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Micro- and macrogeographic allozyme variation in *Littorina fabalis*; do sheltered and exposed forms hybridize?

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Earlier studies of Swedish populations of the marine snail *Littorina fabalis* show that snails from different microhabitats (with a greater and lesser exposure to wave action) have almost diagnostic differences in one allozyme locus (arginine kinase, *Ark*), and differ in adult size. Snails with 'sheltered' and 'exposed' *Ark* genotypes occur in sympatry in intermediary exposed sites and here adult sizes remain distinct. Approaching the microgeographic differentiation we studied the parts of two populations where the frequency of *Ark* changes dramatically over zones 50–120 m wide. The aim was to test if the transitional zones are best described as areas of mixing of two genetically separate populations, or if hybridization between the exposed and sheltered groups occurs. Heterozygotes were in deficiency along both clines but were still roughly twice as common as expected from a pure mixing of 'sheltered' and 'exposed' groups suggesting hybridization. Hybridization was also supported by the observation that snails homozygous for sheltered and exposed alleles mated at random with each other in both populations. On the macrogeographic scale, we found populations from exposed and sheltered sites in France and Wales being fixed for the same exposed and sheltered *Ark* alleles as found in Sweden. However, variation in three other highly polymorphic loci indicated geographic affinity rather than habitat similarity being the main factor of genetic coherence. These observations support a hypothesis of gene flow between exposed and sheltered populations of *L. fabalis*. Two Spanish populations were remarkably different with unique alleles at high frequencies in three of four strongly polymorphic loci.

ADDITIONAL KEY WORDS:—arginine kinase – hybridization – heterozygote deficiency – genetic clines – gene diversity – mate choice.

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INTRODUCTION

Allozyme differentiation may often show clear and consistent patterns over numbers of polymorphic loci. This makes the interpretation in terms of gene flow, evolutionary pathways and genetic relationships among populations, fairly simple (e.g. Hellberg, 1994; Johnson & Black, 1995). When the patterns of variation among polymorphic loci are either inconsistent, or contradict what is observed from other characters, the interpretation may be much more complex and mechanisms such as selection, preceding evolutionary history and hybridization may be invoked. For example, strong selection in one locus may introduce aberrant patterns which contrast with the pattern of variation in accompanying loci (e.g. Koehn *et al.*, 1983), and 'molecular leakage' may eliminate stochastic differentiation between species confusing species boundaries (Clarke *et al.*, 1996).

Genetic clines between populations are usually explained as a result of selection and/or hybridization. Selection gradients produce hybrid zones associated with environmental transitions (e.g. Endler, 1977; Moore & Price, 1993). Tension zones, on the other hand, are hybrid zones between genetically partly incompatible groups which are maintained by decreased hybrid vigour, and these zones may be trapped in regions of low population density or pushed aside by uneven migration (Barton & Hewitt, 1985). A cline may also be best described as a combination of a tension zone and a selection model (Bert & Arnold, 1995). If no hybrids are found, a genetic cline is the result of a mixing of two isolated populations. Evidences of genetic clines over compressed geographic scales (<100 m) are most often found in non-molecular characters, but recent studies disclose striking examples of clinal selection in molecular characters as well (Day, 1990; Nevo *et al.*, 1994; Johannesson *et al.*, 1995; Lönn *et al.*, 1996).

The marine snail *Littorina fabalis* (senior synonym to *L. mariae*, see Reid, 1996) was described as a separate species from *L. obtusata* on only one diagnostic character (male penis tip-length) (Sacchi & Rastelli, 1966). More recently, a diagnostic female character and distinct allozyme alleles have confirmed that the two species are well separated (Reid, 1990; Tatarenkov, 1995). Both species, however, show extensive shell polymorphism, including both a latitude and a habitat component of the shell variation (Reid, 1996). In both species northern snails have projecting spires while southern snails have flat spires. In addition, ecotypes of *L. fabalis* which differ in shell size and colour inhabit shores with contrasting environments (Reimchen, 1981; Rolán & Templado, 1987; Reid, 1996).

Morphological differentiation among populations of *L. fabalis* has twice lead to suggestions that several taxa are involved (Reimchen, 1981; Rolán & Templado, 1987). Furthermore, we recently showed the presence of a reproductive barrier

between snails from moderately wave exposed ('sheltered') sites and wave exposed ('exposed') sites (Tatarenkov & Johannesson, 1998). The evidence for a barrier is that variation in a single allozyme locus is strongly linked to variation in morphological character in snails from truly sympatric samples (Tatarenkov & Johannesson, 1998).

In this study we analyse the microscale transitions between exposed and sheltered parts of two continuous populations of *L. fabalis*. In trying to discriminate between a model of pure mixing and one of hybridization between the exposed and sheltered groups, we examine the distribution of exposed/sheltered heterozygotes along each cline. We also compare the allozyme variation in Sweden with that of populations from exposed and sheltered sites in Wales, France and Spain to test if the habitat related variation in *Ark* found in Sweden is general throughout large parts of the species' distribution. If so, the exposed and sheltered groups may represent evolutionary units of more general validity.

MATERIAL AND METHODS

Microgeographic variation

In Sweden *Littorina fabalis* lives associated with fucoid algae (mainly *Fucus vesiculosus*, but also *F. serratus* and *Ascophyllum nodosum*) at and slightly below mean water level. Continuous, and essentially one-dimensional, populations of *L. fabalis* are present along shores with belts of these algae, occupying the littoral zone from exposed to moderately sheltered parts of shores. We use the presence of *Ascophyllum* as an indicator of 'sheltered' shores, while its absence indicates 'exposed' shores. We sampled *L. fabalis* populations at two small islands (Lökholmen and Jutholmen) off the west coast of Sweden. Both populations occupied a wave-exposure gradient ranging from sheltered to exposed parts of the shore. We have previously screened 30 loci from the exposed and sheltered ends of these clines and knew that there were major differences in allele distributions of arginine kinase between the ends of each cline (Tatarenkov & Johannesson, 1994). In contrast, minute or no differences were found between the cline ends in five other polymorphic loci, and 22 loci were fixed for the same alleles at both ends (Tatarenkov & Johannesson, 1994).

In the present study we sampled several small areas (1–2 m^2) along one-dimensional populations ranging from exposed to sheltered parts of the shore. On Lökholmen we sampled seven areas at intervals of 20 m (subpop. L1–L6); on Jutholmen, 8 km away, six areas (subpop. J1–J6) at intervals of 5–7 m were sampled, except for J6 which was 28 m from J5 (density of snails in the small area between J5 and J6 was too low to allow sampling. Both islands were sampled in August 1993. The shore on Lökholmen has an extended and smooth exposure gradient over 120 m with a steeper gradient between L4 and L5 (Fig. 1). On Jutholmen, there is a rather abrupt increase in exposure over 10 m at the entrance of the small embayment, between sites J4 and J5 (Fig. 2). All samples were screened for the four most polymorphic loci (see below) with average sample sizes of 45.5 snails in Lökholmen and 49.8 snails in Jutholmen.

Mate choice

We sampled a total of 40 copulating pairs of *L. fabalis* from areas of 3×3 m in the middle part of the cline at both Lökholmen (L4: 10, 12 and 14 July 1995) and

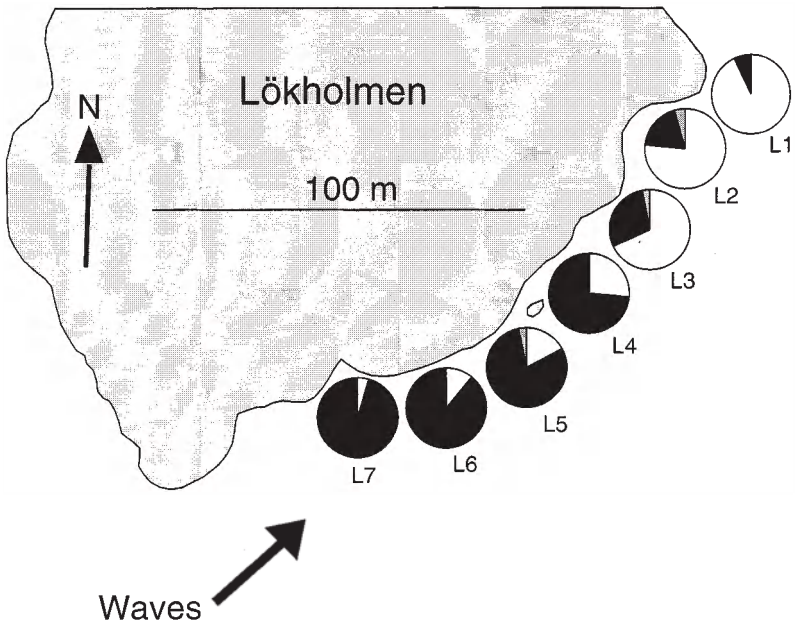

Figure 1. The allozyme cline in *Littorina fabalis* at Lökholmen (SW Sweden). L1 is the most and L7 the least sheltered site (see text). Distances between neighbouring sites are 20 m. Frequencies of the compound allele E (Ark^{80} and Ark^{100}) (black) and the compound allele S (Ark^{130}, Ark^{110}, and Ark^{120}) (white) are shown, as well as the frequency of one rare allele Ark^{70} (hatched) earlier found in both exposed and sheltered samples (Tatarenkov & Johannesson, 1994).

Jutholmen (J3–J4 13 and 17 July 1995). We sexed each snail in the laboratory prior to analysis of *Ark*-genotype and pairs including sexually immature or parasitized snails (often sterilized) were discarded. We compared the observed numbers of copulating pairs (defining each snail by their arginine kinase genotype — SS, SE and EE), with those expected from complete random mating using a χ^2 contingency test in each island separately.

Macrogeographic variation

To assess geographic and habitat-related differentiation on a larger geographic scale we analysed samples of *L. fabalis* from France, Wales and Spain. The French and Welsh samples were taken in September 1993, while the Spanish samples were from December 1995. The French populations were from Bretagne: two from a moderately exposed bay on the Ile de Batz, and two from the relatively sheltered shores close to the marine station in Roscoff. Distances between samples at both locations were about 100 m. One Welsh sample was from a very protected shore (Gann Flats, Dale) and the other was from a fairly exposed shore (Great Castle Bay, Dale). Likewise, the Spanish samples were from a sheltered site (Playa de Canido) and an exposed site (Punta de Estay), both at the mouth of Ría de Vigo in Galicia. Both the exposed and sheltered individuals were of the 'normal' Galician form of *L. fabalis* (*sensu* Rolán & Templado, 1987). In the geographic comparison only the

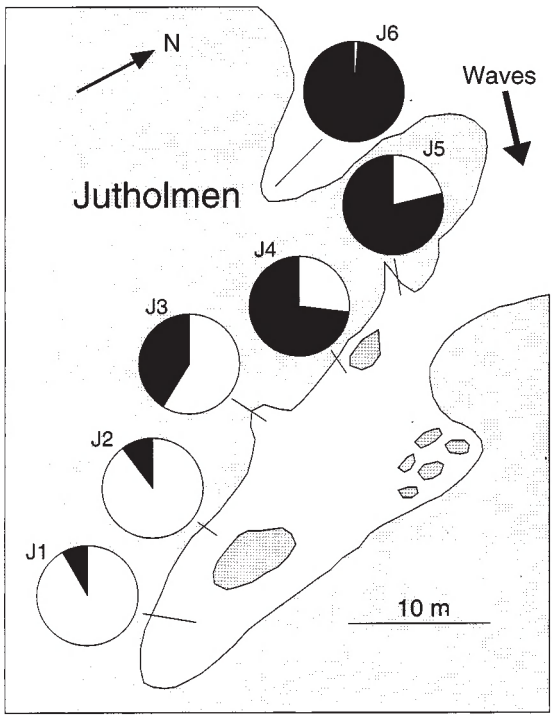

Figure 2. The allozyme cline in *Littorina fabalis* at Jutholmen (SW Sweden). J1 is the most and J6 the least sheltered site (see text). Distances between neighbouring sites are 6 m, except J6 which is 28 m from J5. Allele designations as in Fig. 1.

most exposed and the most sheltered sites from the two Swedish islands (Lökholmen and Jutholmen) were included.

Electrophoresis

Using horizontal starch gel electrophoresis (methods as in Tatarenkov & Johannesson, 1994) we scored snail genotypes at four polymorphic loci; arginine kinase (*Ark*; E. C. code 2.7.3.3), peptidase (*Pep-1*; 3.4.-.-), phosphoglucomutase (*Pgm-2*; 5.4.2.2), phosphoglucose isomerase (*Pgi*; 5.3.1.9). Out of 30 loci screened these were the only ones showing extensive polymorphisms (Tatarenkov & Johannesson, 1994). In many of the analyses we pooled five of the six *Ark*-alleles into two compound alleles (S and E). The 'sheltered' (S-) allele included two rare alleles (Ark^{130} and Ark^{110}) and one allele (Ark^{120}) which is the most common allele in sheltered sites (Tatarenkov & Johannesson 1994, 1998). The 'exposed' (E-) allele included two alleles which are both common in exposed sites (Ark^{80} and Ark^{100}). The allele Ark^{70} has been found, albeit at low frequencies (<0.03), in both exposed and sheltered Swedish sites; genotypes including this allele were excluded from further analysis.

Statistics

Observed genotype frequencies of the compound genotypes of *Ark* (SS, SE and EE) were tested for consistency with Hardy–Weinberg expected frequencies in each

sample from the two clinal habitats at Lökholmen and Jutholmen. To avoid problems with low expected numbers we used a pseudoprobability test (Hernández & Weir, 1989) using the program CHIHW (Zaykin & Pudovkin, 1993). We carried out 13 tests in total, but did not compensate for multiple testing as this tends to increase the risk of Type-II error (Rothman, 1990); also, we did not want to over-estimate the number of non-significant samples.

To avoid the problem of small expected numbers in the test of random mating of snails of different *Ark*-genotype, we used a pseudoprobability program (Zaykin & Pudovkin, 1993) to estimate the observed probabilities of the contingency table.

We used a modified version of Nei's gene diversity analysis (Nei, 1973; Chakraborty, 1980) to evaluate the effects of geographic isolation and habitat on the total genetic variation. The original gene diversity analysis requires an hierarchical sampling design and from this analysis the total variation is partitioned into components of variation generated at different levels. Johannesson & Tatarenkov (1997) modified the gene diversity analysis using an orthogonal sampling structure. The orthogonal model allowed evaluation of the effects of two (or more) factors simultaneously, as well as their interaction, and Johannesson & Tatarenkov (1997) used this to study the effects of habitat and gene flow (population subdivision). We used basically the same design in this study with the two factors habitat (H) and area (A) and their interaction. Johannesson & Tatarenkov (1997) used replicate samples from each combination of the two orthogonal factors and were able to evaluate the effects of the factors and the interaction using an analysis of variance. In this study we lacked replicate samples of the Welsh and Spanish samples and therefore we had no estimate of the residual variation and could not apply an ANOVA.

RESULTS

Variation at micro scale

Both islands exhibited smooth genetic clines in *Ark*; for example, the exposed compound allele ('E') decreasing gradually from a nearly complete dominance (>95%) at the exposed ends of the clines to low frequencies (<9%) at the sheltered part of the clines. Interestingly, the steepest parts of the genetic clines did not exactly coincide with the parts of the environmental gradients which were sharpest. While at Lökholmen the most pronounced change from exposed to sheltered micro environments was between L4 and L5, the genetic switch was most prominent between L3 and L4. Also at Jutholmen the genetic switch (around J3) was located on the sheltered side of the steepest environmental transition (between J4 and J5) (Figs 1 and 2).

In contrast to *Ark*, the three other loci (*Pep-1*, *Pgi* and *Pgm*-2) showed much less, if any, heterogeneity among samples at this microscale. In *Pgi* there was variation among samples within islands ($P=0.013$ for Lökholmen and $P<0.001$ for Jutholmen) and this variation tended to be related to microhabitat. That is, the frequency of Pgi^{100} seemed to increase going from sheltered to exposed sites in both islands, but this trend was only significant in Jutholmen ($P<0.01$ for the regression). In Jutholmen, *Pep-1* revealed heterogeneity over samples, although not in Lökholmen.

We furthermore found no signs of deviation from Hardy–Weinberg expected

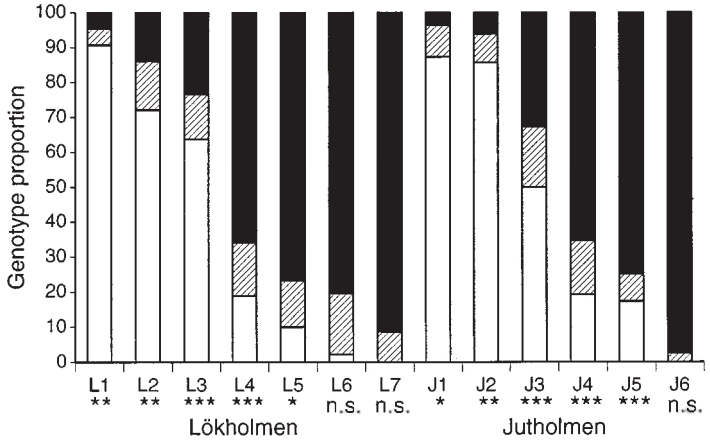

Figure 3. Frequencies of the three different *Ark* genotypes, SS (white), SE (hatched), and EE (black)—along the clines at Lökholmen and Jutholmen. Genotype distributions of each sample were tested against Hardy–Weinberg distributions using a pseudoprobability χ^2 test (see text); $*P<0.05$; $**P<0.01$; $***P<0.001$.

genotype frequencies in these three loci, whereas in *Ark* heterozygote deficiency was the rule rather than the exception (Fig. 3). This population genetic pattern of *Ark* suggested either selection against heterozygotes or a mixing of two genetically separate populations. From an earlier study (Tatarenkov & Johannesson, 1998) there are suggestions that selection against heterozygotes of *Ark* is not substantial and if so, does not explain the deficiency found (see Discussion). Mixing of two isolated gene pools may, however, be an alternative. But one observation did not fit very well with a model of pure mixing and that was the higher proportion of heterozygotes towards the middle of both clines (Fig. 3). We assumed that the most sheltered and exposed sample of each cline represented 'pure' parental groups each including a small fraction of *Ark* heterozygotes. Furthermore we assumed that all heterozygotes in the end samples belonged to the group of homozygotes which dominated. From this we calculated the number of *Ark* heterozygotes that would have been present in the different samples of the clines, if the samples were simply mixtures of two non-hybridizing groups. In most samples the number of observed heterozygotes was more than twice that expected from mixing of two non-hybridizing taxa (Table 1). These calculations, however, were critically dependent on the sampling frequencies of heterozygotes at the cline ends. To get some sort of statistical evaluation of the calculated values we recalculated the expected number of heterozygotes using both the upper and the lower values of the estimated 95% confidence interval of heterozygote frequencies at cline ends. Using the lower estimates gave, of course, extremely low expected numbers of heterozygotes, while using the upper estimates resulted in expected numbers close to those observed. This last result may suggest that we did not indeed observe more heterozygotes than expected and that we could not reject a model of pure mixing. However, this estimate was very conservative as we had used upper confidence limits of heterozygotes of both exposed and sheltered parental groups to calculate the expected number of heterozygotes in a sample: it is extremely unlikely that both values were at the extreme end at the same time. Thus we concluded that there were more heterozygotes than might be expected

TABLE 1. Observed numbers of hybrids along the Lökholmen and Jutholmen clines versus hybrid numbers expected from mixing the end-populations (L1 and L7 for Lökholmen, and J1 and J6 for Jutholmen) in proportions given by the relative distribution of EE and SS individuals in each site

Population	Lökholmen					Jutholmen			
	L2	L3	L4	L5	L6	J2	J3	J4	J5
Obs. No. of SE	6	6	8	4	8	4	9	8	4
Using observed numbers of heterozygotes in L1/L7 and in J1/J6:									
Exp. No. of SE	2.0	2.4	3.5	2.1	3.3	3.9	2.8	1.8	1.8
Obs./Exp.	3.0	2.6	2.3	1.9	2.5	1.0	3.2	4.5	2.2
Using upper value of 95% confidence interval of heterozygote estimates in L1/L7 and in J1/J6:									
Exp. No. of SE	4.7	5.4	7.1	4.2	6.3	7.2	5.8	4.7	5.0
Obs./Exp.	1.3	1.1	1.1	0.9	1.3	0.6	1.5	1.7	0.8
Using lower value of 95% confidence interval of heterozygote estimates in L1/L7 and in J1/J6:									
Exp. No. of SE	0.3	0.4	0.9	0.6	0.9	1.3	0.8	0.3	0.3
Obs./Exp.	19.8	14.1	8.5	6.6	8.4	3.1	11.2	25.8	14.3

TABLE 2. Mate choice with respect to *Ark*-genotype (sheltered homozygote, SS; exposed homozygote, EE and heterozygote, SE) in two intermediary populations of *L. fabalis* (L4 from Lökholmen and J3–J4 from Jutholmen). Only copulating pairs including mature individuals were considered. Observed numbers of pairs are shown along with expected numbers (in brackets). Deviation from random mating is tested by contingency chi-square tests using a pseudo-probability programme (Zaykin & Pudovkin, 1993) to estimate the probabilities of significant deviations (df = 4 in both tests)

Male genotype		Female genotype SS	SE	EE	χ^2	P
Lökholmen:	SS	9 (9.0)	4 (4.8)	7 (6.2)		
	SE	1 (1.3)	1 (0.7)	1 (0.9)	1.09	0.93
	EE	3 (2.7)	2 (1.4)	1 (1.9)		
Jutholmen:	SS	4 (4.4)	1 (0.7)	3 (2.9)		
	SE	1 (1.1)	0 (0.2)	1 (0.7)	1.26	1.0
	EE	1 (0.5)	0 (0.1)	0 (0.4)		

from a model of pure mixing, while at the same time there were far fewer heterozygotes than expected from a population in Hardy–Weinberg equilibrium (Fig. 3). This indicates that although the exposed and sheltered groups of *L. fabalis* hybridize, there is either selection against heterozygotes or a partial genetic isolation.

Mate choice

The mid-cline populations at both islands (L4 and J3–J4) contained high proportions of both sheltered and exposed *Ark* genotypes, with far fewer heterozygotes than expected (as discussed above, Fig. 3). The sampling of copulating pairs from this area did not show any tendencies, however, for non-random mating (Table 2). Pooling the heterozygotes with the rarest group of homozygotes, the EE homozygotes, we calculated Yule's V index of sexual isolation between the two groups (Gilbert &

TABLE 3. Orthogonal gene diversity analysis (Johannesson & Tatarenkov, 1997) indicating the relative importance of habitat and geographic subdivision and their interaction in four enzyme loci of *Littorina fabalis*. Only one population from each habitat (exposed and sheltered, see text for definitions) and each geographic area (France, Spain, Sweden, and Wales) are included

	H_T	H_S	G_S	G_{ST}	G_{AT}	G_{HT}	$G_{H\times A}$
Ark	0.681	0.098	0.144	0.856	0.388	0.337	0.131
Pgi	0.418	0.371	0.887	0.113	0.033	0.015	0.065
Pep-1	0.575	0.473	0.822	0.178	0.156	0.000	0.022
Pgm-2	0.422	0.306	0.725	0.275	0.270	0.000	0.004

H_T total heterozygosity
H_S heterozygosity within populations
G_S differentiation within populations
G_{ST} among populations
G_{AT} among areas
G_{HT} between habitats
$G_{H\times A}$ habitat-area interaction

Starmer, 1985). This index ranges from −1 to 1 with 0 indicating a perfect random mating. Yule's V was 0.005 for Lökholmen and −0.15 for Jutholmen, both values obviously very close to zero.

Variation at macro scales

All the four loci studied were strongly polymorphic over the geographic area covered (France, Spain, Sweden, Wales) with total heterozygosities (H_T) in the range of 0.4–0.7 (Table 3). Similarly, all loci revealed strong differentiation among the sampled populations (all values of G_{ST} in Table 3 are different from zero, $P<0.001$); however, only in *Ark* was this differentiation to a large extent generated by variation between exposed and sheltered populations (G_{HT}). In contrast, *Pep-1* and *Pgm-2* had variations largely due to geographic separation, while in *Pgi* the main contribution to the differentiation among populations seemed to be the interaction between habitat and area ($G_{H\times A}$). Also in *Ark* the interaction was pronounced, reflecting the presence of a unique allele (Ark^{90}) in both the exposed and the sheltered Spanish populations (Fig. 4). Excluding the Spanish populations from the gene diversity analysis gave no interaction in *Ark* ($G_{H\times A}=0.018$) and instead practically all (97%) of the variation among populations was attributed to variation between sheltered and exposed habitats ($G_{HT}=0.759$). Excluding the Spanish samples did not more than marginally change the results of the gene diversity analysis of the other three loci. Interestingly, however, both in *Pep-1* and *Pgm-2* the Spanish populations had unique (or nearly so) alleles at high frequencies ($Pep\text{-}1^{115}$ and $Pgm\text{-}2^{87}$) (Fig. 4).

DISCUSSION

The genetic variation in the four polymorphic loci assayed in this study of *Littorina fabalis* is not consistent on either a microgeographic or a macrogeographic scale. While *Pgi* exhibited no obvious pattern of differentiation, irrespective of geographic scale, *Pep-1* and *Pgm-2* were clearly macrogeographically structured (Fig. 4, Table

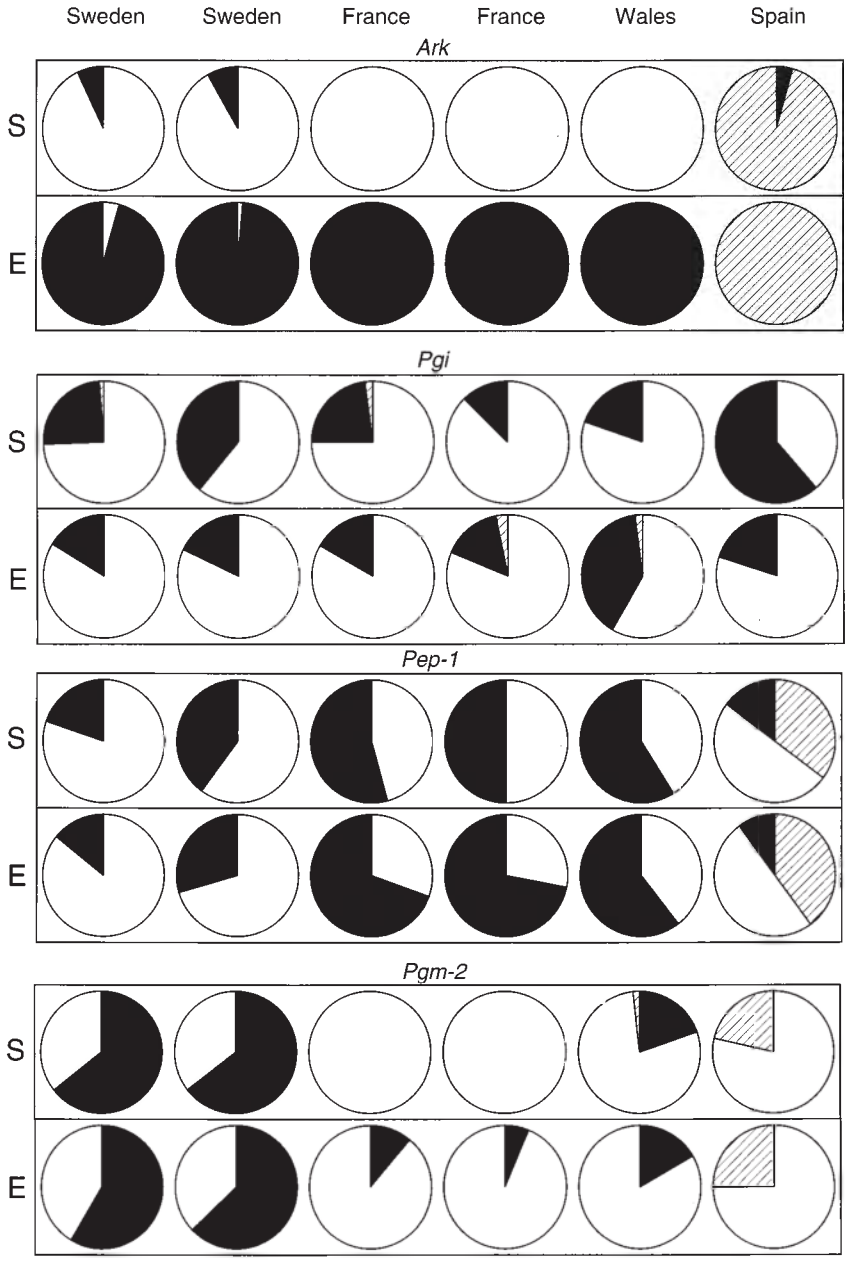

Figure 4. Allele frequencies of four polymorphic loci (*Ark*, *Pgi*, *Pep-1* and *Pgm-2*) in populations of *Littorina fabalis* from sheltered (S) and exposed (E) habitats in four different geographic areas. The alleles present are: *Ark*-E, (■), -S (□), Ark^{90}, (▨); Pgi^{100} (□), Pgi^{93}, (■), miscellaneous rare *Pgi* alleles (▨); $Pep\text{-}1^{80}$ (■), $Pep\text{-}1^{100}$ (□) $Pep\text{-}1^{115}$ (▨); $Pgm\text{-}2^{87}$ (▨), $Pgm\text{-}2^{93}$ (□), and $Pgm\text{-}2^{100}$ (■).

3). *Ark*, on the other hand, was strongly differentiated over habitats, much less so over geographic areas when comparing the French, Welsh and Swedish populations. In contrast, it was the other way round in the Spanish populations (Fig. 4, Table

3). Any habitat-related variation in *Ark* may have been overlooked in the Spanish snails as only two populations were included. However, Rolán-Alvarez (1993) analysed the variation in *Ark* in three populations of *L. fabalis*, one very exposed, one moderately exposed and one sheltered and his results support ours in that one allele was almost fixed in all three populations. The genetic structure of the four polymorphic loci of our study suggests that the Spanish populations are clearly separated from the NW European ones, although this suggestion needs to be confirmed using additional genetic markers and other Spanish populations.

In the group of NW European populations there is both a clear-cut differentiation in *Ark* in accordance with habitat type, and a strong coupling between *Ark* genotype and adult size in allopatric as well as sympatric Swedish sites (Tatarenkov & Johannesson, 1998). Furthermore, we have found small but statistically significant differences in allele frequencies in *Pgi* and *Pgm-2*, and in shell colour frequencies when large samples of SS and EE homozygotes from truly sympatric sites are compared (Tatarenkov & Johannesson, 1998).

The differences in *Pgi*, *Pgm-2* and colour, as well as the deficiencies of *Ark* heterozygotes, are readily explained if the gene flow between the exposed and sheltered groups is, at least partly, interrupted. At the same time we have observations suggesting that there is no assortative mating barrier between the exposed and sheltered groups, and that hybrids are produced, albeit at low rates, through cross-mating. This suggests a post-mating gene flow barrier, either a pre- or a post-zygotic one. Conspecific sperm precedence results in prezygotic barriers which commonly prevent gene flow between closely related animal species in spite of multiple matings (Howard *et al.*, 1997). Another possibility may be cryptic female choice (Birkhead & Møller, 1993). Alternatively, low hybrid viability and/or low fecundity may contribute to the gene flow barrier.

Although we do not know the mechanisms of the gene flow barrier the result is something similar to a mosaic hybrid zone (*sensu* Harrison & Rand, 1989). The deficiency of hybrids in mid-cline populations and the lack of support for selection against heterozygotes (Tatarenkov & Johannesson, 1998) suggest this a tension zone rather than a selection gradient zone between the sheltered and exposed groups, but this needs confirmation.

The interpretation of the results is complicated by the incongruity in genetic structure between *Ark* on the one hand, and *Pgi*, *Pep-1* and *Pgm-2* on the other. Possibly, however, *Ark* is involved in a paracentric inversion which prevents recombination (e.g. Ayala & Kiger, 1980). If loci affecting snail growth and adult size are part of the same inversion, these characters will be linked with *Ark* genotype, and selected variation in growth or size will generate habitat-related variation in the *Ark* locus. If the other three allozyme loci are not involved in the inversion, this will explain their genetic variation being unrelated to *Ark* and habitat. The alternative hypothesis is habitat-related selection acting directly on *Ark* (or on a close locus), but we have so far failed to find evidence which supports this (Tatarenkov & Johannesson, 1998).

An inversion linking *Ark* with selected genes on the same chromosome may therefore explain the habitat-related variation in this locus. Indeed genetic differences, in a range of loci, between subpopulations of *L. fabalis* living in different microhabitats is a quite plausible suggestion. Such differentiations are found in a number of marine snails of rocky shores which lack effective means of larval or adult dispersal (e.g. Boulding *et al.*, 1993; Johannesson & Johannesson, 1996; Rolán-Alvarez *et al.*, 1997; Day *et al.*, 1993; Kirby *et al.*, 1997; Parsons, 1997). However, the evolutionary

mechanisms which lead to strong intraspecific differentiation resulting in microhabitat adaptations are numerous, and include secondary hybridizing lineages (*Nucella lapillus*; Kirby *et al.*, 1997), strong assortative mating combined with phenotypic selection gradients (Galician *Littorina saxatilis*; Johannesson *et al.*, 1995; Rolán-Alvarez *et al.*, 1997), hybrid reproductive dysfunction (British *Littorina saxatilis*; Hull *et al.*, 1996), and choosing non-overlapping microhabitats during the mating season (*Littorina brevicula*; Takada & Rolán-Alvarez, pers. comm.). As in *L. fabalis*, all of these species (with the possible exception of *N. lapillus)* are substructured by partial reproductive barriers which prevent gene flow between groups of snails from different microhabitats.

If the inversion hypothesis is the correct explanation of the incongruous variation in *Ark* and the other polymorphic allozyme loci, *Ark* will not be particularly useful in tracing the amount of gene-flow between exposed and sheltered groups of *L. fabalis*. However, it will still remain a useful marker of the exposed and sheltered forms. Reimchen (1981) as well as Tatarenkov & Johannesson (1998) suggested that the two groups of *L. fabalis* may represent different taxa. If we disregard the geographic pattern of variation in *Ark*, as this locus is biased by some way of habitat-tied variation (either through linkage or through direct selection), we may conclude that the variation in the other three polymorphic loci best fits an isolation by distance model. Thus the conclusion is that the exposed and sheltered forms represent intraspecific forms of *L. fabalis*, and that the gene flow between exposed and sheltered subpopulations in the same geographic area, albeit impeded by a partial barrier, is greater than the gene flow among geographic areas.

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