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Genetic differentiation on multiple spatial scales in an ecotype-forming marine snail with limited dispersal: *Littorina saxatilis*

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The population genetic structure of marine species lacking free-swimming larvae is expected to be strongly affected by random genetic drift among populations, resulting in genetic isolation by geographical distance. At the same time, ecological separation over microhabitats followed by direct selection on those parts of the genome that affect adaptation might also be strong. Here, we address the question of how the relative importance of stochastic vs. selective structuring forces varies at different geographical scales. We use microsatellite DNA and allozyme data from samples of the marine rocky shore snail *Littorina saxatilis* over distance scales ranging from metres to 1000 km, and we show that genetic drift is the most important structuring evolutionary force at distances > 1 km. On smaller geographical scales (< 1 km), divergent selection between contrasting habitats affects population genetic structure by impeding gene flow over microhabitat borders (microsatellite structure), or by directly favouring specific alleles of selected loci (allozyme structure). The results suggest that evolutionary drivers of population genetic structure cannot a priori be assumed to be equally important at different geographical scales.

ADDITIONAL KEYWORDS: allozymes – gene flow – genetic structure – isolation by distance – microsatellites – parallel evolution.

INTRODUCTION

The genetic structure of species depends on various factors, including selection, mutation, genetic drift and migration. It is usually held that marine species with limited migration potential show strong population structure (Bohonak, 1999) as a consequence of increased genetic drift in isolated populations, but also because species with restricted dispersal exhibit more pronounced local adaptation as a result of natural selection (reviewed by Lenormand, 2002). The notion that population structure depends on dispersal capability has been supported in several empirical studies comparing closely related species pairs with similar ecology, but differing dispersal ability (Janson, 1987; Hellberg, 1996; Watts & Thorpe, 2006).

A simple form of population structure is increased genetic distance with increased geographical distance (isolation by distance, IBD), which occurs when the species range is larger than the individuals' dispersal distance (Wright, 1943). The specific shape of the IBD relationship depends on the relative strength of random genetic drift and gene flow (Rousset, 1997; Hutchison & Templeton, 1999). However, population structure and IBD could also be influenced by strong barriers to gene flow (Koizumi, Yamamoto & Maekawa, 2006; Johannesson & André, 2006) or by natural selection, either directly, or indirectly by linkage (Nielsen, Hansen & Meldrup, 2006). Thus, the type of genetic marker may influence the patterns of genetic structure, either because of different

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selection regimes or because of different mutational dynamics at the locus. It is expected, however, that all neutral loci should be affected in a similar way by genetic drift and migration, whereas selection may affect some loci differently (Slatkin, 1987). Previous studies reporting different levels of divergence between allozyme and other nuclear genetic markers suggest that either balancing (Pogson, Mesa & Boutilier, 1995) or directional selection (Dufresne, Bourget & Bernatchez, 2002) acts on the allozymes. Studies showing that some nuclear microsatellites may be affected by selection are also accumulating (Vasemägi, Nilsson & Primmer, 2005; Nielsen *et al*., 2006; Larsson *et al*., 2007).

Littorina saxatilis is an intertidal rocky shore snail found in the northern Atlantic. It lacks pelagic larvae, has a very variable morphology, and shows strong population structure in allozymes (Janson, 1987; Johannesson, Johannesson & Lundgren, 1995), RAPD (Johannesson *et al*., 2004), and microsatellite DNA markers (Panova, Hollander & Johannesson, 2006). This pattern is thought to result from low dispersal, leading to strong genetic drift in neutral genetic markers and local habitat specialization (Johannesson, 2003). Johannesson *et al*. (1995) showed that allele frequencies for some allozyme loci correlated with habitat type, indicating that natural selection acts on those loci. Morphologically distinct ecotypes adapted to local microhabitats can be found throughout most of the range of the species (Reid, 1996). Two ecotypes of *L. saxatilis* are common on the Swedish west coast: a small thin-shelled ecotype on exposed cliffs ('E') and a larger thick-shelled ecotype ('S') on sheltered boulder shores (Janson, 1983). The differences between these ecotypes are thought to result from different selective forces, with wave action being more important on exposed shores, and crab predation being more important on sheltered shores. Panova *et al*. (2006) investigated the hybrid zones between E and S on two different islands, and showed that the ecotypes were most likely to have evolved independently, in parallel, on the separate islands.

In this study, we test if a marine invertebrate with limited dispersal, *L. saxatilis*, shows similar patterns of strong population structure and IBD when using both presumably neutral genetic markers, microsatellite DNA, and different allozyme loci, some of which are possibly affected by selection. Furthermore, we test the hypothesis of parallel evolution of ecotypes in *L. saxatilis* (Panova *et al*., 2006), by comparing genetic differentiation within and between these ecotypes on different spatial scales. Finally, we test whether gene flow depends on coastal morphology, that is, if populations separated by open water tend to be more divergent than populations separated by shoreline.

MATERIAL AND METHODS

Data sets and sampling

We have used one set of microsatellite DNA data and two sets of allozyme data on population structure in *L. saxatilis*. Samples of *L. saxatilis* for microsatellite analyses were collected on the Swedish west coast in 2001 (Saltö and Öckerö) and in 2003 (Ramsö and Ramsholmen) (Fig. 1; Appendix S1). The 2003 samples were used previously by Panova *et al*. (2006). Ramsö, Ramsholmen, and Saltö are situated in the Koster fjord within 2–5 km of each other, whereas the distance between them and Öckerö is ~130 km. On Saltö and Öckerö, samples were collected in two bays separated by 1 km (Appendix S1); on Ramsö and Ramsholmen, samples were collected at two localities

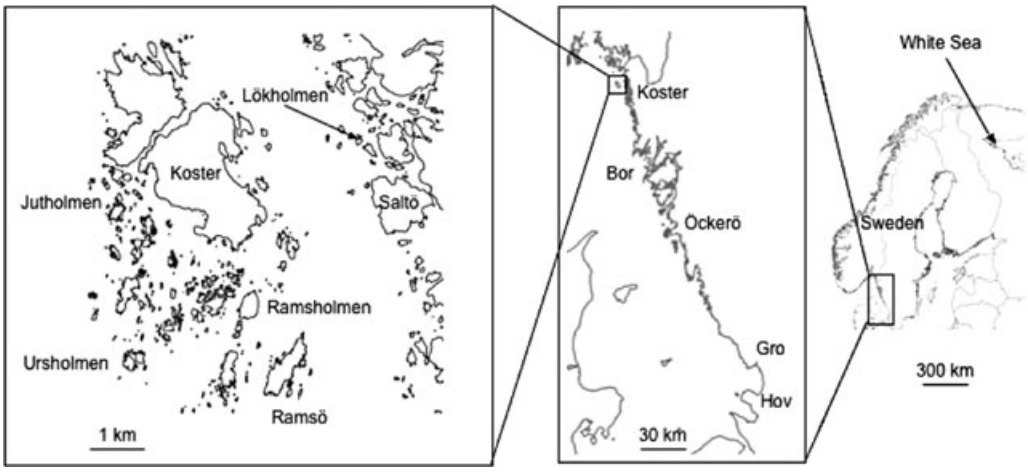

Figure 1. Map showing sampling locations on the west coast of Sweden and in the White Sea.

in one bay. In each locality we sampled the two ecotypes E and S separated by 50 m (Saltö and Öckerö) or by 20 m (Ramsö and Ramsholmen). Finally, we included one sample from a remote geographical population of *L. saxatilis* in the White Sea, collected in 2004 (Fig. 1; Appendix S1). The White Sea population is separated from the Swedish ones by ~3300 km, as measured along the coastline, and does not have ecotypes similar to the Swedish S and E morphs.

The two datasets with allozyme genotypes have partly been used before in earlier studies of *L. saxatilis* on the Swedish west coast (Janson, 1987; Johannesson & Tatarenkov, 1997). The first dataset on small-scale variation included three islands in the Koster area: Lökholmen, Jutholmen, and Ursholmen, separated by distances of 4–9 km (Fig. 1; Appendix S2); the Ursholmen data were previously used by Johannesson & Tatarenkov (1997), whereas the Lökholmen and Jutholmen data have not been previously published. On each island various numbers of samples of each ecotype were collected over distances of 7–150 m (Appendix S2). On Ursholmen, samples of the E ecotype were collected at both low and high shore levels because it had been shown that allele frequencies at one locus (aspartate aminotransferase) correlate with shore level (Johannesson *et al*., 1995; Johannesson & Tatarenkov, 1997). The second dataset, published by Janson (1987), included samples collected at five locations along the Swedish west coast (Fig. 1; Appendix S3) separated by 7–285 km. At each location, two replicate samples were taken over distances of 5–30 m. All samples were taken within a few square metres, and contained only one ecotype.

Allozyme genotyping

We used horizontal starch-gel electrophoresis of the allozymes, as described previously for *L. saxatilis* by Janson & Ward (1984) and Tatarenkov & Johannesson (1994). In the first dataset, eight polymorphic loci were genotyped: arginine kinase (*Ark*, 2.7.3.3), aspartate aminotransferase (*Aat-1*, 2.6.1.1), mannose-6-phosphate isomerase (*Mpi*, 5.3.1.8), peptidase (Gly Leu) (*Pep*, 3.4.–.–), phosphoglucomutases (*Pgm-1* and *Pgm-2*, 5.4.2.2), phosphoglucose isomerase (*Pgi*, 5.3.1.9), and purine-nucleoside phosphorylase (*Pnp*, 2.4.2.1). These loci are found to be the most variable ones in *L. saxatilis* (Janson & Ward, 1984). As it was previously shown that allele frequencies at two allozyme loci, *Aat* and *Pgm-2*, in *L. saxatilis* correlate strongly with habitat type, and thus are likely to be under selection (Johannesson & Tatarenkov, 1997), these loci were excluded in IBD analyses. Clustering analysis was performed both including and excluding *Aat* and *Pgm-2*.

The original dataset from Janson (1987) included five loci that were the same as in the above dataset (*Aat*, *Mpi*, *Pgm-1*, *Pgm-2*, and *Pgi*), and ten other loci that were either monomorphic or showed very low variability. From this dataset we used only three assumed neutral loci (*Mpi*, *Pgm-1*, and *Pgi*) to calculate genetic IBD; IBD was analysed separately in the two allozyme datasets.

Microsatellite genotyping

DNA was extracted from snail foot tissue with the DNeasy Plant Mini Kit (QIAGEN). The snails were genotyped at five microsatellite loci: Lsub62, Lsub32, Lsub16, and Lsub8 (Tie, Boulding & Naish, 2000), and Lsax6 (Sokolov, Sokolova & Pörtner, 2002).

Polymerase chain reaction (PCR) conditions were identical to those described in Panova *et al*. (2006). Sizing of microsatellite fragments was performed on ALFexpress and Beckman CEQ 8000 automatic sequencers. A set of individuals representing a broad range of alleles in each locus was genotyped on both sequencers to calibrate allele sizes produced by the two systems.

Statistical analysis

Gene diversity was calculated in MICROSATELLITE ANALYSER (Dieringer & Schlötterer, 2003) for microsatellite data, and in GDA ver.1.1 (Lewis & Zaykin, 2001) for the allozymes. GENEPOP ON THE WEB (Raymond & Rousset, 1995; http://genepop.curtin.edu.au/) was used to estimate the inbreeding coefficients, F_{IS} (Weir & Cockerham, 1984), and to perform exact tests for Hardy–Weinberg equilibrium at each locus. Allelic diversity was expressed as the number of alleles per locus, A, and allelic richness, A_R, was calculated in FSTAT (Goudet, 2001). Some microsatellite loci showed consistent heterozygote deficiency in several samples, probably as a result of null alleles (Panova *et al.*, 2008). Allele frequencies for these loci were corrected for putative null alleles using Brookfield's (1996) method implemented in MICROCHECKER (van Oosterhout *et al*., 2004). The corrected allele frequencies were subsequently used to calculate genetic distances from the microsatellite data (see Appendix S1).

Pairwise measures of genetic differentiation, F_{ST}, (Weir & Cockerham, 1984) over all loci were calculated both for microsatellite and allozyme data using GENEPOP. Regression of $F_{ST}/(1 - F_{ST})$ was calculated against the natural logarithm of geographical distance according to a two-dimensional migration model (Rousset, 1997): the slope, the standard error of the slope, and the determination coefficient, r^2, were calculated in MS EXCEL. The statistical significance of the genetic IBD relationships was calculated using

Table 1. Parameters for regression of genetic isolation $F_{ST}/1–F_{ST}$ (see text) against geographical distance (ln km) in *Littorina saxatilis*

	Slope	SE (slope)	r^2	P_{Mantel}
Microsatellite DNA	0.0028	0.0007	0.12	0.005
Within ecotypes	0.0036	0.0010	0.20	ND
Between ecotypes	0.0025	0.0009	0.10	ND
Allozymes	0.0097	0.0011	0.30	0.001
Within ecotypes	0.0088	0.0010	0.45	ND
Between ecotypes	0.0076	0.0023	0.10	ND
Janson-87	0.0079	0.0021	0.24	0.001

The isolation by distance relationships are also depicted in Figure 2. The allozyme relationships were based on six loci. ND: not determined; statistical significance was not calculated for the within- and between-ecotypes data subsets because the distance matrices are not balanced.

the Mantel test within the R-package (http://www.r-project.org/).

The relative importance of the factors geographical distance and habitat (ecotype) for the microsatellite genetic distances among samples was investigated using an orthogonal modification of Nei's gene diversity analysis [see Johannesson & Tatarenkov (1997) for a detailed description of the method, and a similar analysis for allozymes in *L. saxatilis*]. In the analysis, the overall variation among samples, G_{ST}, is divided into several components: G_{SHI}, average variation among samples within combinations of islands and habitats; G_{HT}, variation among habitats; G_{IT}, variation among islands; $G_{H\times I}$, variation resulting from an interaction between habitats and islands. Statistical significance of the factors and their interaction were calculated using ANOVA.

Neighbour-joining (NJ) clustering was performed with PHYLIP (Felsenstein, 1989) using Nei's genetic distance (Nei, 1972) and 100 bootstraps. Multidimensional scaling (MDS) plots of samples based on Nei's genetic distance were produced with the software R.

RESULTS

Genetic diversity

Heterozygosity levels were higher in microsatellites than in allozymes, as expected from the greater allele numbers in microsatellites (Supplementary Material, Appendices S1, S2). Janson (1987) reported levels of average expected heterozygosity in her allozyme data that are similar to ours (Supplementary Material, Appendix S3). The remote White Sea population had the highest microsatellite allelic richness; this population also had the highest number of private alleles (three in Lsub8 and Lsax6, two in Lsub62, and one in Lsub32). All other populations had at most one private allele in one locus.

Isolation by distance

Littorina saxatilis showed a clear pattern of IBD on the Swedish west coast, although the genetic distances between populations were very variable (Fig. 2). Mantel tests were significant for both microsatellites and allozymes (Table 1). The IBD within and between ecotypes showed similar patterns, except on the smallest scales, where there was a higher differentiation between ecotypes (Fig. 2a). The IBD based on Janson's (1987) data, comprising fewer allozyme loci, showed a similar slope, but with a lower intercept. Slope, r^2, and statistical significance were all higher for allozymes compared with microsatellites (Table 1).

Population structure

The overall differentiation among samples was very similar ($G_{ST} \approx 0.05$) and highly significant for all microsatellite loci (Table 2). The MDS plot of microsatellite data show that the geographically distant White Sea sample was clearly genetically distinct (Fig. 3a). Excluding the White Sea sample revealed a weak grouping by location (Fig. 3b). This was supported in the orthogonal analysis of gene diversity where island (G_{IT}), but not habitat (G_{HT}; ecotype), was a significant source of genetic variation (Table 2). The small-scale allozyme data showed a strong effect of habitat (ecotype) if genetic distances were based on eight loci (Fig. 4a). When the two loci, *Aat* and *Pgm-2*, potentially affected by selection were removed, the samples instead grouped by location (island) (Fig. 4b).

The NJ trees demonstrated essentially the same pattern as the MDS analyses for both microsatellite and allozyme data (Figs 5, 6). For the microsatellite data, neighbouring samples on the same island mostly grouped together (Fig. 5), and grouping by habitat was less evident, compared with the MDS ordination (Fig. 3b). The NJ tree based on eight alloz-

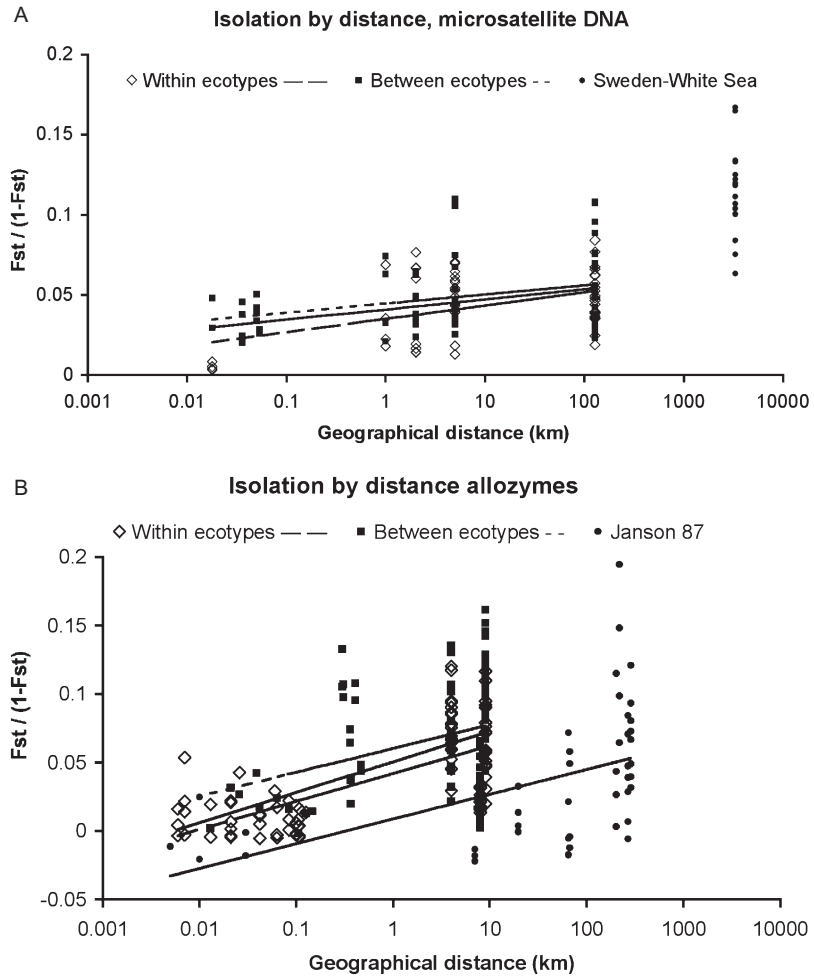

Figure 2. Isolation by distance. Pairwise comparisons of genetic differentiation in different genetic markers are plotted against geographical distance. Regression parameters are summarized in Table 1. A, microsatellites. The solid trend line indicates isolation by distance between 16 samples on the west coast of Sweden (see Appendix S1), based on five microsatellite loci. Within- and between-ecotype relationships, as well as the relationship between the Swedish and the White Sea samples, are also shown. B, allozymes. The upper solid trend line indicates isolation by distance between 20 samples on the west coast of Sweden (see Appendix S2), based on six allozyme loci. Within- and between-ecotype relationships are also shown. The lower solid trend line indicates isolation by distance between 10 samples on the west coast of Sweden (see Supplementary Appendix S3) based on data from Janson (1987) on three allozyme loci.

yme loci (Fig. 6a) showed a clear sign of selection, with all exposed (E) ecotype samples grouped together on one long branch. In contrast, in the NJ tree based on six allozyme loci (Fig. 6b), samples were mainly grouped by island.

DISCUSSION

Genetic structure and IBD

Snail populations on the Swedish west coast show a pattern of IBD (Fig. 2). The higher degree of differentiation between ecotypes at the smallest scales (Fig. 2a) can be explained by partial reproductive isolation between the ecotypes at the contact zone (Hollander, Lindegarth & Johannesson, 2005; Panova *et al*., 2006). Ecotypes are formed independently on each island by parallel evolution (Panova *et al*., 2006), and hence ecotype differences are expected to break down at longer distances, as changes in allele frequencies at neutral loci are not correlated. As gene flow between habitats of the same type is expected to be somewhat higher at short distances (because of the higher fitness of rafting snails when they land in a proper habitat), general genetic differences between ecotypes may still be found at nearby islands. Such genetic differences will disappear with increasing distances. The variance in genetic differentiation also increases with increasing geographical distance, as

Table 2. Orthogonal analysis of microsatellite gene diversity for 16 samples of *Littorina saxatilis* distributed over four islands and two habitats (exposed and sheltered)

Locus	H_S	H_T	G_{ST}	G_{SHI}	G_{HT}	G_{IT}	$G_{H\times I}$
Lsax6	0.862	0.906	0.048***	0.016	0.005	0.017	0.010
Lsub32	0.726	0.772	0.059***	0.016	0.010	0.022†	0.011
Lsub62	0.679	0.708	0.040***	0.014	0.002	0.017†	0.007
Lsub8	0.881	0.921	0.043***	0.012	0.002	0.022*	0.008
Lsub16	0.845	0.900	0.061***	0.016	0.009	0.022†	0.014
Overall	0.799	0.841	0.050***	0.015	0.006	0.020**	0.010

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
†$0.05 < P < 0.1$.
H_S, average heterozygosity within samples; H_T, total heterozygosity; G_{ST}, overall variation among samples. G_{ST} (equivalent to F_{ST}) is statistically higher than zero for all loci (GENEPOP; exact test). When G_{ST} is partitioned into habitat and island components, only island (G_{IT}) is statistically significant.

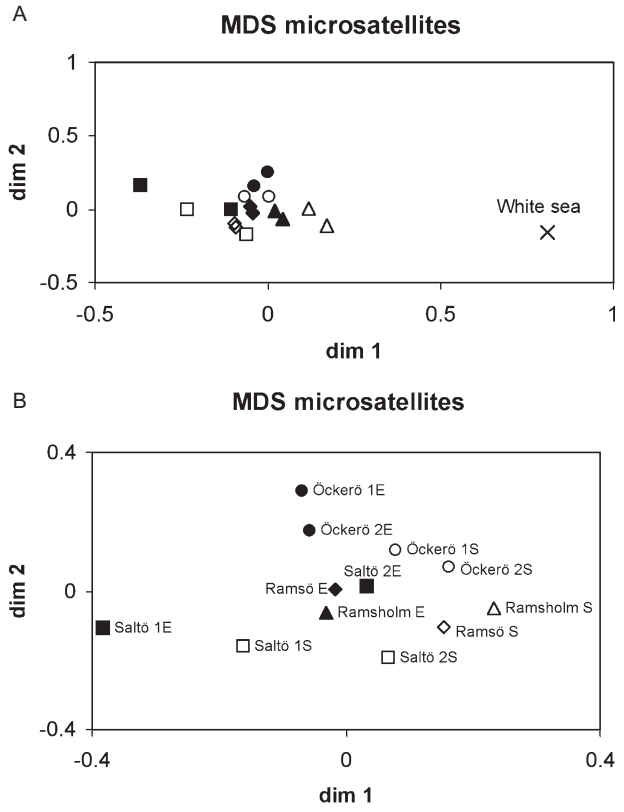

Figure 3. Multidimensional scaling (MDS) plots of samples based on pairwise Nei's distances using five microsatellite loci. A, seventeen samples on the west coast of Sweden and the White Sea (see Appendix S1). Labels: as in (B). B, Twelve samples on the west coast of Sweden. For clarity, samples of the same ecotype on the same island have been pooled for Ramsö and Ramsholmen.

would be expected if populations diverge by random drift. Only the 'island effect' was significant in the gene-diversity analysis, indicating that populations on the same island are on average more similar than populations on different islands (Table 2). It should be mentioned, however, that geographical distance measured in metres may have different effects depending on whether the distance is measured over water or along the shoreline.

It is notable that the marker types show different slopes of the IBD relationship (Table 1), although the slope is often taken to reflect species-specific migration processes (Rousset, 1997). The White Sea population has differentiated from the Swedish west coast populations further than could be expected by extrapolating the IBD relationship in Sweden (Fig. 1). Allelic richness was also higher in the White Sea population compared with the Skagerrak population. Mutational processes may play a greater role over distances of thousands of kilometres, where even infrequent migration between populations is unlikely; another reason may be that the convoluted Norwegian shoreline is longer, from the point of view of the snails, than our measurement straight across the coastline. It is also possible that some of the genetic basis of the White Sea population comes from a different glacial refuge than the Swedish populations, and thus that the divergence occurred before the last glaciation.

MICROSATELLITES VS. ALLOZYMES

Allozymes were in the past presumed to be neutral, but during decades of research, several examples of selection on allozyme loci have been established (Mitton, 1998; De Innocentis *et al.*, 2001). Here, we confirm that at least two loci are under selection (Johannesson & Johannesson, 1989; Johannesson &

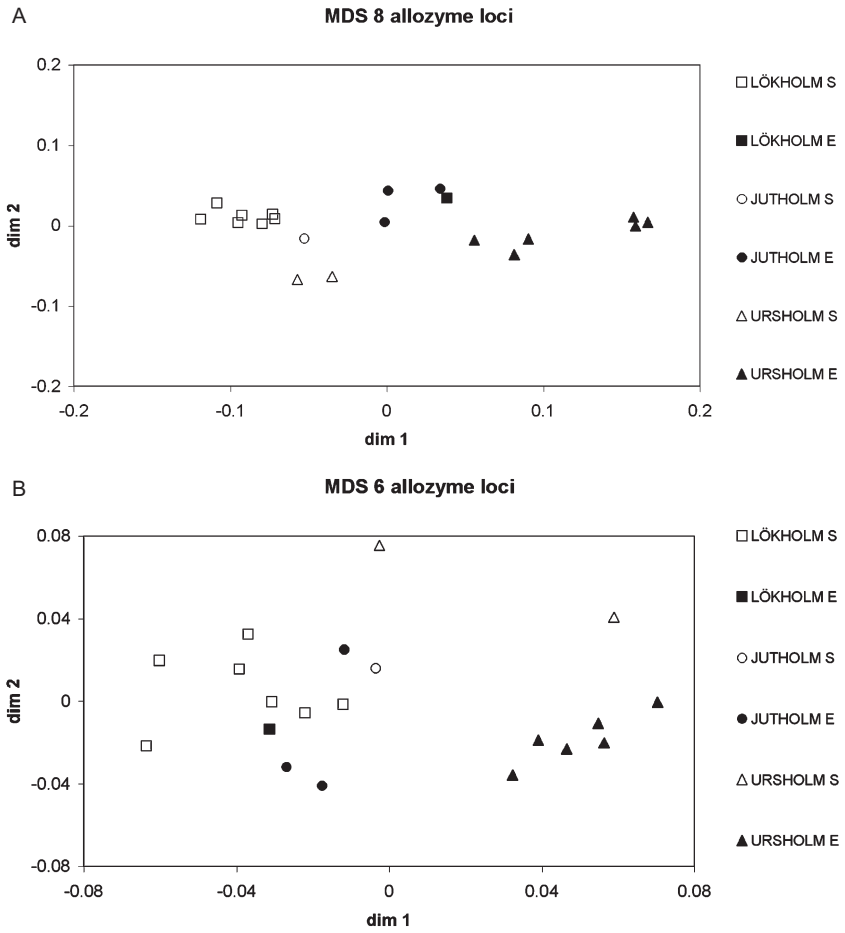

Figure 4. Multidimensional scaling (MDS) plots of 20 samples (see Appendix S2) in the Koster area based on pairwise Nei's distances, using (a) eight allozyme loci and (b) six loci, excluding *Aat* and *Pgm-2*, which are possibly affected by selection. Solid points are exposed sites and open points are sheltered sites.

Tatarenkov, 1997). Including these loci in the analyses gives a very different picture of population structure (Fig. 4a, b). The assumption that microsatellites are neutral does not always hold either: Larsson *et al*. (2007) found evidence of selection at a microsatellite, or a closely linked locus, in herring. Here, however, all microsatellite loci showed similar levels of differentiation, and no correlation to habitat type. If mutation is more important than migration, G_{ST} may be limited by the level of homozygosity (Hedrick, 2005), which could explain the lower levels of differentiation for microsatellites, e.g. the slope of the IBD relationship, which was lower for microsatellites compared with allozymes (Table 1).

Evolution of reproductive barriers

There was no indication that distant populations of the same ecotype would be more similar at neutral loci than populations of different ecotypes, except perhaps for samples clustering by habitat at Öckerö (Fig. 5). Overall, the pattern of population differentiation at scales > 1–2 km is much more random than at the small scales studied by Panova *et al*. (2006). Possibly genetic drift swamps the effects of between-population dispersal, to the extent that the poorer survival (Janson, 1983) and lower mating success (Hollander *et al*., 2005) of ecotypes ending up in the wrong habitat does not result in much differentiation on larger scales.

Dispersal and population biology

The mode of dispersal of *L. saxatilis* is intriguing. Lacking a larval stage, dispersal should mostly be on a very small scale, and observed dispersal rates are normally in the range of about a few metres per month (Janson, 1983). However, *L. saxatilis* has been established on very remote locations, such as the Atlantic island Rockall (Johannesson, 1988). This, and the relatively rapid colonization events observed in new or depleted isolated habitats (Johannesson &

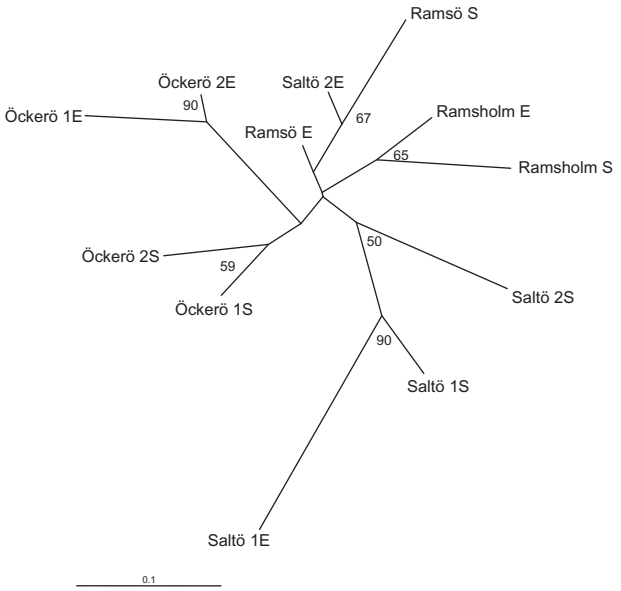

Figure 5. Neighbour-joining tree illustrating the relationship among 12 samples from four islands on the west coast of Sweden (see Appendix S1). The tree is based on pairwise Nei's distances from five microsatellite loci. Bootstrap values of over 50% for 100 replicates are indicated. Exposed and sheltered sites are denoted by E and S, respectively.

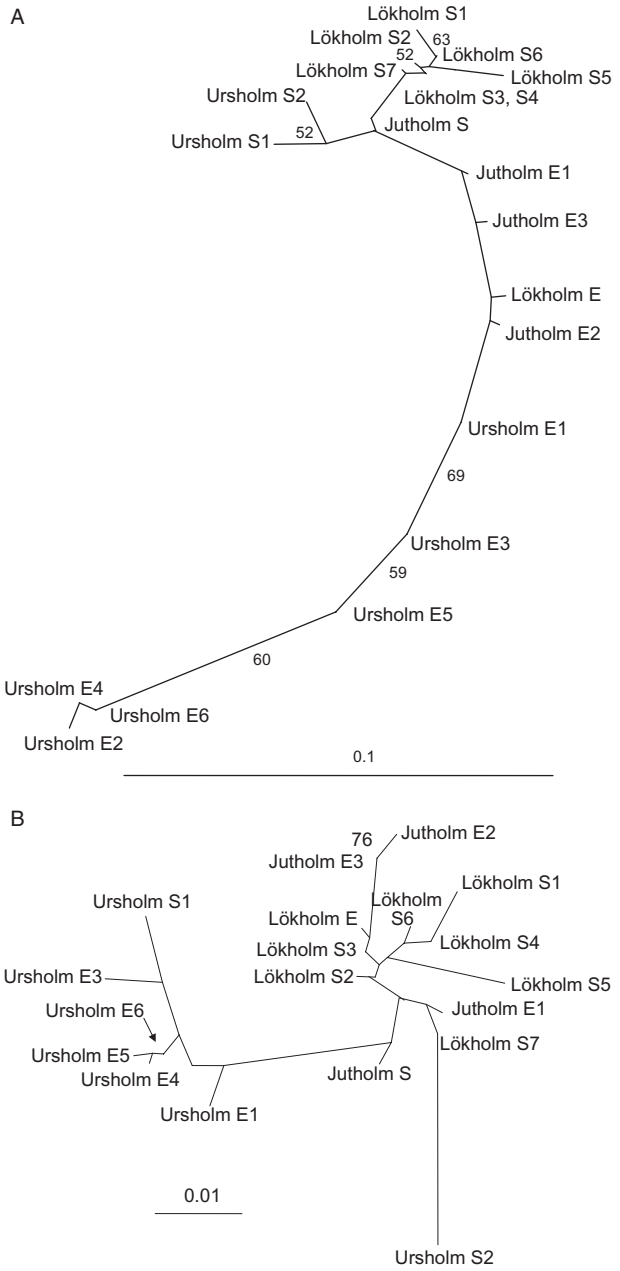

Figure 6. Neighbour-joining trees illustrating the relationship among 20 samples (see Appendix S2) in the Koster area. The trees are based on pairwise Nei's distances using (a) eight allozyme loci and (b) six loci, excluding *Aat* and *Pgm-2*, which are possibly affected by selection. Bootstrap values of over 50% for 100 replicates are indicated.

Warmoes, 1990; Johannesson & Johannesson, 1995), indicate that some form of long-distance dispersal (possibly by birds or floating algae) occurs in this species (cf. Thiel & Gutow, 2005). It has been suggested that single fertilized females may be enough to initiate viable populations in *L. saxatilis*, as females are promiscuous and are able to store sperm from multiple males for months at a time (Johannesson, 1988; Mäkinen, Panova & André, 2007). The genetic buffer provided by sperm storage may partly explain why microsatellite allele diversities are high throughout, even in populations that are expected to have been established relatively recently after the last glaciation (White Sea) and in populations that may have been bottlenecked during a recent toxic algal bloom (Johannesson & Johannesson, 1995).

Is dispersal impeded by large stretches of open water? Neither allozymes nor microsatellites showed a marked distinction over the deep Koster fjord beyond that expected from geographical distance. This is clear from Figure 6b, where Lökholmen clusters closely with Jutholmen, despite being on opposite sides of the fjord. In the microsatellite tree (Fig. 5), such a distinction is not evident either. This is in contrast to the snail *Bembicium vittatum* in Australia, where water gaps where shown to impede dispersal (Johnson & Black, 1995).

CONCLUSIONS

Our results suggest that genetic drift is the most important factor shaping genetic variation at medium and long geographical distances in *L. saxatilis*, which is in contrast with evolution of population genetic structure on shorter spatial scales, i.e. within small

islands (< 1 km). Within islands, *L. saxatilis* adapts to specific habitats and forms genetically distinct ecotypes. The ecological separation of the ecotypes impacts population genetic structure by impeding gene flow between ecotypes, as revealed by microsatellite genetic structure, or through divergent selection on particular allozyme loci. Furthermore, our results give support to the hypothesis that these ecotypes evolve independently in different geographical locations, i.e. through parallel evolution (cf. Panova *et al*., 2006).

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REFERENCES

Bohonak AJ. 1999. Dispersal, gene flow, and population structure. *Quarterly Review of Biology* **74:** 2–45.

Brookfield JFY. 1996. A simple new method of estimating null allele frequency from heterozygote deficiency. *Molecular Ecology* **5:** 453–455.

De Innocentis S, Sola L, Cataudella S, Bentzen P. 2001. Allozyme and microsatellite loci provide discordant estimates of population differentiation in the endangered dusky grouper (*Epinephelus marginatus*) within the Mediterranean Sea. *Molecular Ecology* **10:** 2163–2175.

Dieringer D, Schlötterer C. 2003. MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes* **3:** 167–169.

Dufresne F, Bourget E, Bernatchez L. 2002. Isolation and characterization of microsatellite and allozyme alleles: further evidence for locus-specific selection in the acorn barnacle, *Semibalanus balanoides*. *Molecular Ecology* **11:** 113–123.

Felsenstein J. 1989. PHYLIP: phylogeny inference package, version 3.2. *Cladistics* **5:** 164–166.

Goudet J. 2001. *FSTAT, a program to estimate and test gene diversities and fixation indices* (version 2.9.3.2). Available at http://www.unil.ch/izea/software/fstat.html

Hedrick PW. 2005. A standardized genetic differentiation measure. *Evolution* **59:** 1633–1638.

Hellberg ME. 1996. Dependence on gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution* **50:** 1167–1175.

Hollander J, Lindegarth M, Johannesson K. 2005. Local adaptation but not geographic separation promotes assortative mating in a snail. *Animal Behaviour* **70:** 1209–1219.

Hutchison DW, Templeton AR. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53:** 1898–1914.

Janson K. 1983. Selection and migration in two distinct phenotypes of *Littorina saxatilis* in Sweden. *Oecologia* **59:** 58–61.

Janson K. 1987. Allozyme and shell variation in two marine snails (*Littorina*, Prosobranchia) with different dispersal abilities. *Biological Journal of the Linnean Society* **30:** 245–256.

Janson K, Ward RD. 1984. Microgeographic variation in allozyme and shell characteristics in *Littorina saxatilis* Olivi (Prosobranchia: Littorinidae). *Biological Journal of the Linnean Society* **22:** 289–307.

Johannesson K. 1988. The paradox of Rockall: why is a brooding gastropod *(Littorina saxatilis)* more widespread than one having a planktonic larval dispersal stage (*L. littorea*)? *Marine Biology* **99:** 507–513.

Johannesson K. 2003. Evolution in *Littorina*: ecology matters. *Journal of Sea Research* **49:** 107–117.

Johannesson K, André C. 2006. Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. *Molecular Ecology* **15:** 2013–2029.

Johannesson K, Johannesson B. 1989. Differences in allele frequencies of *Aat* between high- and mid-rocky shore populations of *Littorina saxatilis* (Olivi) suggest selection in this enzyme locus. *Genetical Research* **54:** 7–11.

Johannesson K, Johannesson B. 1995. Dispersal and population expansion in a direct developing marine snail (*Littorina saxatilis*) following a severe population bottleneck. *Hydrobiologia* **309:** 173–180.

Johannesson K, Johannesson B, Lundgren U. 1995. Strong natural selection causes microscale allozyme variation in a marine snail. *Proceedings of the National Academy of Sciences of the USA* **92:** 2602–2606.

Johannesson K, Lundberg J, André C, Nilsson PG. 2004. Island isolation and habitat heterogeneity correlate with DNA variation in a marine snail (*Littorina saxatilis*). *Biological Journal of the Linnean Society* **82:** 377–384.

Johannesson K, Tatarenkov A. 1997. Allozyme variation in a snail *(Littorina saxatilis)* – deconfounding the effects of microhabitat and gene flow. *Evolution* **51:** 402–409.

Johannesson K, Warmoes T. 1990. Rapid colonization of Belgian breakwaters by the direct developer *Littorina saxatilis* Olivi. *Hydrobiologia* **193:** 99–108.

Johnson MS, Black R. 1995. Neighbourhood size and the importance of barriers to gene flow in an intertidal snail. *Heredity* **75:** 142–154.

Koizumi I, Yamamoto S, Maekawa K. 2006. Decomposed pairwise regression analysis of genetic and geographic distances reveals a metapopulation structure of stream-dwelling Dolly Varden charr. *Molecular Ecology* **15:** 3175–3189.

Larsson L, Laikre L, Palm S, André C, Carvalho G, Ryman N. 2007. Concordance of allozyme and microsatellite differentiation in a marine fish, but evidence of selection at a microsatellite locus. *Molecular Ecology* **16:** 1135–1147.

Lenormand T. 2002. Gene flow and the limits to natural selection. *Trends in Ecology and Evolution* **17:** 183–189.

Lewis PO, Zaykin D. 2001. *GENETIC DATA ANALYSIS: Computer Program for the Analysis of Allelic Data* (version 1.1). Available at http://lewis.eeb.uconn.edu/lewishome/software.html

Mäkinen T, Panova M, André C. 2007. High levels of multiple paternity in *Littorina saxatilis* – hedging the bets? *Journal of Heredity* **98:** 705–711.

Mitton JB. 1998. Molecular markers and natural selection. In: Carvalho G, ed. *Advances in molecular ecology*. Amsterdam: IOS Press, 225–241.

Nei M. 1972. Genetic distance between populations. *American Naturalist* **106:** 283–292.

Nielsen E, Hansen M, Meldrup D. 2006. Evidence of microsatellite hitch-hiking selection in Atlantic cod (*Gadus morhua* L.): implications for inferring population structure in non-model organisms. *Molecular Ecology* **15:** 3219–3229.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4:** 535–538.

Panova M, Hollander J, Johannesson K. 2006. Site-specific genetic divergence in parallel hybrid zones suggests non-allopatric evolution of reproductive barriers. *Molecular Ecology* **15:** 4021–4031.

Panova M, Mäkinen T, Fokin M, André C, Johanesson K. 2008. Microsatellite cross-species amplification in the genus *Littorina* and detection of null alleles in *Littorina saxatilis*. *Journal of Molluscan Studies*, in press.

Pogson GH, Mesa KA, Boutilier RG. 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. *Genetics* **139:** 375–385.

Raymond M, Rousset F. 1995. GENEPOP (Version-1.2) Population-genetics software for exact tests and eucumenicism. *Journal of Heredity* **86:** 248–249.

Reid DG. 1996. *Systematics and evolution of Littorina*. London: Ray Society.

Rousset F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145:** 1219–1228.

Slatkin M. 1987. Gene flow and the geographic structure of species. *Science* **236:** 787–792.

Sokolov EP, Sokolova IM, Pörtner H-O. 2002. Polymorphic microsatellite DNA markers from the marine gastropod *Littorina saxatilis*. *Molecular Ecology Notes* **2:** 27–29.

Tatarenkov A, Johannesson K. 1994. Habitat related allozyme variation on a microgeographical scale in the marine snail *Littorina mariae* (Prosobranchia: Littorinacea). *Biological Journal of the Linnean Society* **53:** 105–125.

Thiel M, Gutow L. 2005. The ecology of rafting in the marine environment II. The rafting organisms and community. *Oceanography and Marine Biology* **43:** 279–418.

Tie AD, Boulding EG, Naish KA. 2000. Polymorphic microsatellite DNA markers for the marine gastropod *Littorina subrotundata*. *Molecular Ecology* **9:** 108–110.

Vasemägi A, Nilsson J, Primmer CR. 2005. Expressed sequence tag-linked microsatellites as a source of gene-associated polymorphisms for detecting signatures of divergent selection in Atlantic salmon (*Salmo salar* L.). *Molecular Biology and Evolution* **22:** 1067–1076.

Watts PC, Thorpe JP. 2006. Influence of contrasting larval development types upon the population-genetic structure of cheilostome bryozoans. *Marine Biology* **149:** 1093–1101.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38:** 1358–1370.

Wright S. 1943. Isolation by distance. *Genetics* **28:** 141–138.

SUPPLEMENTARY MATERIAL

The following material is available for this article online:

Appendix S1. Sample information and descriptive statistics of microsatellite genotype data. Sample code, locality, ecotype, and sample size. Expected heterozygosity (H_E), deviation from Hardy–Weinberg expectations (F_{IS}), with statistical significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$), number of alleles (A), and allelic richness (A_R), based on a minimum sample size of $n = 34$. †Denotes that genotypic data have been corrected for null alleles in subsequent analyses.

Appendix S2. Sample information and descriptive statistics of allozyme genotype data. Sample code, locality, ecotype, and sample size. Expected heterozygosity (H_E), deviation from Hardy–Weinberg expectations (F_{IS}), with statistical significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$), number of alleles (A), and allelic richness (A_R), based on a minimum sample size of $n = 25$. Ten out of 160 allozyme F_{IS} values were significantly different from zero, but there was no obvious trend among samples or loci.

Appendix S3. Janson (1987) dataset includes two replicate samples from each of five localities ($n = 33$–37): Ramsökalven, Saltö, Borgmästaren (BOR), Grötvik (GRO), and Hovs hallar (HOV) (Fig. 1). Average expected heterozygosity for the loci *Mpi*, *Pgm-1*, and *Pgi* was 0.42, 0.40, and 0.46, respectively (values per sample were not determined).

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