

# Habitat related allozyme variation on a microgeographic scale in the marine snail *Littorina mariae* (Prosobranchia: Littorinacea)

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The marine snail *Littorina mariae* Sacchi & Rastelli occurs in high numbers in the littoral zone on fucoid macro-algae. The eggs are laid on the seaweed and development to miniature snails takes place without any pelagic larval stage. We have mapped the genetic variation of 30 enzyme loci in populations from eight small islands within 15 km of each other on the Swedish west coast. The original intention was to investigate the magnitude of gene flow within and between islands. However, we soon realized that our basic assumption of neutral genetic variation was forcefully violated in at least one locus, arginine kinase (*Ark*). Allele frequencies of *Ark* were strongly associated with type of habitat. Therefore the main part of this study focused on allele frequency distribution in different habitats. Eight of the 30 loci screened were polymorphic but we mainly considered the four most polymorphic ones (total heterozygosity between 0.32 and 0.57). All four showed significant heterogeneity between subpopulations, *Ark* especially so; 42% of the total variation of *Ark* was explained by differentiation between samples, and 90% of this variation was attributed to differences between different types of habitats (more or less exposed to wave action). In contrast, peptidase (*Pep-1*) and phosphoglucosmutase (*Pgm-2*) varied in a way predicted by neutral theory; between sample variation being mainly attributed to differentiation between islands. The variation in phosphoglucose isomerase (*Pgi*) was less consistent. In some islands there was an obvious difference between different habitats, but on other islands we found no significant difference. Not taking into account the extreme, presumably selected, variation in *Ark*, we concluded that the metapopulation of *Littorina mariae* that we studied was divided into semi-isolated populations, between which the average rate of migration was in the range of a few individuals per generation.

**ADDITIONAL KEY WORDS:**—Allozymes – genetic variation – selection – neutrality – gene flow – genetic differentiation – arginine kinase – wave exposure.

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## INTRODUCTION

Over the last three decades, studies of enzyme variation in natural populations have revealed extensive variation in large numbers of loci. This has generated an intricate debate about the nature of this variation. Perhaps the most controversial problem is the relative importance of the different mechanisms which may generate and maintain genetic variation within species, and there is still no consensus about the relative roles of stochastic and deterministic factors (see e.g. Clarke, 1975; Watt, 1985; Hedrick, 1986; Eanes, 1987; Dykhuizen & Dean, 1990; Kimura, 1991; Lewontin, 1991).

Most species are subdivided into discrete populations. Stochastic factors, essentially random genetic drift, are likely to be more important in species where gene flow among populations is restricted than in species with high dispersal. With a low among-population gene flow, random genetic drift will support differentiation between populations in neutral, or nearly neutral, loci. If, on the other hand, part of the variation within and among populations is adaptive, both species with a restricted dispersal and those with high rates of between-population migration may be differentiated due to differential selection in different habitats (see e.g. Endler, 1977).

One major dilemma is that although neutrality may be suggested when selection is not indicated, it is, in principle, an impossible task to prove neutrality in specific enzyme loci in natural populations. On the other hand, it is possible, although not always easy, to reject neutrality in favour of a model based on selection.

Today combined studies of variation in natural populations and laboratory tests of allozyme activities suggest that only a minor proportion of all polymorphic loci studied at the molecular level deviate substantially from non-neutrality. Furthermore, most genetic variation of natural populations thought to be attributed to selection in specific loci are in the form of large scale clinal variation. That is, the polymorphism is maintained by different alleles being favoured in different geographic regions, possibly as an effect of large scale environmental clines (van Delden, 1982; Koehn, Zera & Hall, 1983; Watt, 1985; Hedrick, 1986; Rank, 1992). Typically this variation is recognized over distances of tens, hundreds or even thousands of kilometres, e.g. the alcohol dehydrogenase (*Adh*) cline of *Drosophila melanogaster* (Oakeshott *et al.*, 1982), the lactate dehydrogenase (*Ldh*) cline in the fish *Fundulus heteroclitus* (Powers & Place, 1978; Place & Powers, 1979).

Variation in the leucine aminopeptidase (*Lap*) locus of the mussel *Mytilus edulis* provides an example of an unusually sharp cline for a species with a long pelagic larval stage and a high dispersal capacity. In *Mytilus*, the frequency of the *Lap*<sup>94</sup> allele increases from 0.12 to 0.55 over 30 km along a salinity gradient (Hilbish, 1985).

One example of selection on a much smaller scale is the glutamate-pyruvate transaminase (*Gpt*) polymorphism of the small rock-pool copepod *Tigriopus californicus*. Extensive differentiation is present at several loci of this species between populations on different rocky-outcrops separated from each other by sandy beaches (Burton, Feldman & Curtsinger, 1979; Burton & Feldman, 1981). Although much of this variation may be due to stochastic factors, the differentiation in *Gpt* is thought to be adaptive (Burton & Feldman, 1983; Burton, 1986). Likewise, Johnson & Black (1982) showed that within the pulmonate limpet (*Siphonaria*), which has good dispersal potential (planktonic larvae), significant genetic heterogeneity over 50 m of shore or less may be found. Part of this variation they attributed to differential selective mortality among swarms of recruits during the planktonic or early post-settlement stages (Johnson & Black, 1984).

An extreme example of microscale clinal variation is the variation in the aspartate aminotransferase (*Aat*) locus in the direct developing marine snail *Littorina saxatilis* (Johannesson & Johannesson, 1989). In this species, one of two alleles is dominant in the high littoral zone and the other allele is dominant a few meters below, in the mid littoral zone, and this differentiation is present in populations from widespread areas of NW Europe.

The developmental strategy of benthic marine invertebrates characterizes species as either having a high dispersal potential (planktotrophic larvae, dispersed in the pelagic zone for weeks to months), a moderately high dispersal potential (lecitotrophic larvae, dispersed in the pelagic for hours to days), or a low dispersal potential (no larvae, direct development). Obviously, a primary hypothesis is that a short larval stage or no larvae at all will increase the overall level of differentiation between populations due to stochastic processes, while species with long-lived larvae will be less differentiated. Most studies support this hypothesis (Berger, 1973; Janson, 1987a; Todd, Havenhand & Thorpe, 1988; Carvalho, 1989; Grant & Utter, 1988; Johnson & Black, 1991; Grant & Lang, 1991). However, incorporating divergent selection on the system may impose substantial differentiation even in species with a high rate of larval dispersal (Koehn *et al.*, 1983).

In this paper we report on small scale variation (over 10 m to 15 km) in 30 enzyme loci of a marine prosobranch gastropod, *Littorina mariae*, which lacks a pelagic dispersal stage. This species is widely distributed over the coasts of the North Atlantic (Fretter & Graham, 1980) where adult and juvenile snails are epiphytic grazers on littoral macro algae. On British shores, *L. mariae* is mainly confined to the canopy of *Fucus serratus* (Reimchen, 1979; Williams, 1990) but along the atidal Swedish west coast the snails are also frequent on plants of *Fucus vesiculosus* and *Ascophyllum nodosum* (pers. obs.). *Littorina mariae* lacks free-swimming larvae and the eggs develop into post-metamorphosis miniature snails in egg masses attached to the seaweed. Williams (1990) reports a random movement of adult snails in the range of 30 cm per day often within the frond structure of a single plant. Thus this seems truly to be a species of low vagility.

Populations are typically rather dense (tens to hundreds per m<sup>2</sup>, pers. obs.), and the distribution on a local scale is essentially one-dimensional along the littoral zone of a shore. On a larger scale, however, particularly in archipelagos, one may consider the distribution as being patchy in a two-dimensional space.

The aims of our present study were to compare the levels of genetic variation

at microscale and moderate scale distances, that is, within continuous populations and among populations of different islands. From the patterns of genetic differentiation we hoped to draw conclusions about the rate of gene flow at different scales in a species of low vagility. This objective was however complicated by the fact that one, or perhaps two, of the most polymorphic loci that we analysed varied in a predictable way among samples from different microenvironments. The potential of strong habitat related variation in one of four polymorphic loci weakened the conclusions concerning gene flow but suggested that a substantial part of the allozyme variation at this locus was due to differential selection in a heterogeneous habitat.

## MATERIAL AND METHODS

### *Sampling design*

The original aim of this study was to estimate the small scale genetic variation within a number of allozyme loci of *Littorina mariae*. The objective of this was to compare the degree of within-species differentiation with that of other species of *Littorina* (the patterns of which have already been analysed) which have other modes of reproduction. However, on the first few gels we found a pattern of variation in one enzyme system, arginine kinase (*Ark*), which seemed to be strongly correlated with the habitat type of a subpopulation. So we re-designed our sampling programme to include samples from mainly two types of microenvironments; those more and those less exposed to wave action.

We sampled a total of 1044 winkles from 21 localities, all within 15 km of each other, in the northern part of the Swedish west coast (Fig. 1). Although we initially made a subjective decision of the relative exposure to wave action of a locality based on topography and position in relation to the main wind direction and to the open sea, we subsequently used the presence or absence of an indicator species, the macro algae *Ascophyllum nodosum*, to assess the degree of exposure. Dense stands of this algae are typical of areas protected from heavy wave action, while the absence of this species indicates strong wave exposure (Lewis, 1972). Using the presence or absence of *Ascophyllum* is a method that also groups habitats of intermediate exposure into one or the other of the two discrete groups, although there is evidently a smooth gradient from exposed to protected habitats.

In most cases (except from the islands Saltö and Långholmen) we sampled one locality with *Ascophyllum*, which we defined as a 'sheltered' locality, and close to it another locality lacking *Ascophyllum*, which we defined as an 'exposed' locality. The distances between two adjacent localities of different habitats ranged from 7 to 100 m and these localities were in all cases except two (Grundskär and Svängen) parts of the same continuous populations of snails (Fig. 1). Some samples from different parts of continuous populations were of the same habitat type (see Saltö and Lökholmen, Fig. 1). In each locality we collected two replicate subsamples (a and b) from sites 8 to 10 m apart, with the exception of the four localities: 9S, 10S, 13E and 15E. In 13E we collected three subsamples (a, b and c), and in 9S, 10S and 15E, only one. Replicate subsamples were in general of the same habitat type. The few exceptions to this are described below.

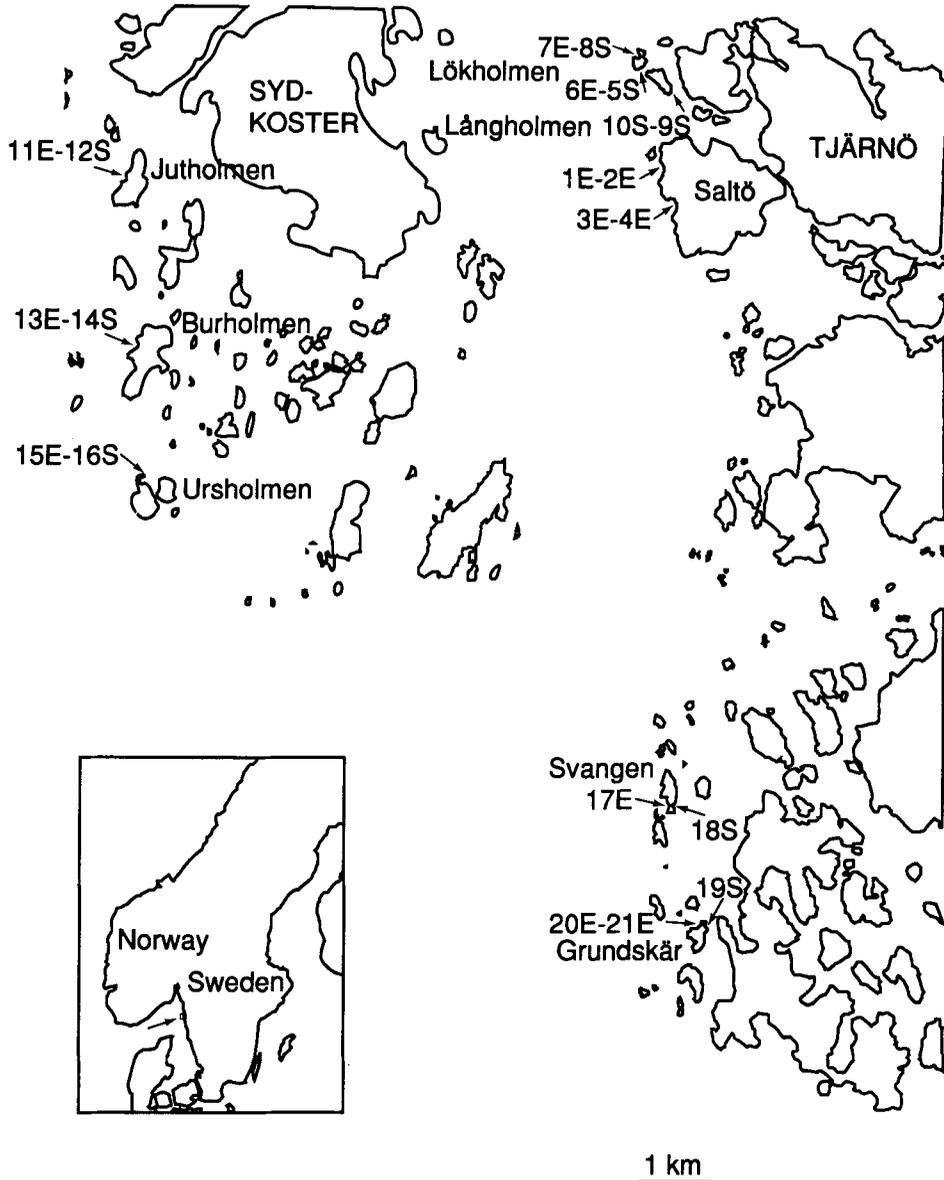


Figure 1. Map of sampling localities in the northern part of the Swedish west coast. The localities are defined as exposed (E) or sheltered (S) to wave action. A hyphen indicates that two samples are from the same continuous population.

### *Electrophoresis*

The snails were kept alive until electrophoresis. We ran horizontal electrophoresis on gels made of 13% hydrolysed starch. Two buffer systems were employed: (1) Tris-citric acid, pH 7.0 (Shaw & Prasad, 1970), (2) Tris-EDTA-boric acid, pH 8.6 (Boyer *et al.*, 1963) (stock solution: 0.9 M Tris, 0.5 M Boric acid, 0.1 M EDTA, 0.1 M  $MgCl_2$ ; electrode buffer: stock solution diluted 1 : 7 for cathode and 1 : 5 for anode; gel buffer: stock solution diluted

TABLE 1. Enzyme loci scored. For the monomorphic loci the number of samples (subsamples pooled) in which a locus have been scored is indicated as well as the mean sample size. We have as far as possible followed the nomenclature of Murphy *et al.* (1990)

Name	E.C. code	No. of samples	Mean N per sample*
Arginine kinase ( <i>Ark</i> )	2.7.3.3	polymorphic, see Appendix	
Aspartate aminotransferase ( <i>Aat</i> )	2.6.1.1	6	17.2
Aconitase hydratase ( <i>Acoh</i> )	4.2.1.3	8	12.8
Alanopine dehydrogenase ( <i>Alpdh</i> )	1.5.1.17	polymorphic but unreliable, see text	
Cytosol aminopeptidase ( <i>Cap-1</i> )	3.4.11.1	12	11.3
Cytosol aminopeptidase ( <i>Cap-2</i> )		3	11.3
Esterase-D ( <i>Est-D</i> )	3.1.1.-	19	14.3
Formaldehyde dehydrogenase ( <i>Fdh</i> )	1.2.1.1	11	15.2
Fructose biphosphate aldolase ( <i>Fba</i> )	4.1.2.13	20	20.3
Guanine deaminase ( <i>Gda-1</i> )	3.5.4.3	5	13.0
Guanine deaminase ( <i>Gda-2</i> )		5	13.0
Glycerol-3-phosphate dehydrogenase ( <i>G3phd-1</i> )	1.1.1.8	8	12.8
Glycerol-3-phosphate dehydrogenase ( <i>G3phd-2</i> )		5	13.6
L-Iditol dehydrogenase ( <i>Iddh</i> )	1.1.1.14	polymorphic but unreliable, see text	
Inorganic pyrophosphatase ( <i>Ipp</i> )	3.6.1.1	polymorphic, see Appendix	
Isocitrate dehydrogenase ( <i>Idh-1</i> )	1.1.1.42	20	17.0
Isocitrate dehydrogenase ( <i>Idh-2</i> )		20	17.0
Malate dehydrogenase ( <i>Mdh</i> )	1.1.1.37	3	11.3
Mannose-6-phosphate isomerase ( <i>Mpi</i> )	5.3.1.8	polymorphic, see Appendix	
NADP dehydrogenase ( <i>Ndh</i> )	-.-.-.-	18	23.4
Peptidase ( <i>Pep-1</i> )	3.4.-.-	polymorphic, see Appendix	
Peptidase ( <i>Pep-2</i> )		21	49.7
Phosphogluconate dehydrogenase ( <i>Pgdh</i> )	1.1.1.44	20	20.2
Phosphoglycerate kinase ( <i>Pgk</i> )	2.7.2.3	20	15.3
Phosphoglucomutase ( <i>Pgm-1</i> )	5.4.2.2	17	35.8
Phosphoglucomutase ( <i>Pgm-2</i> )		polymorphic, see Appendix	
Phosphoglucose isomerase ( <i>Pgi</i> )	5.3.1.9	polymorphic, see Appendix	
Purine-nucleoside phosphorylase ( <i>Pnp</i> )	2.4.2.1	16	17.0
Superoxide dismutase ( <i>Sod-1</i> )	1.15.1.1	9	15.1
Superoxide dismutase ( <i>Sod-2</i> )	1.15.1.1	9	15.1

1 : 20). Specimens were prepared for electrophoresis by removing the shells and rinsing the bodies in cold distilled water. The soft parts were then homogenized in 100  $\mu$ l of 0.1 M Tris-HCl, pH 8.0. The homogenates were centrifuged at 6000 revolutions/min for 20 minutes. Enzyme extracts were absorbed on wicks of Whatman 3 filter paper, which were placed in the slots of the gel. A direct current of 27 mA was applied overnight for 12 hours through the buffer system (1) and a DC of 31 mA for 14 hours through buffer system (2).

We stained 23 enzyme systems which gave patterns for 30 loci (Table 1). Enzyme stains were prepared according to Aebersold *et al.* (1987) except for alanopine dehydrogenase (*Alpdh*), arginine kinase (*Ark*), and inorganic pyrophosphatase (*Ipp*). Stains for *Alpdh* were prepared according to Manchenko & Balakirev (1985) and for *Ipp* according to Harris & Hopkinson (1976). Ingredients of the stains used for detection of *Ark* were the same as in the ultra-violet method for creatine kinase (Harris & Hopkinson, 1976) except for creatine which was replaced with arginine. The substrate used for peptidase was glycyl-leucine.

*Littorina mariae* has a close relative in *L. obtusata*. Juveniles of the two species may be confused in field sampling due to overlapping shell morphologies. However, previous studies (Zaslavskaya, Sergievsky & Tatarenkov, 1992a and

b) show *Ipp*, *Pgm-2*, and *Got-1* to be diagnostic between these two species in the White Sea. Through side-by-side running against over one hundred mature *L. obtusata* specimens we were able to confirm that *Ipp* and *Pgm-2* were diagnostic in our study area too (we did not run *Got-1*). Furthermore, we found diagnostic differences in *Pgm-1* and *Pep-2*, which were not, however, diagnostic in the White Sea (Zaslavskaya *et al.*, 1992b). As all these four loci (*Ipp*, *Pgm-1*, *Pgm-2* and *Pep-2*) were analysed in all specimens of *L. mariae* of this study, we can be sure that no individuals of *L. obtusata* has been included by mistake.

### Statistics

We resolved the products of 30 loci in 6 to 40 samples. Phenotypes observed at variable loci segregating for codominant alleles were analysed for consistency with Hardy-Weinberg expectations in each subsample. The conventionally used chi-square test for testing homogeneity between observed and expected random mating phenotypic proportions would not be applicable to many of the samples because of small expected numbers. Therefore, in order to improve the reliability of the tests, we applied a pseudo-probability programme (Zaykin & Pudovkin, 1993) which estimates the probability of agreement with Hardy-Weinberg distributions using Monte-Carlo simulations (a method originally developed by Roff & Bentzen, 1989; see also Hernández & Weir, 1989). We also estimated Selander's  $D$   $((H_{\text{obs}} - H_{\text{exp}})/H_{\text{exp}})$ , a negative value of which indicates heterozygote deficiency. The significance of  $D$  was tested using the pseudo-probability programme mentioned above.

Tests for homogeneity of allele frequencies among subsamples and at higher levels were performed with chi-square statistics. To avoid small expected numbers we pooled the frequencies of the rarest alleles.

When testing for homogeneity of allele frequencies among samples we usually had to make a number of separate tests (for individual loci and for sample combinations). In applying multiple although independent tests 5 out of 100 tests are expected to result in  $P$  values less than 0.05 by chance only. To avoid type I errors, we used a correction for multiple tests described by Sokal & Rohlf (1981, p. 728) in which the critical values of the chi-square distribution is based on Sidák's multiplicative inequality (see also Rohlf & Sokal, 1981: table 15). We used the programme MULTTEST (Zaykin & Pudovkin, 1991) to find the correct critical values of  $\chi^2$  for each replicate test considering that it was a part of a group of analogous and independent tests.

The relative amount of genetic variation among populations was analysed using Nei's (1973) measure of gene diversity.

For each locus, we calculated matrices of genetic identities (Nei, 1972) between pairs of samples, in order to compare the average identities between samples from the same and from contrasting habitats.

### RESULTS

Twenty two of the 30 loci stained were fixed for the same allele in different locations, and although we used small sample sizes and did not sample all loci in all locations we consider these loci as monomorphic, or nearly so (Table 1). Two other loci, *Alpdh* and *Iddh*, were polymorphic when applying the 0.95 criterion of

polymorphism, but the resolution of enzyme bands of these loci was very often indistinct and we have chosen to refrain from using these loci as population markers. The two loci *Ipp* and *Mpi* were polymorphic but the frequencies of the most common alleles in these loci were in many samples more than 0.95 (Appendix), and we did not analyse the differentiation in these two loci further. We discovered clear, easily interpretable polymorphisms and high variation at four loci, *Ark*, *Pgi*, *Pep-1* and *Pgm-2* (Appendix). These loci were used as population markers for all subsamples, except for the ones from Saltö which were not scored for *Pgm-2*.

#### *Variation in arginine kinase (Ark)*

Among the four highly polymorphic loci, variation between localities in *Ark* was extraordinary and obviously strongly related to sample habitat (Appendix). The overall distribution of allele frequencies varied dramatically between sheltered and exposed shores (Fig. 2). In exposed habitats, the most common allele was *Ark*<sup>100</sup> with a mean frequency of 0.814 (range 0.595 to 0.925). The mean frequency of this allele in sheltered habitats was, however, only 0.181 (range 0.022 to 0.477). The other common allele in exposed habitats was *Ark*<sup>80</sup> with a mean frequency of 0.147 (range 0.038 to 0.405), but the mean frequency of this allele decreased to 0.031 (range 0.000 to 0.118) in sheltered habitats. In sheltered habitats, the allele *Ark*<sup>120</sup> was predominant with a mean frequency of 0.740 (range 0.477 to 0.870), while the mean frequency of this allele in exposed habitats was only 0.035 (range 0.000 to 0.183). The allele *Ark*<sup>110</sup> was only found in two exposed subsamples but was present in 12 of 16 sheltered subsamples (mean frequency 0.042, range 0.000 to 0.103).

If we consider the fact that adjacent exposed and sheltered localities were rather close to each other, and in most cases parts of the same continuous population (Fig. 1), this differentiation is surprising, to say the least.

On the island of Ursholmen three subsamples were taken. Those were all from the same continuous population, were very close together, and were from different parts of a microenvironmental gradient. *Ascophyllum* was present in both the sites 16Sa and 16Sb, and thus both were defined as sheltered sites, but site 16Sa was less protected than 16Sb. Subsample 15E was from the most exposed site (Fig. 3). All four alleles of *Ark* present exhibited clinal variation, with the frequencies of site 16Sa intermediate to those of 15E and 16Sb (Appendix). Chi-square tests further indicate that the allelic distributions in *Ark* of 15E and 16Sa were different ( $\chi^2 = 12.6$ , d.f. = 2,  $P < 0.01$ ), and likewise those of 16Sa were different from 16Sb ( $\chi^2 = 18.6$ , d.f. = 1,  $P < 0.001$ ).

At Jutholmen we had a somewhat similar situation as both the exposed and sheltered subsamples were from slightly different habitats. Subsample 11Eb came from a slightly more exposed site than subsample 11Ea, and subsample 12Sb was the most protected site of the two 12S samples (Fig. 4). In this case the allele frequencies of subsamples 12Sa and 12Sb (10 m apart) differed ( $\chi^2 = 21.1$ , d.f. = 1,  $P < 0.001$ ) but not those of 11Ea and 11Eb (10 m apart) ( $\chi^2 = 4.5$ , d.f. = 2,  $P > 0.1$ ).

Apart from Ursholmen and Jutholmen, we did not recognize differences in habitats between replicate subsamples, but we obviously cannot exclude the

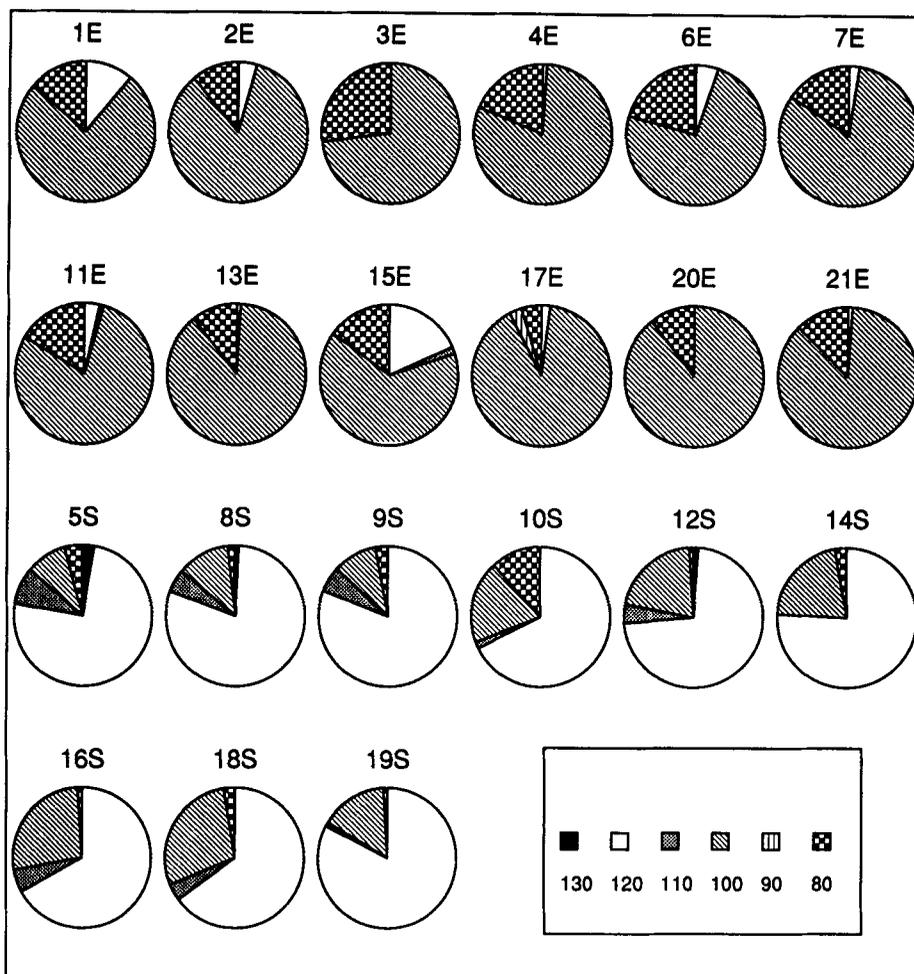


Figure 2. Allelic distributions of the six alleles (80, 90, 100, 110, 120 and 130) in arginine kinase (*Ark*) in exposed (E) and sheltered (S) samples, as in Fig. 1 (subsamples pooled).

possibility that allele frequency differences between other subsamples may in part be related to subtle habitat alterations.

On one occasion, a sample from a locality which we originally rated as a sheltered locality revealed allele frequencies of *Ark* typical of exposed samples. This was sample 21E of the island Grundskär (Fig. 2). Although the presence of *Ascophyllum* in this site made us classify this habitat as sheltered, there were only a few rather tiny plants of *Ascophyllum* present. The other criteria of exposure that we used also indicated that this site was truly intermediate to the extremely exposed 19E and the well protected 20S. As the *Ark* frequencies of this sample (21E) were quite similar to the frequencies of *Ascophyllum*-free sites, we treated this locality as a member of the exposed group in all further analyses, and we will later argue (see Discussion) that this was a proper decision.

Besides strong between-sample variation in *Ark*, we also found a substantial number of subsamples out of Hardy-Weinberg equilibrium for this locus. Nine

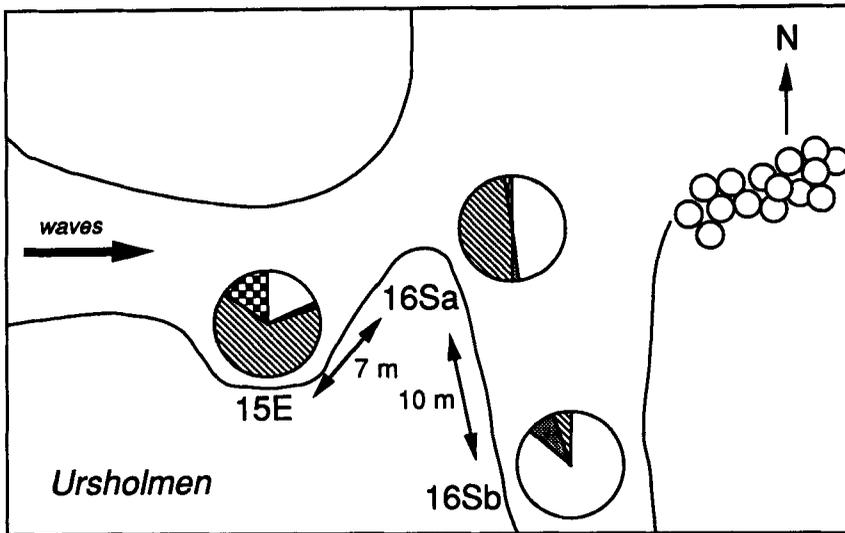


Figure 3. A detailed map of the *Ark* gradient found at the island Ursholmen. Sector patterns used for each allele are the same as in Fig. 2. Smooth contours indicate cliffs and circles boulders.

out of 40 subsamples showed significant deviations from Hardy-Weinberg expectations in *Ark* after applying the pseudo-probability test. In none of the other polymorphic loci did we find more than two of 40 subsamples deviating from Hardy-Weinberg, and these most probably represent type I errors due to multiple testing. Estimates of Selander's  $D$  showed that the deviations in all except one subsample (11Ea) were due to a significant deficiency of mainly 120/100 heterozygotes. In fact, three further subsamples had significant deficiencies according to the pseudo-probability test of Selander's  $D$  (Table 2).

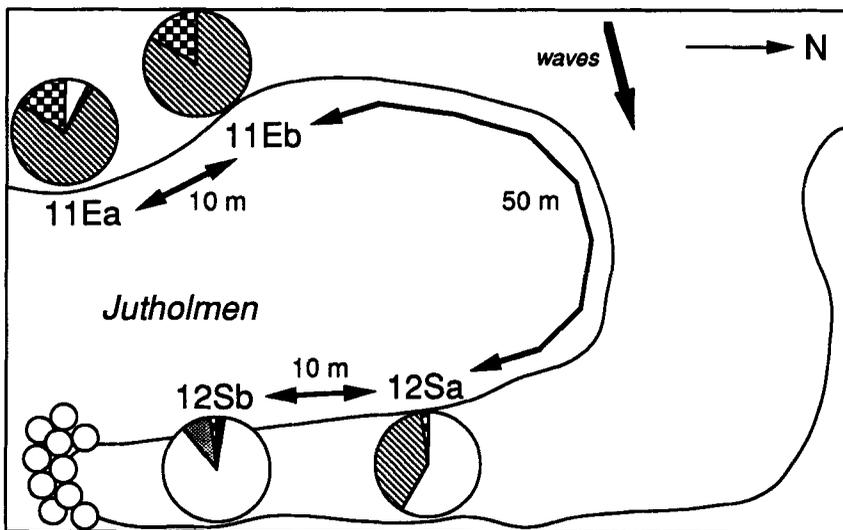


Figure 4. A detailed map of the *Ark* gradient found at the island Jutholmen. Sector patterns used for each allele are the same as in Fig. 2. Smooth contours indicate cliffs and circles boulders.

TABLE 2. Deficiency of heterozygotes, given by Selander's  $D = (H_{obs} - H_{exp})/H_{exp}$ , and deviations from Hardy-Weinberg expected genotype distributions, indicated by a  $\chi^2$  approach, in the locus arginine kinase (*Ark*). Only those subsamples are listed in which significant values of  $D$ , and/or significant departures from Hardy-Weinberg were present. In both cases we used the pseudo-probability program by Zaykin & Pudovkin (1993) to estimate the significance levels. A pseudo-probability test is thought to give reliable probability estimates even in the presence of low expected numbers. The heterozygotes which contribute the most to the deficiency in each sample are indicated. Note that the significant value of  $\chi^2$  in sample 11Ea is completely due to the presence of one 120/110 heterozygote

Sample	Selander's $D$	$P$ of $D$	$P$ of $\chi^2$	Heterozygotes in pronounced deficit
3Eb	-0.506	0.02	n.s.	100/80
5Sb	-0.370	0.007	n.s.	none
8Sa	-0.376	0.02	n.s.	120/100
8Sb	-0.386	0.01	0.03	120/100
9S	-0.467	0	0.001	120/100
10S	-0.402	0.002	0.003	120/80
11Ea	0.142	n.s.	0.03	—
12Sa	-0.751	0	0.001	120/100
15E	-0.232	0.05	0.02	120/100
16Sa	-0.512	0	0.03	120/100
16Sb	-0.329	0.05	0.03	120/100
18Sb	-0.547	0.001	0.04	120/100

It is noteworthy that the three Ursholmen subsamples (15E, 16Sa and 16Sb) all had deficiencies of 120/100 heterozygotes, which was especially pronounced in the intermediate sample (16Sa). Likewise, the 12Sa subsample from the middle part of the cline at Jutholmen showed a pronounced deficiency of 120/100 genotypes.

#### *Variation in other polymorphic loci and sources of differentiation*

The overall chi-square tests for heterogeneity among subsamples were highly significant in each of the four loci ( $P < 0.0001$ ). This indicates that there is indeed differentiation among the sampled populations of *Littorina mariae* for not only *Ark* but also *Pep-1*, *Pgm-2* and *Pgi*. However, replicate subsamples (i.e. a and b subsamples) were mostly not differentiated. Although we found a few cases of significant heterogeneity, all except the two of *Ark* already mentioned (12S and 16S) were non-significant when we corrected for multiple testing using Sidák's inequality.

To study differentiation between localities of contrasting habitats, we thus pooled the subsamples and performed chi-square tests for heterogeneity between neighbouring samples of contrasting habitats from the same continuous populations, or in the cases of Grundskär and Svangen, of the same islands. Apart from the highly significant  $\chi^2$  of *Ark*, we found a number of significant values in *Pgi*, but practically none in *Pep-1* and *Pgm-2* (Table 3). *Pgi* seemed differentiated in relation to wave exposure in three islands, Lökholmen, Jutholmen and Grundskär with *Pgi*<sup>93</sup> in general being more common in sheltered sites than exposed sites of the same island, while in Burholmen, Ursholmen and Svangen we found no significant trends (Table 3 and Appendix).

TABLE 3. Chi-square tests for heterogeneity of allele frequencies between neighbouring samples of contrasting habitats (E, exposed and S, sheltered), but from the same continuous population, except for Svangen and Grundskär where the samples were close but from non-continuous populations. Significance levels are indicated by \*( $0.01 < P < 0.05$ ). \*\*( $0.001 < P < 0.005$ ) and \*\*\*( $P < 0.001$ ).

Island	Localities	Distance between localities	<i>Ark</i> $\chi^2$ (d.f.)	<i>Pep-1</i> $\chi^2$ (d.f.)	<i>Pgi</i> $\chi^2$ (d.f.)	<i>Pgm-2</i> $\chi^2$ (d.f.)
Lökholmen	5S vs 6E	100 m	168.7 (2)***	0.1 (1)	11.9 (1)***	0.6 (1)
Lökholmen	7E vs 8S	60 m	166.2 (2)***	0.6 (1)	8.6 (1)**	0.6 (1)
Jutholmen	11E vs 12S	50 m	111.0 (2)***	2.9 (1)	11.3 (1)***	0.9 (1)
Burholmen	13E vs 14S	80 m	119.1 (2)***	0.0 (1)	2.2 (1)	0.0 (1)
Ursholmen	15E vs 16S	7-17 m	36.9 (2)***	1.6 (1)	0.0 (1)	5.1 (1)*
Svangen	17E vs 18S	40 m	102.5 (2)***	0.0 (1)	0.4 (1)	0.1 (1)
Grundskär	19S vs 20E + 21E	90-120 m	260.8 (2)***	1.8 (1)	6.3 (1)*	0.0 (1)
Total			965.1 (14)***	7.1 (7)	40.8 (7)***	7.3 (7)

We also performed  $\chi^2$ -tests of heterogeneity between samples from similar habitats. These tests revealed that there were in most cases substantial differences even between samples of similar environments (Table 4). This differentiation may be caused by samples being from different islands, but may also originate from deviations within the same island.

To be able to distinguish between the different components of differentiation, we performed a gene diversity analysis (Nei, 1973; Chakraborty, 1980) in which we analysed both the differentiation due to different habitats and the differentiation due to geographic separation between islands. The overall level of heterozygosity was high in all four loci ( $H_T$  range: 0.31-0.57, Table 5). Three loci (*Pep-1*, *Pgi* and *Pgm-2*) had  $G_{ST}$  values of around 5% but *Ark* showed that more than 40% of its total diversity was due to differentiation between subsamples (Table 5). The main part of the between subsample variation in *Ark* was due to differentiation between subsamples of different habitats ( $G_{HT}$  compared with  $G_{SH}$ ), while differentiation among islands was relatively modest ( $G_{IT}$  compared with  $G_{SI}$ ). Although the overall level of between subsample variation was much lower in *Pgi*, this locus was similar to *Ark* in that differentiation within islands ( $G_{SI}$ ) was larger than that between islands ( $G_{IT}$ ). But in contrast to the situation in *Ark*, the differentiation between habitats ( $G_{HT}$ ) was no greater than differentiation within habitats ( $G_{SH}$ , Table 5). The two loci *Pep-1* and *Pgm-2* appeared to reflect a similar population genetic structure. The

TABLE 4. Chi-square test for heterogeneity of allele frequencies among all samples of *Littorina mariae* of similar habitat types (sheltered or exposed)

Locus	Habitat type:		
	Exposed $\chi^2$ (d.f.)	Sheltered $\chi^2$ (d.f.)	Total $\chi^2$ (d.f.)
<i>Ark</i>	102.9 (22)***	49.1 (16)***	152.0 (38)***
<i>Pep-1</i>	32.9 (11)***	28.3 (8)***	61.2 (19)***
<i>Pgi</i>	23.0 (11)***	14.1 (8)	37.1 (19)**
<i>Pgm-2</i>	29.1 (8)***	11.6 (8)	40.7 (16)***

TABLE 5. Gene diversity analysis (Nei, 1973) of four polymorphic loci analysed in all 40 subsamples of *Littorina mariae*. The total variation ( $H_T$ ) is first divided into the two components  $G_S$  and  $G_{ST}$  (the proportions of within and between subsample variation respectively).  $G_{ST}$  may be split further into  $G_{SI}$  and  $G_{IT}$  (variation within and between islands), or to  $G_{SH}$  and  $G_{HT}$  (variation within and between habitat types)

Locus	No. of sub-samples	$H_T$	$H_S$	$d_m$	$G_S$	Coefficients of gene differentiation:				
						$G_{ST}$	$G_{SI}$	$G_{IT}$	$G_{SH}$	$G_{HT}$
<i>Ark</i>	40	0.572	0.332	0.246	0.580	0.420	0.311	0.108	0.040	0.379
<i>Pep-1</i>	40	0.317	0.305	0.012	0.962	0.038	0.010	0.028	0.035	0.003
<i>Pgi</i>	40	0.367	0.350	0.017	0.954	0.046	0.035	0.011	0.033	0.014
<i>Pgm-2</i>	34	0.511	0.485	0.027	0.949	0.051	0.018	0.033	0.049	0.002

$H_T$ : total diversity (heterozygosity).

$H_S$ ,  $G_S$ : within subsamples.

$d_m$ : average minimum genetic distance between subsamples ( $d_m = s(H_T - H_S)/(s-1)$ , where  $s$  is the number of subsamples).

$G_{ST}$ : between subsamples ( $G_{ST} = 1 - H_S/H_T$ ).

$G_{SI}$ : between subsamples, within islands.

$G_{IT}$ : between islands.

$G_{SH}$ : between subsamples, within habitat types (exposed and sheltered).

$G_{HT}$ : between habitat types.

main part of their between subsample variation was due to between island differentiation ( $G_{IT}$  compared with  $G_{SI}$ ), while the between habitat components were small ( $G_{HT}$  compared with  $G_{SH}$ , Table 5).

The pattern of variation in both *Pep-1* and *Pgm-2* accorded with the pattern expected for neutral loci, that is differentiation caused by stochastic processes among geographically separated populations. The allele distribution in *Pgi* was, however, more complex. Differences between subsamples within islands were larger than differences between islands, as in *Ark*, albeit on a smaller scale. In *Ark*, however, the large differences between subsamples from the same island were explained by the fact that these subsamples belonged to different habitats. In *Pgi*, the coefficient of differentiation between habitats ( $G_{HT}$ ) explained only a minor part of the total differentiation between subsamples ( $G_{ST}$ ) (Table 5).

Another way to find out if there is any pattern of genetic relationship between samples is to compare genetic identity coefficients between all pairs of subsamples. Considering one locus at a time, mean genetic identities between samples of similar habitats were significantly higher than mean genetic identities between samples of dissimilar habitats for both *Ark* and *Pgi*, while in *Pep-1* and *Pgm-2* samples of different habitats were as genetically similar as samples within habitats (Table 6).

TABLE 6. Average values of Nei's (1972) genetic identities ( $I$ ) between pairs of samples of the same habitat type and between pairs of samples of different habitat type (exposed and sheltered). Differences between means are tested with Student's  $t$ -test

Locus	Average $I$ within habitats	Average $I$ between habitats	$t$	d.f.	$P$
<i>Ark</i>	0.986	0.300	58.35	208	< 0.001
<i>Pep-1</i>	0.988	0.986	0.72	208	n.s.
<i>Pgi</i>	0.992	0.978	5.76	208	< 0.001
<i>Pgm-2</i>	0.975	0.973	0.39	151	n.s.

## DISCUSSION

The general pattern of variation in *Littorina mariae* over the study area in Sweden is that of a species with substantial genetic differentiation among populations, especially among island populations. Although this applies for all four polymorphic loci, the differentiation in arginine kinase is extreme. Before we discuss possible explanations for the arginine kinase variation, we may consider the differentiation revealed by the other three loci.

Differentiation between discontinuous populations of *L. mariae* was expected as this species appears to lack an effective method for between-island dispersal. Similar differentiation, due perhaps mainly to genetic drift in discontinuous populations, is found in other marine snails without pelagic larvae, for example, in *Littorina saxatilis* (Janson & Ward, 1984; Janson, 1987b), *Bembicium vittatum* (Johnson & Black, 1991) and *Nucella lapillus* (Grant & Utter, 1988). On the other hand, species of marine snails which have larvae which spend weeks in the pelagic zone may be only moderately differentiated even on a scale of thousands of kilometres (e.g. Grant & Lang, 1991; Johannesson, 1992).

Of course, even species which lack pelagic larvae, such as *Littorina mariae*, do spread between islands at a low rate. There are no studies of long distance dispersal in *L. mariae*, but possible mechanisms include rafting of egg masses and snails attached to seaweed. Active creeping of juveniles and adults may be possible only within continuous populations and is probably only of importance over short shore distances (tens of metres or so).

The level of between population differentiation as indicated by the  $G_{ST}$  coefficients of the polymorphic loci (except *Ark*) indicate that the differentiation in *L. mariae* ( $G_{ST}$  around 0.04–0.05 over 15 km, this study) is similar to that of *L. saxatilis* (mean  $G_{ST}$  of 14 polymorphic loci is 0.05 over 1 km of a continuous population—from Janson & Ward, 1984 with *Aat* excluded). *Littorina saxatilis* lives as a rock dweller and can rarely be dispersed by rafting on seaweed. Egg masses, juveniles and adults of *L. mariae*, on the other hand, live on seaweed fronds and are thus more likely to be passengers on drifting parts of plants. However, care must be taken in comparing the level of differentiation between species as the estimates (especially if based on a small set of different loci) may be crucially dependent on the particular set of enzyme loci chosen (see e.g. Da Silva, Bell & Burt, 1992). Anyway, differentiation in *L. mariae* (disregarding *Ark*), if explained mainly by stochastic evolution, implies an interchange of migrants among island populations in the range of a few individuals per generation (e.g. Hartl, 1980).

The differentiation in *Ark*, which is one order of magnitude greater ( $G_{ST}$ -value of 0.42), cannot be explained by the same evolutionary process. The allele frequencies of *Ark* are more or less directly related to habitat type, suggesting that selection in *Ark* or, possibly, in a closely linked locus, is the primary cause of variation in this locus.

An alternative hypothesis is that two sibling species are present in the area, each species associated with one type of habitat, and each having a distinct distribution of *Ark* alleles. A closely related species to *Littorina mariae* is *Littorina obtusata* (L.). These species have no diagnostic shell characters but mature snails may be distinguished by differences in the reproductive organs (Sacchi & Rastelli, 1966; Reid, 1990). Although we did not dissect all snails of our study,

we can reject the possibility that *L. obtusata* was included in the samples. This is because in the study area these two sibling species have diagnostic differences in the loci *Ipp*, *Pgm-1*, and *Pep-2*, as described earlier.

It remains a possibility that the samples included an as yet undiscovered species of flat periwinkles. This latter hypothesis seems improbable, however, as all populations were fixed for the same alleles for 22 loci, and had similar allele frequencies in at least six of eight polymorphic loci. Sibling species may be genetically very similar, as, for example, the littorinid sibling species pair *Littorina saxatilis* and *L. arcana* (Ward & Warwick, 1980; Ward & Janson, 1985). It seems unlikely, however, that random genetic drift would have generated such a great difference in just one out of 29 loci while the others have remained undifferentiated, or nearly so, since the time of speciation.

However, one observation may point in the direction of two more or less reproductively isolated taxa. This is the striking deficiency of 120/100 heterozygotes, especially in intermediate habitats (e.g. 12Sa and 16Sa). This deficiency may suggest a barrier to genetic exchange between populations inhabiting different microenvironments. Alternatively, the lack of heterozygotes may be due to selection favouring the 120/120 and 100/100 homozygotes over the 120/100 heterozygote even at intermediate degrees of exposure. Population mixing as a result of migration from exposed and sheltered areas into intermediate parts of the clines may contribute to the striking heterozygote deficiency present. Selection, and perhaps, Wahlund mixing, seems to us more likely explanations than the hypothesis of two reproductively isolated taxa.

In spite of the deficiency of 120/100 heterozygotes, we thus believe that the variation in *Ark* is intraspecific and strongly related to the type of microenvironment of a population. We have found that one important aspect of the habitat is the degree of wave exposure. Unfortunately there is no good and simple way of estimating the degree of wave exposure of a microenvironment. We used a rough method in which the presence of the macrophyte *Ascophyllum nodosum* indicated sheltered sites and the absence indicated exposed sites. We had a few sites of intermediate habitat, and these sites were in all cases classified as sheltered, as we found at least some plants of *Ascophyllum* there (12a, 16a and 21a and b). The two sites 12a and 16a had intermediate frequencies of *Ark*, indicating that these sites were close to the middle of the *Ark* cline. At Ursholmen the switch from dominance of the sheltered alleles (110 and 120) to dominance of the exposed alleles (80 and 100) took place over 17 m (Fig. 3), indicating that such a switch may be fairly abrupt. It thus seems unlikely that a switch in *Ark* genotype does in fact coincide with the point on the exposure gradient where *Ascophyllum* is no longer present. That the two sites 21a and b showed frequencies of *Ark* typical of exposed sites in spite of some *Ascophyllum* plants being present is thus not at all surprising, given the pattern of abrupt switches between the dominance of sheltered and exposed *Ark* genotypes.

At the molecular level, the possibility of selective survival of different *Ark* genotypes in different microenvironments seems at least plausible. Arginine phosphate in marine gastropods is an energy rich molecule analogous to creatine phosphate in vertebrates. Arginine kinase catalyses the translocation of one phosphate group from the high-energy phosphate reservoir (arginine phosphate) to ADP to replenish the supply of ATP during anaerobic muscular work (e.g. Hochachka & Somero, 1984). It is known that the rate of energy production

depends upon the amount of arginine kinase available (Hochachka & Somero, 1984), and it seems likely that the specific enzyme activity of different allozymes may be important. Genetic variation at the *Ark* locus in snails may be closely related to physiological performance. One may imagine that the demands of muscular activity of a snail may differ between areas of intense wave stress and areas protected from heavy wave action.

Perhaps the most interesting finding of this study is not the presumably selected differentiation in *Ark* in itself, but the scale of this differentiation. As briefly reviewed in the first part of this paper, genetic variation attributed to selection has been reported for individual loci of a wide range of organisms, but usually this variation is evident over tens of kilometres or even much more. The steepest cline that we found in *Ark* of *L. mariae* was at Ursholmen where, for example, *Ark*<sup>120</sup> increased from 0.18 in the exposed site to 0.86 in the most sheltered site only 17 m away. In the remaining four populations (Jutholmen, Burholmen, L oxholmen north and south sides) where we sampled both sheltered and exposed habitats in the same continuous populations we found the allele *Ark*<sup>120</sup> to increase from about 0.05 or less in exposed sites to about 0.75 or more in sheltered sites over distances of between 50 and 100 m.

The presence of allelic differentiation over only tens of metres of distance is unexpected. However, there are a few other examples in direct developing marine gastropods of similarly compressed, presumably selected, allozyme clines. In *Littorina saxatilis*, one of two *Aat* alleles is favoured in the high intertidal while the other allele is favoured in the mid intertidal zone (Johannesson & Johannesson, 1989, 1990). This cline is extremely sharp with, sometimes, the frequency of one allele dropping from 0.80 to 0.01 over 2 metres of a dense continuous population. The appearance of similar clines in scattered geographic areas (Sweden, Iceland, Norway, Isle of Man) strongly supports the suggestion of selection in this or a closely linked locus.

Populations of *Nucella lapillus* along 21 km of shore in SW. England are highly differentiated at four loci (*Pep*, *Est*, *Lap* and *Mdh*). Differences may be substantial even between sites only 50 m away, and the frequencies of alleles at these four loci correlate with the degree of wave exposure (Day, 1990).

Two facts are noteworthy here: *L. mariae*, *L. saxatilis* and *Nucella lapillus* all have low vagility and they are all confined to a harsh and gradient rich environment; the littoral zone of rocky shores. This makes them likely candidates for genetic divergence in general, and strongly selected variation in particular.

Finally, we should like to stress the importance of microscale sampling when studying the genetic variation in species. We suggest that large scale studies should be accompanied by an analysis of microscale variation within each geographic area. We furthermore believe that microscale variation may be more common than earlier studies suggest, because the scale of sampling may often have been too large.

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## APPENDIX

Genetic variation in six polymorphic loci scored in 40 subsamples from 21 locations (samples) of *Littorina mariae* from the northern part of the Swedish west coast. The localities are numbered 1 to 21. E indicates wave exposed sites and S sheltered sites. Subsamples (within 8 to 10 m of each other) from the same locality are indicated by low case letters (a, b and c)

Locus/ allele	Saltö								Lökholmen					
	1Ea	1Eb	2Ea	2Eb	3Ea	3Eb	4Ea	4Eb	5Sa	5Sb	6Ea	6Eb	7Ea	7Eb
<i>Ark</i>														
(n)	26	20	29	20	22	21	30	28	29	29	34	34	33	26
130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.017	0.000	0.000	0.000	0.000
120	0.096	0.125	0.034	0.050	0.000	0.000	0.000	0.018	0.724	0.776	0.044	0.059	0.030	0.019
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.103	0.069	0.000	0.000	0.000	0.000
100	0.750	0.750	0.793	0.900	0.864	0.595	0.767	0.839	0.121	0.069	0.750	0.735	0.848	0.788
90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
80	0.154	0.125	0.172	0.050	0.136	0.405	0.233	0.143	0.017	0.069	0.206	0.206	0.121	0.192
<i>Ipp</i>														
(n)	26	20	29	20	23	21	30	28	29	29	34	34	33	26
110	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.962	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.914	0.971	0.971	0.985	0.962
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.069	0.029	0.015	0.000	0.038
90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.015	0.015	0.000
<i>Mpi</i>														
(n)	13	13	10	6	10	9	14	12	12	12	8	8	17	12
150	0.038	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000
110	0.038	0.038	0.000	0.000	0.050	0.000	0.071	0.125	0.042	0.000	0.000	0.000	0.000	0.000
100	0.923	0.962	1.000	1.000	0.950	1.000	0.893	0.875	0.958	1.000	1.000	1.000	1.000	1.000
90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Pep-1</i>														
(n)	27	20	29	20	23	21	30	27	29	29	34	34	33	26
107	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.833	0.825	0.828	0.850	0.652	0.762	0.817	0.722	0.862	0.828	0.897	0.824	0.894	0.827
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
80	0.167	0.175	0.172	0.150	0.348	0.238	0.183	0.278	0.138	0.172	0.103	0.176	0.106	0.173
<i>Pgi</i>														
(n)	27	20	29	20	23	21	30	28	29	29	34	34	33	26
100	0.722	0.800	0.862	0.925	0.783	0.714	0.833	0.839	0.759	0.638	0.926	0.824	0.773	0.808
93	0.278	0.200	0.138	0.075	0.217	0.286	0.167	0.161	0.241	0.328	0.074	0.176	0.227	0.192
85	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000
<i>Pgm-2</i>														
(n)	5	—	5	—	—	—	9	9	29	29	34	34	33	26
102	0.000	—	0.000	—	—	—	0.000	0.056	0.000	0.034	0.059	0.059	0.000	0.058
100	0.600	—	0.800	—	—	—	0.667	0.611	0.707	0.500	0.603	0.618	0.606	0.635
93	0.400	—	0.200	—	—	—	0.333	0.333	0.293	0.466	0.338	0.324	0.394	0.308

## Appendix—continued

Locus/allele	Långholmen				Jutholmen				Burholmen				
	8Sa	8Sb	9S	10S	11Ea	11Eb	12Sa	12Sb	13Ea	13Eb	13Ec	14Sa	14Sb
<i>Ark</i>													
(n)	31	27	34	34	28	24	24	23	20	20	21	14	18
130	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000
120	0.855	0.741	0.809	0.676	0.071	0.000	0.583	0.870	0.000	0.000	0.024	0.821	0.694
110	0.032	0.074	0.059	0.015	0.018	0.000	0.000	0.087	0.000	0.000	0.000	0.000	0.000
100	0.097	0.148	0.103	0.191	0.750	0.833	0.396	0.022	0.850	0.925	0.881	0.179	0.250
90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
80	0.000	0.037	0.029	0.118	0.161	0.167	0.021	0.000	0.150	0.075	0.095	0.000	0.056
<i>Ipp</i>													
(n)	31	27	34	34	23	21	30	28	29	29	34	34	33
110	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.984	0.963	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Mpi</i>													
(n)	17	12	8	10	8	10	8	8	—	—	—	—	—
150	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	—	—	—	—	—
110	0.029	0.042	0.000	0.000	0.000	0.000	0.000	0.000	—	—	—	—	—
100	0.941	0.958	1.000	1.000	0.938	1.000	1.000	1.000	—	—	—	—	—
90	0.000	0.000	0.000	0.000	0.062	0.000	0.000	0.000	—	—	—	—	—
<i>Pep-1</i>													
(n)	31	27	34	34	28	24	24	23	20	20	21	14	18
107	0.000	0.019	0.000	0.000	0.018	0.000	0.000	0.000	0.050	0.000	0.000	0.036	0.000
100	0.790	0.833	0.765	0.706	0.732	0.688	0.667	0.543	0.875	0.900	0.857	0.857	0.889
95	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
80	0.194	0.148	0.235	0.294	0.250	0.313	0.333	0.457	0.075	0.100	0.143	0.107	0.111
<i>Pgi</i>													
(n)	31	27	34	34	28	24	24	23	20	20	21	14	18
100	0.694	0.519	0.735	0.706	0.804	0.896	0.688	0.587	0.800	0.775	0.786	0.714	0.667
93	0.290	0.481	0.265	0.294	0.196	0.104	0.313	0.413	0.200	0.225	0.214	0.286	0.333
85	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Pgm-2</i>													
(n)	31	27	34	34	28	24	24	23	20	20	21	14	18
102	0.000	0.000	0.059	0.059	0.089	0.000	0.104	0.087	0.000	0.000	0.000	0.071	0.139
100	0.597	0.796	0.647	0.544	0.589	0.542	0.521	0.652	0.675	0.800	0.738	0.643	0.639
93	0.403	0.204	0.294	0.397	0.321	0.458	0.375	0.261	0.325	0.200	0.262	0.286	0.222

## Appendix—continued

Locus/allele	Ursholmen			Svängen				Grundskär					
	15E	16Sa	16Sb	17Ea	17Eb	18Sa	18Sb	19Sa	19Sb	20Ea	20Eb	21Ea	21Eb
<i>Ark</i>													
(n)	30	22	18	26	26	25	28	26	26	33	33	26	26
130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
120	0.183	0.477	0.861	0.000	0.038	0.620	0.679	0.827	0.827	0.000	0.000	0.000	0.019
110	0.017	0.023	0.083	0.000	0.000	0.060	0.018	0.000	0.019	0.000	0.000	0.000	0.000
100	0.650	0.477	0.056	0.942	0.865	0.320	0.250	0.173	0.135	0.864	0.924	0.904	0.827
90	0.000	0.000	0.000	0.019	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
80	0.150	0.023	0.000	0.038	0.058	0.000	0.054	0.000	0.019	0.136	0.076	0.096	0.154
<i>Ipp</i>													
(n)	31	27	34	34	23	21	30	28	29	29	34	34	33
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Mpi</i>													
(n)	15	11	7	5	8	6	11	—	—	8	8	9	9
150	0.000	0.000	0.000	0.000	0.000	0.083	0.000	—	—	0.000	0.000	0.000	0.000
110	0.000	0.000	0.000	0.000	0.125	0.083	0.000	—	—	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	0.875	0.833	0.955	—	—	1.000	1.000	1.000	1.000
90	0.000	0.000	0.000	0.000	0.000	0.000	0.045	—	—	0.000	0.000	0.000	0.000
<i>Pep-1</i>													
(n)	30	22	18	26	25	25	28	26	26	33	33	26	26
107	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000
100	0.850	0.705	0.833	0.750	0.840	0.780	0.786	0.750	0.846	0.894	0.924	0.692	0.923
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
80	0.150	0.295	0.167	0.250	0.160	0.220	0.214	0.231	0.154	0.106	0.076	0.308	0.077
<i>Pgi</i>													
(n)	30	22	18	26	26	25	28	26	26	33	33	26	26
100	0.767	0.750	0.778	0.769	0.865	0.880	0.696	0.712	0.519	0.682	0.727	0.808	0.808
93	0.233	0.250	0.222	0.231	0.135	0.120	0.304	0.288	0.481	0.318	0.273	0.192	0.192
85	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Pgm-2</i>													
(n)	28	22	18	26	26	25	28	26	26	33	33	26	25
102	0.036	0.068	0.000	0.077	0.058	0.060	0.018	0.038	0.038	0.167	0.121	0.077	0.060
100	0.839	0.614	0.750	0.673	0.615	0.660	0.643	0.558	0.481	0.394	0.470	0.481	0.440
93	0.125	0.318	0.250	0.250	0.327	0.280	0.339	0.404	0.481	0.439	0.409	0.442	0.500