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Evolution

Strong natural selection causes microscale allozyme variation in a marine snail

(aspartate aminotransferase/selection coefficient/genotype fitness/microevolution/*Littorina saxatilis*)

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ABSTRACT **Natural selection is one of the most fundamental processes in biology. However, there is still a controversy over the importance of selection in microevolution of molecular traits. Despite the general lack of data most authors hold the view that selection on molecular characters may be important, but at lower rates than selection on most phenotypic traits. Here we present evidence that natural selection may contribute substantially to molecular variation on a scale of meters only. In populations of the marine snail *Littorina saxatilis* living on exposed rocky shores, steep microclines in allele frequencies between splash and surf zone groups are present in the enzyme aspartate aminotransferase (allozyme locus *Aat*; EC. 2.6.1.1). We followed one population over 7 years, including a period of strong natural perturbation. The surf zone part of the population dominated by the allele Aat^{100} was suddenly eliminated by a bloom of a toxin-producing microflagellate. Downshore migration of splash zone snails with predominantly Aat^{120} alleles resulted in a drastic increase in surf zone frequency of Aat^{120}, from 0.4 to 0.8 over 2 years. Over the next four to six generations, however, the frequency of Aat^{120} returned to the original value. We estimated the coefficient of selection of Aat^{120} in the surf zone to be about 0.4. Earlier studies show similar or even sharper *Aat* clines in other countries. Thus, we conclude that microclinal selection is an important evolutionary force in this system.**

The nature, frequency, and strength of natural selection have been much debated since the days of Darwin, but we still know very little about the microevolution of most characters, especially biochemical ones (1, 2). Thus, the controversy between selectionists and neutralists remains largely unresolved (2–5). Statistical surveys suggest that some part of the allozyme variation cannot be due to random genetic drift (6–10). However, good examples of natural selection acting on particular loci are few (e.g., refs. 11–14) and then most often come from studies of large-scale latitudinal clines (15, 16). Although selected variation at microscales has been documented for morphological and physiological traits (17–19), it has rarely been considered in detail for molecular traits. Few studies include analysis of genetic differentiation of molecular traits over distances of tens of meters or less. Among those that do, substantial levels of presumably selected differentiation are sometimes reported (20–24), although the actual rate of selection has seldom been estimated (1).

In this study we have estimated the allozyme differentiation in a Swedish population of the marine snail *Littorina saxatilis* over a 25 × 30 m area of rocky shore before and following a natural perturbation of the population. We found evidence of high rates of selection in the allozyme locus aspartate aminotransferase (*Aat*; EC 2.6.1.1). This result not only is at odds with the belief that biochemical traits are neutral or close to neutral but also shows that biochemical differentiation on a meter scale can be of the same order of magnitude as is usually found over distances of tens of kilometers or much more.

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MATERIALS AND METHODS

The intertidal snail *Littorina saxatilis* (Olivi) is one of the most common invertebrates on rocky shores in western Europe, occurring in densities of hundreds or more per m^2 toward the top of the shore. It lacks a pelagic larva and young are produced as tiny benthic snails in the immediate vicinity of the female. This and an adult vagility in the range of a few meters per 3 months (25) suggest a generally low dispersal of members of this species, although occasionally long-distance migration takes place (26).

This study aimed to analyze microscale genetic differentiation over a heterogeneous habitat (the littoral zone of an exposed rocky shore), and we chose a population in a wave-exposed area of the rocky island Ursholmen, on the Swedish west coast (58°50′ N, 11° E). The morphological variation of this population has been documented and, as expected due to slow dispersal and a varying habitat, there is a slight gradual change in, for example, spire height, aperture size, and shell thickness from the surf to the splash zone (27). We analyzed five polymorphic allozyme loci—i.e., mannose-6-phosphate isomerase (*Mpi*; EC. 5.3.1.8), phosphoglucose isomerase (*Pgi*; EC. 5.3.1.9), phosphoglucomutase (*Pgm*; EC. 5.4.2.2), purine-nucleoside phosphorylase (*Pnp*; EC. 2.4.2.1), and aspartate aminotransferase (*Aat*). We used conventional horizontal starch gel electrophoresis to analyze the variation of these loci, in which Mendelian inheritance had been confirmed earlier by rearing experiments (28, 29). Sample sizes ranged between 23 and 84, but most samples were in the range 38–42. We used χ^2 contingency table statistics to test for variation over years and within years among samples. If more than two alleles were present we pooled them to avoid low expected frequencies. All significance levels were adjusted for multiple testing using the program MULTTEST (30).

We took samples from two transects 20 m apart, extending from mean tide level (0 m) up to +6 m. Each sample was from within 1 m^2. The first sampling was made in August 1987. In May 1988 a bloom of a toxin-producing microflagellate (*Chrysochromulina polylepis*) killed all surf zone snails, while splash zone snails survived because they were not immersed at the time. In the summers of 1988 and 1989, very few snails were found in the surf zone, but by 1990 snails had attained densities of hundreds per m^2 again, and in June 1990 we resampled exactly the same spots as in 1987. We continued yearly sampling until 1993 (sampling dates: September 1991, September 1992, September 1993).

RESULTS AND DISCUSSION

In 1987 we found dramatic differences in *Aat* between surf zone (lower shore) and splash zone (higher shore) snails, but

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Table 1. Temporal and spatial variation at five allozyme loci in a population of *Littorina saxatilis*

Sample	Year 1987	1990	1991	1992	χ^2	df	P'*
	Mannose-6-phosphate isomerase (*Mpi*)†						
Pool	‡	0.72	0.64	0.67	1.4	2	NS
High A	0.57	0.59	‡	0.56	0.1	2	NS
Mid A	0.57	0.61	0.51	0.66	3.7	3	NS
Upper low A	0.60	0.58	0.55	0.62	1.3	3	NS
Low A	0.54	0.62	0.59	0.52	2.0	3	NS
High B	0.58	0.53	0.59	0.46	3.4	3	NS
Mid B	0.58	0.52	0.50	0.56	1.3	3	NS
Low B	0.49	0.46	0.55	0.45	1.9	3	NS
χ^2	7.0	14.9	4.1	17.0			
df	6	7	6	7			
P'	NS	NS	NS	NS			
	Phosphoglucose isomerase (*Pgi*)§						
Pool	‡	0.87	0.86	0.94	3.1	2	NS
High A	0.89	0.74	‡	0.92	11.5	2	NS
Mid A	0.84	0.85	0.76	0.78	3.7	3	NS
Upper low A	0.90	0.79	0.76	0.88	9.8	3	NS
Low A	0.90	0.93	0.75	0.88	13.7	3	NS
High B	0.92	0.86	0.86	0.94	4.5	3	NS
Mid B	0.82	0.89	0.88	0.93	4.8	3	NS
Low B	0.87	0.78	0.81	0.87	3.1	3	NS
χ^2	7.1	17.5	9.5	18.0			
df	6	7	6	7			
P'	NS	NS	NS	NS			
	Phosphoglucomutase (*Pgm*)¶						
Pool	‡	0.95	0.84	0.92	3.2	2	NS
High A	0.89	0.86	‡	0.88	0.4	2	NS
Mid A	0.95	0.93	0.89	0.95	2.7	3	NS
Upper low A	0.87	0.84	0.85	0.89	1.3	3	NS
Low A	0.88	0.94	0.96	0.82	9.6	3	NS
High B	0.92	0.88	0.92	0.89	1.2	3	NS
Mid B	0.93	0.98	0.86	0.84	7.4	3	NS
Low B	0.92	0.81	0.86	0.89	4.8	3	NS
χ^2	6.4	16.8	8.4	8.6			
df	6	7	6	7			
P'	NS	NS	NS	NS			
	Purine-nucleoside phosphorylase (*Pnp*)‖						
Pool	‡	0.86	0.88	0.93	1.9	2	NS
High A	0.81	0.79	‡	0.75	0.9	2	NS
Mid A	0.74	0.70	0.70	0.66	1.1	3	NS
Upper low A	0.76	0.68	0.85	0.77	6.7	3	NS
Low A	0.76	0.87	0.85	0.81	4.2	3	NS
High B	0.79	0.79	0.76	0.66	5.1	3	NS
Mid B	0.80	0.80	0.80	0.84	0.8	3	NS
Low B	0.90	0.68	0.68	0.67	17.1	3	<0.05
χ^2	11.2	20.9	17.9	40.5			
df	6	7	6	7			
P'	NS	NS	NS	<0.001			
	Aspartate aminotransferase (*Aat*)**						
Pool	‡	0.40	0.51	0.42	2.4	2	NS
High A	0.94	0.96	‡	0.98	1.4	2	NS
Mid A	0.95	0.93	0.88	0.97	5.6	3	NS
Upper low A	0.52	0.92	0.62	0.78	38.7	3	<0.001
Low A	0.38	0.82	0.74	0.50	42.6	3	<0.001
High B	0.93	1.00	0.99	0.96	9.6	3	NS
Mid B	0.90	0.88	0.84	0.82	3.1	3	NS
Low B	0.40	0.79	0.54	0.43	30.8	3	<0.001
χ^2	176.9	160.5	78.1	193.4			
df	6	7	6	7			
P'	<0.001	<0.001	<0.001	<0.001			

Samples are from different microhabitats of two crevices (A and B, see Fig. 1) sampled in 1987, 1990, 1991, and 1992. Data from 1993, which were only obtained for *Aat*, are shown in Fig. 1. Only the frequencies of the most common alleles of each loci are given. NS, not significant.

essentially no differences at the other four loci (Table 1). The frequencies of Aat^{120} ranged from 0.9 in the splash zone to 0.4 in the surf zone. However, after the catastrophic kill in 1988 and the subsequent recruitment of new surf zone snails the pattern was radically different in 1990. Now Aat^{120} dominated also the surf zone with frequencies of around 0.8 (Fig. 1 and Table 1). But this pattern was unstable and the frequency of Aat^{120} in the surf zone declined rapidly and by 1992 settled to its original surf zone level (Fig. 1 and Table 1). While the surf zone samples of *Aat* showed dramatic variation over time, the *Aat* frequencies of the splash zone samples (including a rock pool sample) as well as the frequencies of the other four loci remained stable over time (Table 1). [Allele frequency differences between samples of 0.2–0.3, or greater, in any locus were detected with 80% power (31) but we cannot exclude the existence of smaller differences between sites or between sampling occasions in any loci. Such differences, if present, may have been due to genetic drift or to low levels of selection, or both.]

Reproductive isolation between splash and surf zone snails as an explanation for the large spatial differentiation in 1987 may be rejected as we found no important differentiation at any of the other loci studied (Table 1) and almost no deviations from Hardy–Weinberg equilibrium in *Aat* (Table 2). Thus we conclude that the variation is intraspecific. [We admit, however, that the power of the Hardy–Weinberg tests was low—that is, in samples where both alleles were common, we could obtain significant deviations from Hardy–Weinberg with a power of 80% only if we observed about twice as many or more heterozygotes as we expected (32). However, the Hardy–Weinberg tests would most probably have unveiled a reproductive barrier between surf zone and splash zone snails if present, because such a barrier would have generated large heterozygote deficiencies in most surf zone samples.]

As nowhere on the island (or on adjacent islands; ref. 26) did surf zone snails survive the toxic bloom, we may exclude the possibility that the Aat^{100} allele increased due to gene flow from persisting low shore populations. Thus the explanation for the dramatic increase in Aat^{120} in the surf zone between 1987 and 1990 is that snails from the splash zone dominated by Aat^{120} spread down into the surf zone after the elimination of the original surf zone snails in May 1988. The rapid decline to original surf zone frequencies in 1990–1992 is explained by strong microhabitat-related selection.

The conclusion of strong microhabitat-related selection is convincing. However, the target locus (or, perhaps, loci) of the selection may not be *Aat* but a locus in linkage disequilibrium with *Aat*. But we believe selection on a linked locus is unlikely for two reasons. (*i*) The same type of microscale clines have been found in populations of the species from Iceland, the British Isles, and Norway (33), although not from Spain (34). (*ii*) The Aat^{100} allele, which increased in frequency after 1990, originated in the splash zone. Extremely tight linkage, perhaps kept by a chromosomal inversion, is needed to explain why we found the same phenomenon in geographically remote populations as well as in the splash zone habitat where a linkage between two surf zone-favored alleles would split more easily by recombination.

The most dramatic difference in the relative frequency of *Aat* alleles occurred between 0 and +2.5 m and we found fluctuating trends in the "upper low" site at +1 (Fig. 1). Indeed, the frequency of Aat^{120} in an additional sample 2 m

*Probability adjusted for multiple testing (see text).
†Allele 120; other allele is 100.
‡Missing value.
§Allele 100; other alleles are 90 and 110.
¶Allele 100; other alleles are 85 and 105.
‖Allele 65; other alleles are 35 and 100.
**Allele 120; other allele is 100.

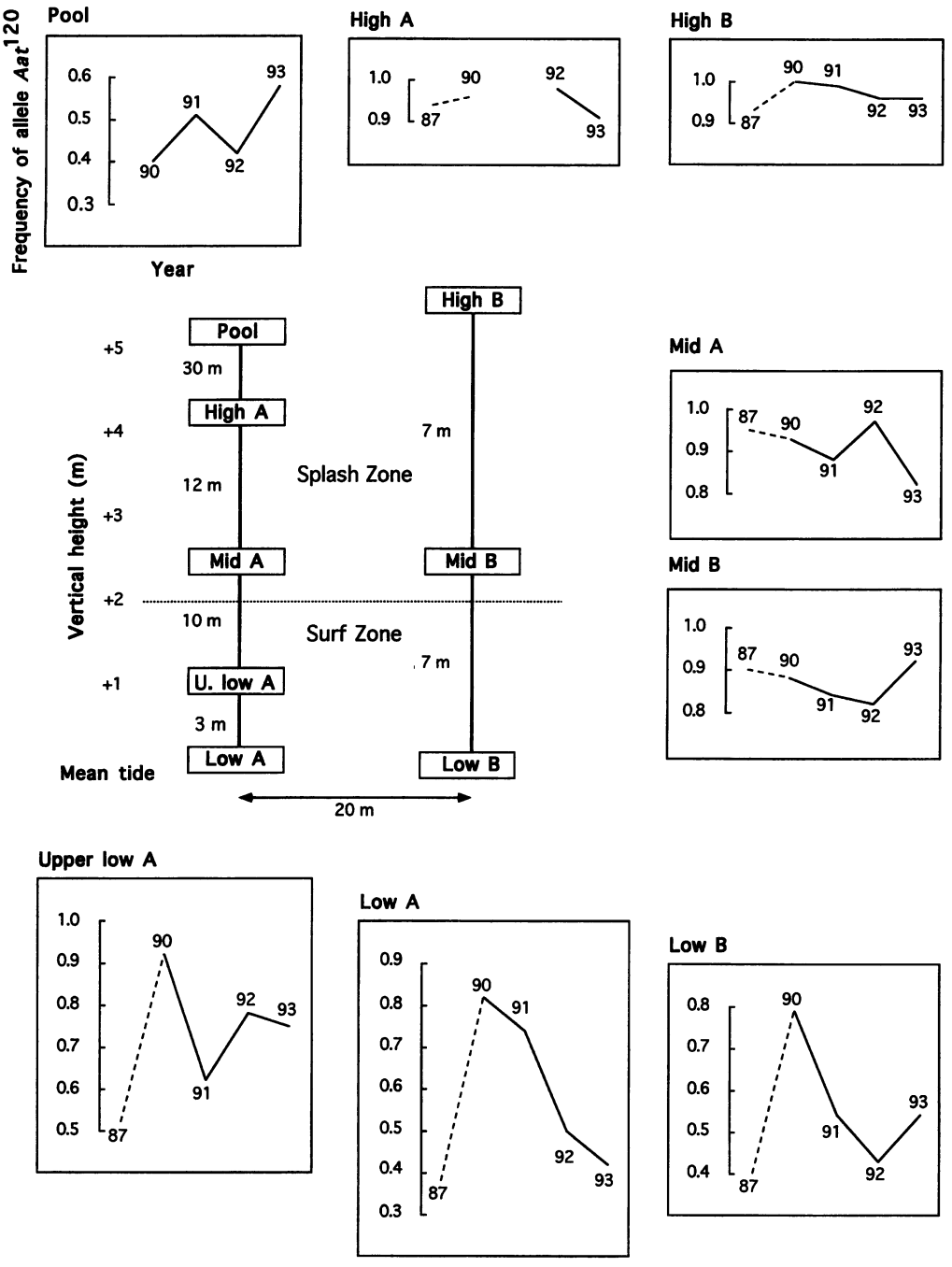

FIG. 1. Spatial and temporal variation in Aat^{120} in different microhabitats (surf zone and splash zone) of a rocky shore population of *Littorina saxatilis*. Heights from mean tide level and distances between samples are indicated. Snails were continuously distributed in the surf zone, while in the splash zone no snails were present between the two crevices A and B. The tidal range is ≤0.3 m.

away and at +0.5 m was 0.51 in 1992, which was significantly different from 0.78 of the upper low site ($\chi^2 = 18.1$, df = 1, $P < 0.001$). It seems likely that the exact position of the cline may change because physical factors vary, and snails move about.

Earlier studies have shown that Aat^{120} is rare in populations on boulder shores (35), sea-grass beds (36), and small offshore skerries (37). The frequency of this allele also remained low from 1987 to 1993 in the splash zone rock pool (Table 1 and Fig. 1). The snails often live submerged in these habitats in which Aat^{120} is less common, while snails in the splash zone (except those submerged in rock pools) often experience desiccating conditions. Interestingly, splash zone snails of *Littorina saxatilis* have a more heat stable form of *Aat* than low shore snails (38). These circumstances suggest that wetness and temperature may be important selective agents.

The approximate strength of the selection may be estimated from the decline in Aat^{120} in the surf zone between 1990 and 1993. From the formula (39)

$$W_{III} = [(u_0/u_1)u_0]W_I + [(u_0/u_1) - u_0] \quad [1]$$

we obtain fitness values for the two homozygotes 120/120 and 100/100 (W_I and W_{III}, respectively) in relation to the fitness of the heterozygote that is set to 1.0 ($u = p/q$; p is the frequency of Aat^{120} and $q = 1 - p$). A solution of W_{III} in terms of W_I is possible with data from at least two successive generation sequences (e.g., $u_0 \rightarrow u_1$ and $u_1 \rightarrow u_2$) (39). Although the assumption of discrete generations is not fully met within this species, we assume generation time to be equal to the age of reproduction—that is, about 6 months (40). Solving this equa-

Table 2. χ^2 values of deviation from Hardy–Weinberg expected genotype frequencies in *Aat*

Sample	Year				
	1987	1990	1991	1992	1993
Pool	*	0.41	4.36	0.20	3.82
High A	0.17	0.06	*	42.0†	10.9‡
Mid A	0.10	0.28	0.66	0.03	0.10
Upper low A	2.35	0.46	1.21	0.01	1.60
Low A	1.02	2.36	0.00	0.01	2.07
High B	3.34	0.00	0.01	0.06	0.06
Mid B	0.46	3.53	1.51	1.98	0.26
Low B	3.99	0.72	5.32	0.20	0.04

There is only one degree of freedom and P' is the probability adjusted for multiple testing (see text). The presence of a few (≤2) 100/100 homozygotes caused the significant values.
*Missing value.
†$P' < 0.001$.
‡$P' < 0.05$

tion for seven successive generations between 1990 and 1993 suggested that the allele Aat^{100} is approximately dominant ($W_{II} \approx W_{III} \approx 1.0$). Furthermore, the fitness of the 120/120 homozygote at mean tidal level is ≈0.6, which corresponds to a coefficient of selection of 0.4 (Fig. 2).

This is indeed an extremely strong coefficient of selection for an allozyme allele. We do not, however, think that this impressive amount of selection is unique to a Swedish rocky shore. In Iceland, for example, we have found even more extremely compressed *Aat* clines—for example, a decline in Aat^{120} from 0.71 to 0.30 over 3 m of vertical shore, from 0.70 to 0.10 over 1 m in two replicate transects of one area (Sandgerdi), and from 0.76 to 0.31 over 1 m and 0.82 to 0.20 over 2 m in another area (Akranes) (41). It seems evident that the selection coefficients that maintain these sharp clines need at least to be as strong as in Sweden and perhaps even stronger.

The neutral model is popular among taxonomists (42) because of the assumption that genetic differentiation reflects genetic relatedness among taxa. Indeed, with low to moderate

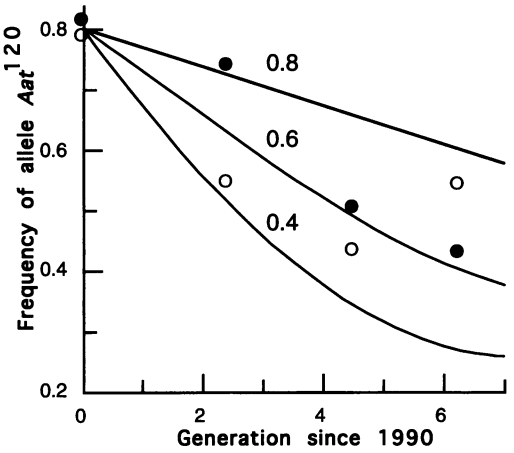

FIG. 2. Predicted and observed declines in frequency of Aat^{120} from June 1990 onward in surf zone samples (Low A and Low B of Fig. 1). The curves are predicted frequency changes if the 100/100 homozygote and the heterozygote both have fitness 1.0 (i.e., $W_{II} = W_{III} = 1.0$; see text), while the fitness of the 120/120 homozygote (W_I) is set to 0.4, 0.6, and 0.8 for the different curves, respectively. We assume a generation time of 6 months. The predicted frequency changes per generation were obtained by rearranging Eq. **1** into

$$u_1 = [W_I (u_0)^2 + u_0]/(u_0 + W_{III}) \qquad [2]$$

and setting u_0 to 4 ($u = p/q$, $p_0 = 0.8$, and $q_0 = 0.2$). The closed and opened circles indicate the observed values for the two surf zone sites Low A and Low B (see Fig. 1), respectively.

levels of selection, gene flow estimates, like Wright's F_{ST} statistics, are relatively robust. But if the selection coefficient is of the same order as or larger than the rate of migration between demes, F_{ST} will be more related to selection than to gene flow (43).

In *Littorina saxatilis Aat* is an example of a locus that would easily yield irrelevant and misleading information in studies of phylogenies and gene flow. The problem is that without careful small-scale sampling, effects of strongly selected microscale differentiation may not be discovered, and differences found on a much larger scale may be interpreted as reflecting low gene flow. Today, few studies of biochemical variation include microscale sampling. Among these, however, strong microscale selection seems quite likely in some cases (20–24), and we suggest that further studies of microscale variation will provide a valuable contribution to our understanding of genetic patterns of population and of short-term evolution.

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