmental heterogeneity (e.g., Hedrick et al. 1976; Hedrick

1986). This process is not dependent on local adaptation of populations. For example, if selection favors one homozygous genotype in one type of environment and the alternative homozygote in another, the heterozygous genotype may be of highest arithmetic or harmonic mean fitness (marginal overdominance: Prout 1967; Wallace 1968; Gillespie 1976). Thus, the heterozygous state may act as a buffer against environmental variance, and the presence of environmental heterogeneity results in the realization of a higher heterozygote fitness potential (Maynard Smith 1998). Similarly, Levene (1953) proposed a model of balancing selection predicated on the existence of multiple environmental niches with distinct selection regimes. When alternative genotypes exhibit higher fitness in alternative environments, spatially variable selection can result in the adaptive maintenance of genetic polymorphism in natural populations.

In marine species with high dispersal and negligible predicted population structure, balanced genetic polymorphisms may be a common reflection of the interaction between organismal fitness and variation in the physical environment (e.g., Hilbish and Koehn 1985; Hedgecock 1986; Karl and Avise 1992; Schmidt et al. 2000). In the northern acorn barnacle, *Semibalanus balanoides*, individual larvae can travel more than 70 km from their source (Flowerdew 1983) during an approximately five- to eight-week tenure in the plankton (Lucas et al. 1979). Planktonic larval dispersal in this species predicts high levels of gene flow among populations (Crisp 1978), which has been supported by both mtDNA genealogies (Brown 1995) and allele frequency distributions for hypervariable microsatellite markers (A. D. Kern, P. S. Schmidt, and D. M. Rand, unpubl. ms.).

Barnacles experience a great deal of environmental het-

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erogeneity at a variety of spatial scales. In particular, the effect of variation in physical stress on barnacle survivorship and distributional patterns has been extensively studied. Temperature and/or desiccation stress determines the upper distributional limit of barnacles in the high intertidal zone (Connell 1961; Wetthey 1984). The magnitude of this stress and resulting patterns of mortality are affected by spatial orientation (Barnes 1958), barnacle density (Bertness 1989), the presence of ephemeral algae (Minchinton and Scheibling 1993), and protective cover offered by a canopy of the brown alga *Ascophyllum nodosum* (Bertness et al. 1999; Leonard 1999).

The intertidal region can be considered a mosaic of patches that greatly differ in environmental parameters that affect barnacle survivorship and fitness. Because barnacles are sessile, the intertidal environment is discrete and coarse grained. A given individual will only experience the environmental conditions of the microhabitat in which it settled. Unless barnacle larvae recruit to environments similar to those experienced by their parents, any response to spatially variable selection among microhabitats in a given generation will be obscured by annual recruitment of larvae from external sources. No cumulative genetic differentiation or local adaptation to microenvironments would be predicted. However, spatial variation in selection pressures may yet result in the active maintenance of allelic diversity at particular loci in this species.

Previously, we documented a consistent association between genotype frequencies at the mannose-6-phosphate isomerase (*Mpi*, E.C. 5.3.1.9) locus and the degree of physical stress experienced by barnacles in distinct habitat types (Schmidt and Rand 1999). Two lines of reasoning suggested this pattern was the result of selection and not random genetic drift and/or habitat-specific recruitment from different source populations. First, the pattern of genotype frequency variation was consistent across multiple sampled sites in two river estuaries and in two separate yearly cohorts. Second, genotype frequencies at another allozyme locus (glucose-6-phosphate isomerase [*Gpi*], E.C. 5.3.1.8) and a presumably neutral mtDNA marker (Brown 1995) were homogeneous among all samples.

When the level of temperature and/or desiccation stress experienced by barnacle recruits was experimentally manipulated in field transplants, genotype frequencies for the *Gpi* and mtDNA markers were unaffected. In contrast, *Mpi* homozygotes demonstrated reciprocal patterns of differential mortality in high-stress and low-stress microhabitats (Schmidt et al. 2000). The variation in genotypic viability estimates among microhabitats demonstrated a surprisingly strong fit to the Levene (1953) model of balancing selection. The link between *Mpi* genotype and organismal fitness is apparently due to the combination of mannose containing compounds in the barnacle diet and exposure to variable levels of physical stress in intertidal habitats. Experimental manipulations in the laboratory demonstrated that supplementing mannose in the diet and exposing barnacles to physical stress results in phenotypic differences among *Mpi* genotypes that predict patterns of survivorship (Schmidt 2001).

The present study is the fourth in a series designed to investigate the potential adaptive significance of allelic var-

iation at the *Mpi* locus in barnacle populations, using aspects of the methodology originally outlined in Clarke (1975). Here, genotype frequencies for three genetic markers are tracked over time in the plankton as well as in four distinct habitat types. This cohort analysis had three primary objectives. First, predictions regarding patterns of differential survivorship of *Mpi* genotypes were tested. We were particularly interested in evaluating the effects of environmental mediation by the algal canopy on genotype-specific patterns of mortality in the field. Second, we attempted to determine when selection acts during the barnacle life cycle: Does the differential mortality of genotypes occur gradually over time, or does it result from intense selection at a particular stage of barnacle development? Finally, we used viability estimates from temporal genotype frequency change in natural habitats to further assess whether the Levene (1953) model predicts the stable maintenance of the *Mpi* polymorphism in this species.

## MATERIALS AND METHODS

### *Sampling Design*

Planktonic larvae were sampled by sweeping a particular location with a 1-m<sup>2</sup> aperture net towed behind an outboard motorboat at a depth of approximately 0.5 m. *Semibalanus balanoides* cyprids were sorted in the laboratory, immersed in liquid nitrogen, and then transferred to a -80°C freezer for storage. Only cyprids were processed to observe any potential temporal stratification of genotype frequencies in the water column. The plankton were sampled on 27 March and 9 April from two locations in the Damariscotta River Estuary, Maine (44°54'N, 69°35'W): a midriver site (Hodgson Cove, approximately 6 km from the open coast) and an upper-river site (Glidden Ledge, approximately 10 km from the open coast).

Barnacle settlers and recruits were sampled at two previously characterized sites (E1 and E2 in Schmidt and Rand 1999) from exposed substrate (X) and from underneath the *Ascophyllum nodosum* algal canopy (A) in both the high (H) and low (L) intertidal zones. Thus, barnacles were collected from two replicates of four distinct habitat types: exposed-high (XH), exposed-low (XL), algae-high (AH), and algae-low (AL). Temperature data loggers (Onset Computer Corp., Pocasset, MA) were used to monitor surface substrate temperature in each habitat at each site. Thermal profiles were used as indicators of the degree of physical stress experienced by barnacles in each habitat. Consistent with temperature measurements in previous years (Schmidt and Rand 1999; Schmidt et al. 2000), the relative order of stress level among habitats, from high to low stress, was XH > XL > AH > AL. Approximately 100 barnacles were collected from each site by habitat area at four dates: recently settled cyprid larvae were collected on 9 April, metamorphs were collected on 21 April, and juveniles were collected on 5 May (early juveniles) and 30 June (midseason juveniles). After collection, individuals were stored in liquid nitrogen in the field and subsequently transferred to a -80°C freezer in the laboratory.

### Electrophoresis

The genotype of each collected individual was determined according to the protocols outlined in Schmidt and Rand (1999) for three genetic markers: the *Gpi* and *Mpi* allozyme loci and the mtDNA control region. Allozyme genotype was determined using cellulose acetate electrophoresis (Hebert and Beaton 1989). The two common alleles for both *Mpi* and *Gpi* are designated according to relative mobility, F for the fast allele and S for slow. The polymerase chain reaction was used to amplify a 401-bp fragment of the mtDNA control region using the *Iso-2* primer from Simon et al. (1994) and a species-specific primer designed from barnacle sequence near the 12S ribosomal gene (Brown 1995). A *DdeI* restriction enzyme polymorphism defined two mtDNA haplotypes (A and B) that were visualized on 3% agarose gels.

### Statistical Analyses

The procedure CATMOD (SAS ver. 6.12, SAS Institute, Cary, NC; see also Stokes et al. 1995) was used to model the relationship between the response variable (genotype) and the predictors. CATMOD fits a model for nominal, categorical variables using a maximum-likelihood estimation of generalized logits. For the two allozyme loci, which have three genotypes, two logits were formed for each combination of predictor variables. Parameter estimates yielded the differential effects of each predictor variable on each logit. For both *Mpi* and *Gpi*, the SS homozygous genotype was used as the reference genotype. The two logits were therefore the log probability of sampling the FF genotype relative to the log probability of sampling the SS genotype ( $\text{logit}_1$ ), and the log of the probability of sampling the SF genotype relative to the SS genotype ( $\text{logit}_2$ ). For the mtDNA control region, which has two haplotypes, only one logit was formed by CATMOD. Separate regressions were performed for each locus. In each analysis, the saturated model was used initially and predictor variables were subsequently excluded to generate the most parsimonious regression model that adequately fit the observed genotype counts. Model fit was evaluated by the likelihood ratio goodness-of-fit statistic.

The purpose of the employed sampling design was threefold: (1) to evaluate genetic homogeneity in the water column prior to settlement; (2) to assess whether genotype frequencies were homogeneous among larvae settling in the four habitats; and (3) to determine whether genotype frequencies changed in these habitats after settlement had occurred. Site, date, and the interaction term were included as predictor variables for the plankton data. The settler and recruit collections also had two primary components, habitat and date. Rather than including date of collection as another factor, separate sets of regressions were run for each of the four sampling dates. The focus was placed on modeling the genotype frequency variation among habitats and then comparing results from different time periods rather than modeling a simple interaction between habitat and date of sampling.

One concern with analyzing genotype frequencies in the same habitats at multiple points in time is the potential for statistical nonindependence (e.g., Koch et al. 1977) due to a shared history of selection. To account for this effect in the analyses, any differences in genotype frequencies among hab-

itats observed at a particular time point were evaluated in light of frequencies from previous sampling times. This was done in two ways. First, the genotype counts from a previous sample were also included in the settler/recruit analyses. The CATMOD-generated parameter estimates were used to calculate odds ratios, which outlined how the odds of sampling a given genotype had changed from the initial to the later sample in each of the habitat types. Second, odds ratios were compared among separate regression analyses. This made it possible to determine which temporal collection was responsible for significant effects in subsequent models. Thus, both the variation among habitats and change in genotype frequencies over time were analyzed.

### Evaluation of the Levene Model

The mean change in frequency over the sampling interval was used to estimate habitat-specific viability of *Mpi* genotypes (Prout 1965; Spiess 1989). These empirically derived fitness estimates were then evaluated in the context of the Levene (1953) model. Assuming that genotypic fitness is constant within but variable among habitats, the net fitness of the entire barnacle population was estimated as the geometric mean of fitnesses over  $k$  habitats (Spiess 1989):

$$\bar{W} = (\bar{W}_1)^{c_1} (\bar{W}_2)^{c_2} \dots (\bar{W}_k)^{c_k}, \quad (1)$$

where  $W_k$  is the average fitness in habitat  $k$  and  $c_k$  is the proportion of the population that exists in habitat  $k$ . This equation can be simplified and rearranged to

$$0 = \sum c_i \frac{(ps_1 - qs_2)_i}{\bar{W}_i}, \quad (2)$$

where  $s_1 = 1 - W_1$ ,  $s_2 = 1 - W_3$ ,  $W_1$  is the niche-specific fitness of the AA homozygote standardized by the fitness of the heterozygote,  $W_2$  is the standardized fitness of the Aa heterozygote (1.0),  $W_3$  is the standardized fitness of the aa homozygote,  $p$  and  $q$  are allele frequencies,  $\bar{W}_i$  is average fitness of the population in the  $i$ th habitat, and  $c_i$  is defined as the proportional reproductive output of the population occupying the  $i$ th habitat (Spiess 1989). Defining habitat proportions ( $c$ -values) and solving equation (2) yields an equilibrium allele frequency that reflects a balance between selection pressures among habitats. For a given set of  $c$ -values, the stability of any calculated equilibrium allele frequency was evaluated by graphically plotting the net fitness of the population over all habitats as a function of allele frequency (Li 1955). If the equilibrium represented a local fitness maximum, such that selection would restore any deviation in allele frequencies, the polymorphism was considered stable, or "protected."

## RESULTS

### Logistic Regression Analysis

For both the *Gpi* and mtDNA data from each temporal sample, all of the predictor variables could be dropped without compromising the fit of the model to the observed genotype counts. The average generalized logit across all statistical subpopulations, the intercept term, was by itself an adequate predictor (Table 1). No significant variation in ge-

TABLE 1. Likelihood ratio goodness-of-fit tests for regression models containing only the intercept term ( $P > 0.05$  indicates an adequate fit of the model to observed genotype counts).

Model	df	$\chi^2$	$P$
<i>Mpi</i> : settlers	22	9.75	0.989
<i>Mpi</i> : metamorphs	30	15.34	0.988
<i>Gpi</i> : plankton	6	3.80	0.704
<i>Gpi</i> : settlers	22	14.65	0.877
<i>Gpi</i> : metamorphs	30	24.70	0.739
<i>Gpi</i> : early juveniles	30	25.22	0.714
<i>Gpi</i> : midseason juveniles	30	20.48	0.904
mtDNA: plankton	3	2.10	0.551
mtDNA: settlers	11	6.02	0.872
mtDNA: metamorphs	15	8.85	0.885
mtDNA: early juveniles	15	12.99	0.603
mtDNA: midseason juveniles	15	5.97	0.980

nototype frequencies among plankton samples or habitats was detected in any of the collections (see Table 2).

Initial samples for *Mpi* resembled the patterns observed at the other loci. However, there was some indication of genetic differentiation in the plankton. The frequency of the *Mpi*-SS genotype varied more than 10% between sampling localities in March but this pattern was not present in April (Table 2). The parameter estimates for site, date, and the interaction term were all nonsignificant (not shown). For the settler and metamorph sample regressions, all predictor variables could be eliminated without significantly reducing model fit (Table 1). No significant variation in genotype frequencies was detected at settlement and after metamorphosis in any of the habitats (see Table 2).

A very different pattern, however, was observed for barnacles collected two weeks after metamorphosis (the early juvenile stage). The XH habitat had a positive and significant effect on  $\text{logit}_1$ , and the differential effects of the AH habitat were negative and significant for both logits (Table 3).

Whereas the *Mpi*-FF genotype increased and the *Mpi*-SS genotype decreased in frequency in the XH habitat, the opposite pattern was observed in the AH habitat (Fig. 1). The odds of sampling the *Mpi*-FF genotype relative to the *Mpi*-SS genotype (odds ratio for  $\text{logit}_1$ ) were 3.46 times higher in the XH compared to the AH habitat (Table 4). Furthermore, genotype frequencies changed in both these habitats subsequent to the cyprid collection at settlement. The odds of a sampled barnacle being the *Mpi*-FF genotype rather than the *Mpi*-SS genotype was significantly higher than 1.0 in the XH habitat at the early juvenile stage when compared to the cyprid sample, but was significantly lower than 1.0 in the AH-settler comparison. The XH and AH habitats had opposite effects on genotypic probabilities.

The results for the midseason juvenile sample demonstrated the same patterns observed at the early juvenile stage. The XH habitat again had a significant and positive effect on  $\text{logit}_1$ , and the differential effects of the AH habitat were negative for both logits (Table 5). As in the previous sample, the effects of the XL and AL habitats were nonsignificant. Odds ratios comparisons among habitats and between samples at the midseason juvenile stage and cyprids at settlement yielded the same general patterns as in the previous temporal sample (Table 6). No additional effects on genotypic probabilities were observed during the two-month interval between the early and midseason juvenile collections.

*Maintenance of Polymorphism at Mpi*

The similarity in *Mpi* genotype frequencies among the early juvenile sample, midseason juvenile sample, and postreproductive adults sampled in two preceding generations (Schmidt and Rand 1999) suggests that no further differential mortality of genotypes would be predicted. No selection was observed in the XL and AL habitats; in this portion of the intertidal environment the fitness of each genotype was sta-

TABLE 2. Observed genotype frequencies for each locus in each temporal sample.

Stage	Site	<i>Mpi</i>				<i>Gpi</i>				mtDNA		
		<i>N</i>	SS	SF	FF	<i>N</i>	SS	SF	FF	<i>N</i>	A	B
Larvae 27 March	GL	89	0.112	0.528	0.360	90	0.600	0.311	0.089	68	0.456	0.544
	HC	110	0.227	0.455	0.318	116	0.500	0.405	0.095	87	0.563	0.437
Larvae 9 April	GL	87	0.184	0.437	0.379	88	0.500	0.432	0.068	64	0.516	0.484
	HC	85	0.165	0.459	0.377	99	0.545	0.384	0.071	72	0.556	0.444
Settlers	XH	149	0.148	0.503	0.349	152	0.474	0.467	0.059	134	0.500	0.500
	XL	135	0.156	0.489	0.356	142	0.556	0.380	0.063	123	0.528	0.472
	AH	151	0.146	0.497	0.358	152	0.493	0.434	0.072	134	0.545	0.455
	AL	153	0.178	0.474	0.349	153	0.536	0.379	0.085	128	0.492	0.508
Metamorphs	XH	143	0.175	0.420	0.405	145	0.448	0.428	0.124	115	0.565	0.435
	XL	131	0.137	0.588	0.275	145	0.531	0.372	0.097	124	0.492	0.508
	AH	144	0.174	0.486	0.340	144	0.521	0.403	0.076	127	0.528	0.472
	AL	143	0.147	0.497	0.357	144	0.528	0.389	0.083	134	0.493	0.507
Early juveniles	XH	142	0.106	0.430	0.464	142	0.500	0.401	0.098	118	0.492	0.508
	XL	144	0.132	0.493	0.375	142	0.585	0.338	0.077	133	0.474	0.526
	AH	144	0.229	0.479	0.292	144	0.458	0.403	0.139	119	0.538	0.462
	AL	145	0.145	0.510	0.345	144	0.569	0.347	0.083	127	0.496	0.504
Midseason juveniles	XH	141	0.092	0.461	0.447	139	0.518	0.380	0.102	124	0.492	0.508
	XL	144	0.153	0.500	0.347	142	0.507	0.394	0.099	130	0.523	0.477
	AH	144	0.229	0.465	0.305	142	0.556	0.359	0.085	131	0.534	0.466
	AL	142	0.148	0.493	0.359	144	0.542	0.396	0.062	118	0.525	0.475

TABLE 3. Maximum-likelihood parameter estimates for the two *Mpi* logits modeled in the regression for early juveniles. The parameter estimates are relative to the genotype counts in the four habitats at settlement.

Variable	Interpretation	logit <sub>1</sub> (FF/SS)					logit <sub>2</sub> (SF/SS)				
		Parameter	Estimate	SE	Wald $\chi^2$	P	Parameter	Estimate	SE	Wald $\chi^2$	P
Intercept	average generalized logit	a1	0.8891	0.1078	68.08	0.0001	a2	1.1692	0.1040	126.5	0.0001
Habitat	differential effect for XH	b1	0.5925	0.2463	5.79	0.0162	b2	0.2337	0.2462	0.90	0.3425
	differential effect for XL	b3	0.1554	0.2330	0.44	0.5048	b4	0.1491	0.2255	0.44	0.5084
	differential effect for AH	b5	-0.648	0.2100	9.53	0.0020	b6	-0.4316	0.1941	4.94	0.0262
	differential effect for AL	b7	-0.0216	0.2284	0.01	0.9245	b8	0.0768	0.2182	0.12	0.7249

tistically indistinguishable from 1.0. Although all fitness values were estimated with considerable error, these environments would not be predicted to contribute to the conditions necessary for a stable polymorphism. Viability estimates for *Mpi* genotypes in the four habitat types are given in Table 7. Considering only those habitats in which genotypic fitness was significantly variable (i.e., XH and AH), a nontrivial equilibrium allele frequency ( $0 < q < 1$ ) was found to exist when the proportional reproductive output of the XH habitat was set between 0.315 and 0.450. Only some of the equilibrium allele frequencies were stable, however. One such stable point is shown in Figure 2, in which the relative proportion of reproductive output from the XH and AH habitats was 0.45 and 0.55, respectively. Under these conditions, selection would maintain both the *Mpi*-S and *Mpi*-F alleles in the population when the frequency of the *Mpi*-S allele is above 0.48.

#### DISCUSSION

*Mpi* genotypes demonstrated habitat-specific patterns of differential mortality, whereas no temporal change in genotype frequencies was observed for a presumably neutral mtDNA locus. This contrast suggests the action of spatially variable selection at the *Mpi* locus (Karl and Avise 1992;

Berry and Kreitman 1993; McDonald 1994; Pogson et al. 1995). Genetic homogeneity for all markers was observed in the plankton and at settlement, but statistical models for juvenile samples predict a significant change in the frequency of both *Mpi* homozygotes in two types of habitats in the high intertidal zone: one characterized by a high degree of temperature/desiccation stress (XH) and one in which these stresses were reduced by association of barnacles with algae (AH). Many studies have demonstrated that barnacle larvae explore their environment before committing to settlement, and a variety of physical and chemical cues influence this decision (e.g., Crisp and Meadows 1962; Wethey 1986; Yule and Walker 1987; Jarrett 1997). The lack of variation in genotype frequencies among cyprid larval samples at settlement suggests that habitat selection is not responsible for subsequent associations between *Mpi* genotype and habitat.

Genotype frequencies may vary among daily cohorts of larvae settling in the intertidal zone, and random temporal variation in genotype frequencies at settlement can result in genetic heterogeneity among habitats in subsequent samples (Johnson and Black 1984). Larval organic content and growth capacity in *S. balanoides* vary among daily cohorts of settling cyprids, and larvae attaching earlier tend to grow faster than individuals that attach later in the settlement season (Jarrett

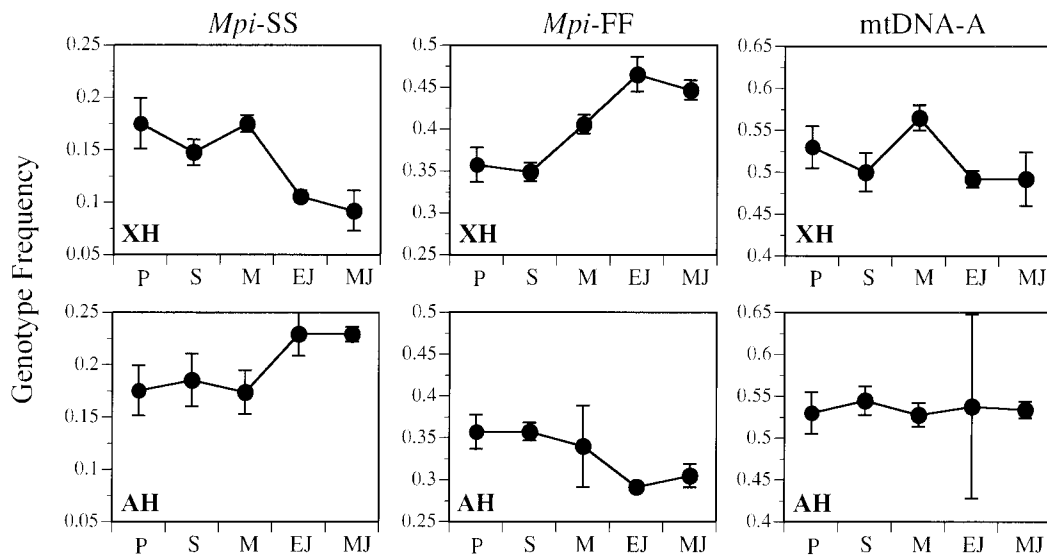


FIG. 1. Mean frequencies ( $\pm$  SE) of the *Mpi*-SS and *Mpi*-FF genotypes and mtDNA-A haplotypes in the XH (top panels) and AH (bottom panels) habitats at each of the five sampling periods. On the x-axis: P, planktonic samples; S, larvae at settlement; M, metamorphosis; EJ, early juveniles; MJ, midseason juveniles.

TABLE 4. Odds ratios calculated from the *Mpi* early juvenile regression parameters. Cells below the diagonal are for  $\text{logit}_1(\text{FF}/\text{SS})$ ; columns are compared to rows. Cells above the diagonal are for  $\text{logit}_2(\text{SF}/\text{SS})$ ; rows are compared to columns.

	XH	XL	AH	AL	Settlers
XH	—	1.09	1.95*	1.17	1.30
XL	1.55	—	1.79*	1.07	1.19
AH	3.46*	0.92	—	0.60	0.67
AL	1.85*	1.19	0.53*	—	1.11
Settlers	1.96*	1.26	0.57	1.06	—

\* The 95% confidence intervals do not include 1.0.

and Pechenik 1997). The probability of larval survivorship may have a strong temporal component. In this study, the metamorph collection represented the cumulative recruitment in the four habitat types over the majority of the settlement period. Genotype frequencies in this sample were homogeneous among habitats and were indistinguishable from those of cyprids sampled at settlement. Thus, any random differentiation in genotype frequencies that may have existed among larval cohorts was effectively averaged across habitats. The variation in *Mpi* genotype frequencies among habitats that was observed in the juvenile samples was not due to preexisting differentiation.

*Selection and Barnacle Development*

Results from a previous study demonstrated the same patterns of genotype-habitat association in postreproductive adult barnacles (Schmidt and Rand 1999). It was unknown whether selection operated at a specific point in time or was a continuous process over the barnacle life span. In this study, selection had not occurred when the metamorphs were collected but had already taken place when the early juveniles were sampled two weeks later. Genotype frequencies in the early juvenile samples closely match those observed for adults collected in similar habitats in the two previous years (Schmidt and Rand 1999). Furthermore, the genotype frequencies essentially did not change between the early and late juvenile stages. Therefore, it appears that selection acts over a short interval between the metamorph and early juvenile stages, during which time the average barnacle diameter increases by less than 1 mm (data not shown).

This is somewhat surprising, given that barnacle mortality rates are much higher during the short period encompassing settlement and metamorphosis (Connell 1961; Wethey 1985; Raimondi 1990; Gosselin and Qian 1996) as well as in the late summer (Southward 1958) than during this short devel-

TABLE 6. Odds ratios calculated from the *Mpi* midseason juvenile regression parameters. Cells below the diagonal are for  $\text{logit}_1(\text{FF}/\text{SS})$ ; columns are compared to rows. Cells above the diagonal are for  $\text{logit}_2(\text{SF}/\text{SS})$ ; rows are compared to columns.

	XH	XL	AH	AL	Settlers
XH	—	1.53	2.80*	1.54	1.60
XL	2.13*	—	1.61	0.98	1.05
AH	3.63*	1.70*	—	0.61	0.65
AL	2.00*	1.05	0.55*	—	1.06
Settlers	2.15*	1.01	0.59*	1.08	—

\* The 95% confidence intervals do not include 1.0.

opmental window. This indicates that the association between *Mpi* genotype and survivorship may depend on a balance between susceptibility and resistance to mortality induced by temperature/desiccation stress. The cypris larval stage that attaches to the intertidal substrate has a relatively high surface area-to-volume ratio and lacks the calcified test that characterizes the adult form. Cyprids are very susceptible to desiccation, and whether an individual larva lives or dies is most likely not dependent on its genetic constitution, much less genotype at one locus. Similarly, during periods when low tides are coincident with unusually high temperatures (such as in the late summer), survivorship may be entirely determined by an individual's settlement location.

In barnacle species of the family Archaeobalanidae, such as *S. balanoides*, a condition of tubiferous or porous plate walls is thought to represent the ancestral state (Newman and Ross 1976). Species that have subsequently colonized the intertidal region have secondarily developed solid plate walls that are thought to confer resistance to water loss and represent an adaptation to intertidal existence (Stanley and Newman 1980). *Semibalanus balanoides* begins development with tubiferous plate walls, which can increase the rate of growth, but juveniles and adults are characterized by solid plate walls. Stanley and Newman (1980) interpret this as a trade-off between competition for space and resistance to desiccation stress. Although the exact timing of this shift between tubiferous and solid plate walls has not been quantified, it occurs at approximately the same time as the change in *Mpi* genotype frequencies.

*Balancing Selection at Mpi*

On exposed substrate in the high intertidal zone, *Mpi*-FF genotypes exhibited increased survivorship relative to the other two genotypes. The differential mortality of *Mpi* genotypes in stressful intertidal environments appears to result

TABLE 5. Maximum-likelihood parameter estimates for the two *Mpi* logits modeled in the regression for midseason juveniles. The parameter estimates are relative to the genotype counts in the four habitats at settlement.

Variable	Interpretation	$\text{logit}_1(\text{FF}/\text{SS})$					$\text{logit}_2(\text{SF}/\text{SS})$				
		Parameter	Estimate	SE	Wald $\chi^2$	P	Parameter	Estimate	SE	Wald $\chi^2$	P
Intercept	average generalized logit	a1	0.8770	0.1085	65.36	0.0001	a2	1.1697	0.1405	125.23	0.0001
Site	differential effect for XH	b1	0.7012	0.2597	7.29	0.0069	b2	0.4398	0.2575	2.92	0.0877
	differential effect for XL	b3	-0.0560	0.2259	0.06	0.8041	b4	0.0159	0.2157	0.01	0.9411
	differential effect for AH	b5	-0.5893	0.2088	7.97	0.0048	b6	-0.4615	0.1951	5.60	0.0180
	differential effect for AL	b7	0.0103	0.2283	0.01	0.9641	b8	0.0343	0.2192	0.02	0.8757

TABLE 7. Habitat-specific viability estimates for *Mpi* genotypes in the midseason juvenile collection. All values are standardized by the estimated fitness of the heterozygous genotype.

Habitat	<i>Mpi</i> -SS	<i>Mpi</i> -SF	<i>Mpi</i> -FF
XH	0.696	1.000	1.424
XL	0.898	1.000	1.012
AH	1.519	1.000	0.880
AL	0.913	1.000	0.976

from a differential ability to process mannose-6-phosphate through the glycolytic pathway (Schmidt 2001). When mannose was supplemented in the diet and barnacles were exposed to temperature and/or desiccation stress, individuals of the *Mpi*-FF genotype grew faster than *Mpi*-SS genotypes; the growth phenotype exhibited by *Mpi* genotypes predicted survivorship both in the laboratory and the field. The heterozygote was intermediate in both respects, suggesting a phenotypic increment of allelic substitution. These results indicate the direct action of selection at the *Mpi* locus (Clarke 1975; Koehn 1978).

It remains unclear what combination of environmental variables results in an increase in the frequency of the *Mpi*-SS genotype and a decrease in frequency for the *Mpi*-FF homozygote. This pattern was previously documented in experimental treatments characterized by the lowest level of environmental stress (Schmidt et al. 2000). The identical pattern was observed in the AH habitat in this study. However, the distinct selection regime created by the interaction between barnacles and algae cannot be fully explained by stress amelioration, because in the present investigation no effect was observed when barnacles were covered by algae in the low intertidal zone. Further examination of the biochemical and physiological properties of barnacle *Mpi* genotypes under a variety of environmental conditions may help resolve this issue.

Regardless of the mechanistic details of selection at the *Mpi* locus, the observed variation in the realized fitness of genotypes among habitats can result in the stable maintenance of the polymorphism. However, genotype frequencies and viabilities were estimated with considerable error. The range of habitat proportions that yield stable equilibria is surprisingly broad, given the extremely restrictive nature of the model (Maynard Smith and Hoekstra 1980). Clearly, the habitats included here are a minority of the many potential environments that occur over the broad geographic range of this species. Selection pressures may also vary over moderate and biogeographical scales (Holm and Bourget 1994). Any existing population structure at large spatial scales, assortative mating by habitat, or habitat selection would all increase the likelihood of maintaining polymorphism by spatial variation in selection pressures (Maynard Smith 1966; Strobeck 1974; Karlin 1982). The Levene (1953) model in the strict sense is an extremely implausible mechanism by which genetic variation is maintained, but it does provide a useful framework for evaluating patterns of genetic variation in nature. The concordance between the theoretical model and the data presented in this study further support the proposition that intertidal habitats are characterized by distinct environmental conditions and selection pressures, and that this het-

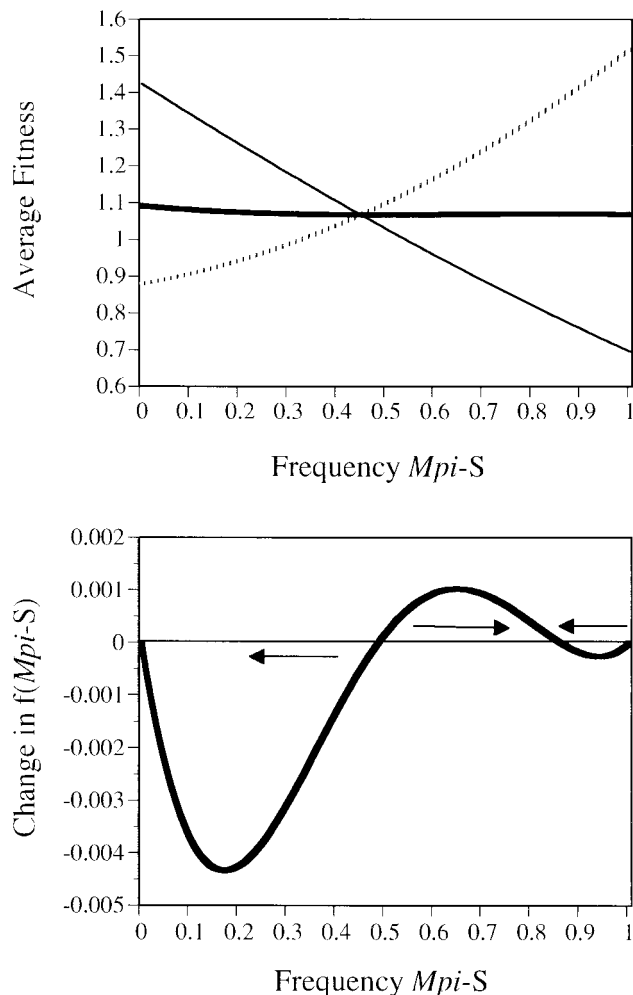


FIG. 2. An example of a stable polymorphism with  $c_{XH} = 0.45$  and  $c_{AH} = 0.55$ . Top panel: Average fitness as a function of *Mpi*-S allele frequency. Estimated viabilities are given in Table 7. The thin solid line represents average fitness in the XH habitat, the broken line average fitness in AH. The thick line defines the geometric mean fitness across the two environments. The slope changes in direction twice, when the frequency of the *Mpi*-S allele is approximately 0.48 and 0.85. Bottom panel: The change in the frequency of the *Mpi*-S allele as a function of its initial frequency and habitat-specific viability of *Mpi* genotypes. When the frequency of the *Mpi*-S allele lies between 0.48 and 1.0, selection drives it to the stable equilibrium point.

erogeneity results in the active maintenance of the *Mpi* polymorphism in barnacle populations.

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