Biological Journal of the Linnean Society, 2004, **82**, 377–384. With 1 figure

Island isolation and habitat heterogeneity correlate with DNA variation in a marine snail (*Littorina saxatilis*)

KERSTIN JOHANNESSON*, JOAKIM LUNDBERG, CARL ANDRÉ and PER G. NILSSON

Göteborg University, Department of Marine Ecology, Tjärnö Marine Biological Laboratory, SE-452 96 Strömstad, Sweden

Received 20 February 2003; accepted for publication 19 January 2004

The null assumption of molecular variation is that most of it is neutral to natural selection. This is in contrast to variation in morphological traits that we generally assume is maintained by selection, and therefore often by selection coupled to environmental heterogeneity in time and space. Examples of molecular variation that vary over habitat-shifts, particularly in allozymes, show that the relative impact of non-neutral variation as compared to neutral variation might be substantial in some systems. To assess the importance of habitat-generated variation in relation to variation generated by random processes in nuclear DNA markers at small spatial scales, we compared the effects of island isolation and habitat heterogeneity on genetic substructuring in a rocky shore snail (*Littorina saxatilis*). This species has a restricted migration among islands owing to the lack of free-floating larvae. Earlier studies show that allozymes vary extensively as a consequence of isolation by water barriers among islands, but also as a consequence of divergent selection among different microhabitats within islands. In the DNA markers we observed genetic differentiation owing to island isolation at three of nine loci. In addition, variation at three loci correlated with habitat type, but the correlation for two of the loci was weak. Overall, isolation contributed slightly more to the genetic variation among populations than did habitat-related factors but the difference was small. It is concluded that both island isolation, which interrupts gene flow, and a heterogeneous habitat cause genetic substructuring at the DNA level in *L. saxatilis* in the studied area, and thus in this species we need to be somewhat concerned about habitat heterogeneity also at DNA loci. *Biological Journal of the Linnean Society*, 2004, **82**, 377–384.

ADDITIONAL KEYWORDS: genetic structure – isolation by distance – neutral and selected variation – RAPD.

INTRODUCTION

The population genetic structure of species is the combined result of stochastic and deterministic processes acting either to increase or decrease genetic variation within and between populations. Drift, for example, tends to reduce genetic variation within populations by elimination of rare alleles, but at the same time drift may increase differentiation among populations by independent and random changes of allele frequencies in semi-isolated populations. Obviously, natural selection may either support or impede genetic differences among populations. Although drift and divergent selection are both important in generating genetic diversity, we have not yet reached a consensus about the relative contribution of each factor to the total genetic variation in molecular traits (Stewart & Excoffier, 1996; Eanes, 1999; Hey, 1999). Many authors advocate the view that allozyme variation, and certainly variation at DNA loci [microsatellites, random amplified polymorphic DNA (RAPD), single-stranded conformational polymorphism (SSCP), amplified fragment length polymorphism (AFLP), etc.] can be considered as neutral and as such be used to indicate gene flows, phylogenetic relationships and the genetic substructuring of species (Eanes, 1987; Thorpe & Solecava, 1994; Allendorf & Seeb, 2000; Merilä & Cronokrak, 2001; McKay & Latta, 2002). However, exceptions are numerous for specific allozyme loci (Koehn, Newell & Immermann, 1980; Sullivan, Atkinson & Starmer, 1990; Riddoch, 1993; Tatarenkov & Johannesson, 1994; Lönn, Prentice & Bengtsson, 1996; Schmidt, Bertness & Rand, 2000). A

*Corresponding author. E-mail: Kerstin.Johannesson@tmbl.gu.se

few examples of selected variation in DNA are also reported (Kashi, King & Soller, 1997; Wilding, Butlin & Grahame, 2001).

Divergent selection among populations of a species is expected if populations occupy contrasting environments (Hedrick, 1986; Kassen, 2002). Indeed, variation in allozyme characters related to habitat is found at various spatial scales in organisms distributed over different habitat types (e.g. Koehn *et al*., 1980; Watt, 1985, 1992; Lönn *et al*., 1996; Johannesson, Johannesson & Lundgren, 1995; Schmidt *et al*., 2000). Few studies have, however, addressed the possibility of habitat-related DNA variation among populations of species occupying contrasting habitats. One interesting exception is the study by Wilding *et al*. (2001) in which the DNA variation in 5% of the 306 scored AFLP loci were shown to correlate with habitat type in the marine gastropod *Littorina saxatilis*. In this study we approached similar problems of habitat-dependent DNA variation in this species using randomly amplified polymorphic DNA, a more compressed geographical scale and populations isolated on islands instead of populations separated by a continuous coast. We scored nine loci in 30 populations distributed over five islands and three habitats. The overall aim was to compare the relative contribution of habitat heterogeneity and island isolation to the genetic structuring of this species at a small geographical scale (islands 2–15 km apart), and to compare the patterns obtained by nuclear (this study) and allozyme (analysis of the same populations; Johannesson & Tatarenkov, 1997) markers.

MATERIAL AND METHODS

THE ORGANISM

Littorina saxatilis is common over northern Atlantic rocky shores with densities of hundreds of snails per m^2 (K. Johannesson, pers. observ.). It has internal fertilization and a direct development, and females raise their embryos beyond the metamorphose stage in a brood pouch from which miniature snails are released directly on the shore. In addition, juvenile and adult dispersal are restricted (1–4 m, Janson, 1983; Erlandsson, Rolán-Alvarez & Johannesson, 1998), and island habitats are slowly re-colonized if local populations become extinct (Johannesson & Johannesson, 1995). The poor dispersal promotes isolation by distance in neutral traits. It also promotes local adaptation in inherited traits that are under divergent selection (Janson, 1987), and indeed most of the variation in quantitative traits is inherited (Janson, 1982; Johannesson & Johannesson, 1996). The species is strongly polymorphic in morphological traits with ecotypes that are characteristic of particular habitats (Reid, 1996). As a comparison, shell shape and size of a closely related species, *Littorina littorea*, that has a pelagic dispersive larva, is essentially monomorphic over the same range of habitats (Janson, 1987; Reid, 1996). Transplant experiments of *L. saxatilis* reveal divergent selection among habitats that maintain shell size and shape differences (Janson, 1983; Rolán-Alvarez, Johannesson & Ekendahl, 1997).

SAMPLING DESIGN

We sampled *Littorina saxatilis* from five islands in a Swedish archipelago of hundreds of small islands, most of them less than 1 km in diameter. In each island we sampled three habitat types (Fig. 1). The islands (Jutholmen, Burholmen, Ursholmen, Arsklovet and Svangen) are 2–15 km apart and each is a few hundred meters across. *Littorina saxatilis* is continuously, or almost continuously, distributed around the shores of each island. We sampled two replicate sites of each habitat per island; boulder shore ('boulders'), upper tidal level of exposed rocky shore ('high rocky') and low-to-mean tide level of exposed rocky shores ('low rocky'). Each sample consisted of five randomly collected individuals. The replicates were spread over each island in order to represent the whole island rather than a particular shore and the same sites were sampled as in an earlier study of

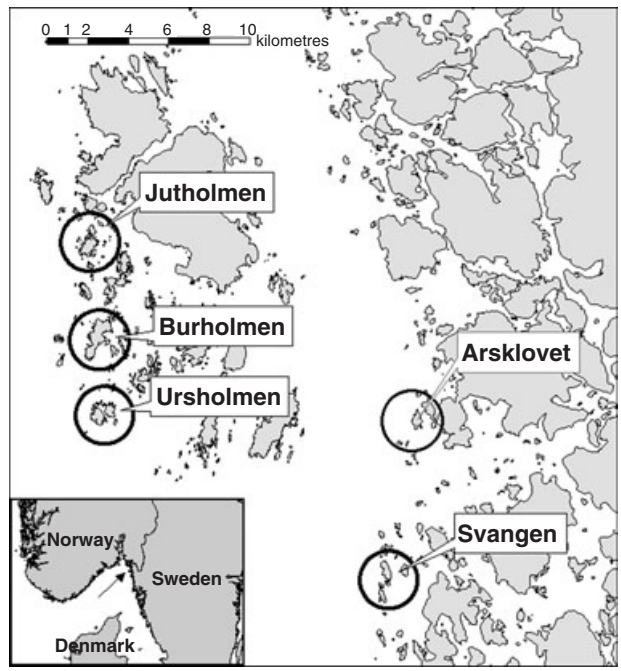

Figure 1. Populations of *Littorina saxatilis* were sampled from five islands in the Koster archipelago on the Swedish west coast. On each island, replicate sites for each of three habitat types were sampled.

allozyme variation (see Johannesson & Tatarenkov, 1997, Table 1, for further details).

DNA POLYMORPHISM

We scored DNA variation using randomly amplified polymorphic DNA (Williams *et al*., 1990), a method that gives a random sample of the genetic variation from the entire genome. DNA was extracted using the hexadecyltrimetyl ammonium bromide (CTAB) method. From each snail, a few mm^3 of frozen muscle tissue from the foremost part of the foot was placed in a 1.5 mL Eppendorf tube with 0.5 mL buffer containing 2% (w/v) CTAB, 1.4 M NaCl, 0.2% (v/v) 2-mercaptoethanol, 20 mM EDTA and 100 mM Tris-HCl (pH 8.0). Fifty micrograms of proteinase K were added, followed by incubation at 60°C for 3 h. There-after we added 0.5 mL chloroform/isoamylalcohol (24/1, v/v) and centrifuged the tubes for 10 min at 6000 *g*. The upper phase was transferred to a new tube, mixed with 350 μL cold isopropanol and left overnight to allow precipitation of the DNA. Centrifugation was carried out as above and the pellet dried and dissolved in 200 μL 0.1 × Tris-EDTA buffer for direct use in the polymerase chain reaction (PCR).

The 25 μL PCR amplifications contained 2 μL template DNA, 4 mM $MgCl_2$, 1 × PCR buffer (Perkin-Elmer), 0.8 mM dNTP mix, 1 μM primer, and 2 units Stoffel-*Taq* Polymerase (Perkin-Elmer). Reactions were carried out in an MJ Research PTC-100 thermocycler with the following temperature and time settings: 94°C for 2 min; followed by 94°C for 1 min, 36°C for 1 min and 72°C for 2 min × 40 cycles.

PCR products, negative controls and 100 bp ladders were loaded on to ethidium bromide-stained 2% agarose gels in 0.5 × TBE buffer and electrophoresed for 3.5 h with 3.8 V cm^{-1}. Gels were photographed under UV-light with Kodak TMAX 400 pro b/w film. Negatives were scanned for subsequent visual band scoring in Adobe Photoshop 3.0.

To ensure repeatability, identical protocols for extraction, PCR, electrophoresis and scoring were used for all samples (Hadrys, Balick & Schierwater, 1992; Grosberg, Levitan & Cameron, 1996; Grundmann *et al*., 1997). Samples were analysed in random order with respect to sample locality to further minimize systematic errors. We furthermore checked the repeatability of the scoring by re-running the same individuals of several of the samples. A subsample of individuals was scanned with several 10-mer RAPD primers (Operon Technologies) for bright and distinct polymorphic bands. We chose three primers (H-6, H-7 and H-14), yielding a total of nine scorable loci. Fragment lengths spanned between 200 and 700 base pairs. An absence/presence matrix was created for all individuals and loci.

DNA DATA ANALYSES

Analysis of dominant markers such as RAPD with traditional population genetic methods requires additional assumptions about, for example, Hardy–Weinberg equilibrium (Lynch & Milligan, 1994). To escape from this problem, and as the purpose was to assess relative rather than absolute amounts of genetic variation, we used two multivariate statistical approaches, the Mantel test and a distance tree constructed by neighbour-joining to resolve the genetic structure.

The Mantel test is a method for computing correlations between distance matrices (Manly, 1986). Correlations are expressed with the statistic z, or its normalized version r, that allows cross-variable comparisons. Statistical significance of r can be assessed by permutations. Two matrices, A and B, are compared in a two-way Mantel test, denoted A*B. A partial Mantel test is used when the relationship between two matrices (A, B) should be analysed keeping the effect of a third matrix (C) constant; this is written A*B | C (Manly, 1986; Fortin & Gurevitch, 1993).

Mantel tests were run with the R Package 3.0 (Vaudor, 1990) and 4.0d1 (Casgrain & Legendre, 1999). We computed a Euclidean distance matrix from the RAPD data set (GEN), and a kilometre distance matrix from geographical positions of each site (GEO). As an alternative to the GEO matrix, a distance matrix describing which samples shared the same island (ISL) was constructed such that any pair of individuals that came from the same island was scored as 0, while pairs not sharing the same island had the value 1. A habitat matrix (HAB) was constructed in a similar way with 0 for shared habitat and 1 for different habitats. We used the normalized Mantel statistic r for interpreting the results of the analysis, and significance was assessed from 999 permutations in all runs.

Firstly, we tested whether the geographical distance (GEO) or the discrete variable island (ISL) was correlated most strongly to genetic distance (GEN). The Mantel tests were performed according to the following design:

GEN*GEO

GEN*ISL

GEN*GEO | HAB

GEN*ISL | HAB

The partial tests revealed whether habitat effects were confounding the effects of geographical variables. The variable most correlated with GEN was then kept constant in the partial Mantel test of GEN and HAB to achieve the strongest test of these two variables (island proved to be the geographical variable strongest correlated to genetic distance):

GEN*HAB

GEN*HAB | ISL

Finally, the resulting Mantel statistics and significance from GEN*HAB | ISL and GEN*ISL | HAB were compared. The latter comparison was also made for each locus and primer separately as a qualitative evaluation of their contributions to the genetic correlations. Significance levels for tests of individual loci were adjusted using the sequential Bonferroni correction (Rice, 1989).

A neighbour-joining distance tree was constructed from the genetic distance matrix with the computer package PHYLIP 3.57c (Felsenstein, 1995). The relative abilities of the variables habitat and island to explain the genetic distance tree topology were assessed in a parsimony analysis. The variable demanding the least number of individual movements between its different habitats or islands, respectively, was considered as having the greatest explanatory power. Habitat and island were entered as characters into a two-character/149 individuals matrix, and the three habitats and five islands were coded as categorical character states. Character state changes were parsimoniously mapped onto the distance tree using the phylogenetic analysis computer programs PAUP 3.1.1 (Swofford, 1993) and MacClade 3.06 (Maddison & Maddison, 1992). The respective fits of the characters on the tree were estimated with the consistency index, *ci* (Kluge & Farris, 1969). The *ci* is calculated as the minimum amount of change possible for the character on any tree (which equals the number of states minus one) divided by the actual number of changes on the tree. It is thus comparable also between characters with a different number of states. *ci* ranges from 0 to 1; a higher *ci* implies a better fit of the character to the tree. The number of changes between the different habitats was checked as an indication of relative interhabitat contact.

We also performed a nested analysis of molecular variance (AMOVA, Schneider, Roessli & Excoffier, 2000) to contrast the levels of genetic variation within and among islands for each habitat type separately, using the two replicate sample populations of each habitat sampled in each island. Significance values were calculated from 10 000 permutations.

RESULTS

DNA was successfully extracted from all but one of the 150 individuals. Island (ISL, defining isolation as '0' for snails from the same island and '1' for snails of different islands) was significantly correlated to genetic distance (see below), whereas geographical distance among islands (GEO) showed a non-significant tendency (two way test, $r = 0.021$, $P = 0.066$; partial test, $r = 0.022$, $P = 0.055$). We therefore used ISL as the matrix indicating isolation in the following analyses.

With all loci included, there was low but significant correlation between genetic distance and habitat in the two-way Mantel test ($r = 0.076$, $P = 0.001$) as well as the partial test ($r = 0.077$, $P = 0.001$, Table 1) where genetic correlation with island was kept constant. Surprisingly, genetic distance correlated less with island, though it was still significant in both tests (two-way $r = 0.034$, $P = 0.002$ and partial $r = 0.035$, $P = 0.003$, Table 1). The partial Mantel tests of individual loci, however, showed that a few loci only were significantly correlated with either habitat or island. The correlation between locus H7-4 and habitat was about one order of magnitude higher than for any other locus-variable combination. A partial Mantel test including all loci except H7-4 gave a significant correlation between genetic distance and island ($r = 0.022$, $P = 0.016$), but not between genetic distance and habitat ($r = 0.0134$, $P = 0.075$, Table 1). Thus RAPD variation among populations was both generated by stochastic (isolation effects) and deterministic (habitat effects) processes, and in particular the variation at one of the nine loci (H7-4) was strongly habitat related.

Table 1. Partial Mantel tests of the importance of habitat (boulders, high rocky shore and low rocky shore) and isolation (island effect) in generating genetic variation in nine RAPD loci among populations of *Littorina saxatilis*. The test GEN*HAB | ISL estimates correlations between a matrix of genetic distances (GEN) and a matrix of habitat differences (HAB) keeping the effect of isolation on different islands (ISL) constant. The test GEN*ISL | HAB estimates correlations between matrices of genetic distance and isolation keeping effects of habitat constant. Probability values for the correlations significant after Bonferroni correction ($\alpha = 0.0057$) are in bold

	GEN*HAB | ISL 'Habitat effect'		GEN*ISL | HAB 'Island effect'	
Locus	*r*	*P*	*r*	*P*
H6-1	0.0009	0.342	–0.0054	0.345
H6-2	0.0171	0.042	0.0064	0.166
H7-1	0.0282	0.024	0.0064	0.207
H7-2	–0.0042	0.377	0.0113	0.108
H7-3	0.0030	0.256	0.0100	0.134
H7-4	0.1792	**0.001**	0.0318	0.012
H14-1	0.0039	0.121	0.0031	0.151
H14-2	–0.0083	0.121	0.0476	**0.004**
H14-3	0.0077	0.153	–0.0081	0.178
All	0.0765	**0.001**	0.0352	**0.003**
All except H7-4	0.0134	0.075	0.0217	**0.016**

The neighbour-joining tree analysis of the genetic distances revealed a consistency index for the parsimoniously mapped habitat changes of 0.034, and for the island changes of 0.047. Thus, in contrast to the Mantel test this analysis showed island isolation to be somewhat more important than habitat heterogeneity to explain the intraspecific phylogeny, despite the H7-4 locus being included. The number of changes between habitats were, regardless of direction, boulders to high rocks 19; high rocks to low rocks 23; and boulders to low rocks 16. This suggests a closer genetic relationship between boulders and low rocks than between high and low rocks, although the latter two habitat types were adjacent on the islands.

Genetic variation at different levels for each habitat (within populations, between populations within islands, among islands) analysed with AMOVA showed no consistent pattern across habitat type (Table 2). Thus, for example, differentiation among islands was only found in the high rocky habitat, while part of the variation in the boulder habitats was between samples within islands. The low rocky habitat did not show significant variation at any of these levels (Table 2).

DISCUSSION

In the study area, *Littorina saxatilis* is distributed over more than a hundred small islands (1–100 m across). Dispersal among islands without a pelagic larva is occasional but not as infrequent as might be expected. Indeed, an assessment of recolonization rate following a toxic algal bloom that completely wiped out populations of small, intertidal islands indicated that 3% of the islands were recolonized per year, on average (Johannesson & Johannesson, 1995). If recolonization takes place through dispersal of single fertilized females, as has been suggested (Johannesson, 1988), dispersal among islands is probably in the range of $Nm \approx 1$ or less. Allozyme differentiation among populations of *L. saxatilis* over islands of the same area suggested Nm to be in the range of 1–2 individuals per generation (Janson, 1987; Johannesson & Tatarenkov, 1997). The significant genetic differentiation among islands (Mantel's test, $P = 0.003$) in the nine RAPD loci of this study supports the conclusion of an overall isolation by distance effect among island populations, supporting the results obtained from the allozyme experiment.

The heterogeneous environment of the snails imposes divergent selection for a range of morphological, behavioural and physiological traits (Janson, 1983; Janson & Ward, 1984; Sundell, 1985; Johannesson & Johannesson, 1996; Sokolova *et al*., 2000) and it seems possible that DNA polymorphisms will be partly generated by linkage to selected loci. Indeed, the habitat gave an overall effect on the DNA variation that was stronger than the island effect. However, the separate loci contributed much more evenly to the island effect than to the habitat effect (r variances 0.0003 and 0.0034, respectively). This suggests the island effect to be a consequence of random genetic drift, while the habitat effect is due to a few of the RAPD loci being linked to loci under divergent selection, or, less probably, that they are targets of selection. In the study by Wilding *et al*. (2001), removing those 5% of the AFLP loci that were linked to type of habitat of the snails, the variation in the remaining loci clearly reflected isolation by distance. Similarly, in this study, removal of the H7-4 locus yields a non-significant habitat effect while the island effect remains significant (Table 1).

Allozyme variation also reveals both an effect of habitat and an effect of island isolation (Johannesson

Table 2. Analysis of molecular variation in populations of the three habitats. The genetic variation is decomposed into three hierarchal levels, within-populations, between-populations-within-islands and among-islands. Significant probabilities are in bold

Source	d.f.	Variance component	% of variation	P
Boulder habitats				
Among islands	4	–0.084	–5.0	0.78
Between populations within islands	5	0.263	15.6	**0.0064**
Within populations	39	1.51	89.4	
High rocky shore habitats				
Among islands	4	0.177	8.6	**0.025**
Between populations within islands	5	–0.032	–1.6	0.60
Within populations	40	1.90	92.9	
Low rocky shore habitats				
Among islands	4	0.032	1.7	0.32
Between populations within islands	5	–0.002	–0.1	0.45
Within populations	40	1.89	98.4	

& Tatarenkov, 1997). Out of eight highly polymorphic allozyme loci, seven showed differentiation owing to island isolation, while the variation in six also showed significant effects of habitat. In a similar way as for the DNA loci, two allozyme loci, *Aat* and *Pgm-2*, showed a much stronger habitat effect than the remaining loci.

The poor dispersal of both juveniles and adults of *L. saxatilis* suggests that genetic substructuring might also occur between replicate samples of the same habitat and island. For allozymes such differentiation was present only in one of the eight loci analysed (*Aat*, see G_{SHI} of table 2 in Johannesson & Tatarenkov, 1997). This study revealed significant differentiation between replicate samples of boulder habitats, while not between replicate samples of low and high rocky shores (Table 2). Thus, overall, we have no strong evidence for within-island and within-habitat variation at this spatial scale and indeed, the bulk of the variation (89–98%) is among individuals within populations. A plausible explanation seems to be that there is stabilizing selection among sites of similar habitats and that gene flow among sites of the same island is high enough to prevent significant effects of drift, although this conclusion is preliminary as the AMOVA indicate substantial differences in spatial structure among the three habitats (Table 2). The habitats showed different patterns of variation with low rocky shores showing no substructuring at all, indicating gene flow over the entire geographical scale studied. On the other hand, high rocky populations differed significantly between islands suggesting high rocky shore populations being more isolated than low rocky shore populations, which might be true if the main mechanism of dispersal among islands is by rafting. More intriguing is that boulder samples seem to be structured at a within-island level, but not between islands. It appears to be impossible to explain these results by any relevant mechanism of gene flow and we are inclined to believe that this suggests the presence of an additional structuring factor not accounted for by our study design.

Geographic distance was slightly less important in generating genetic differences among populations but this correlation showed a tendency in the same direction as the island separation. Perhaps a wider range of geographical distances than the ones used in this study would have produced a significant isolation by distance effect similar to what is found for allozyme differentiation over a scale of 1–300 km in Swedish populations of this species (Janson, 1987).

The results of this study show that not only allozyme variation, but also nuclear DNA variation, in populations of *Littorina saxatilis* is genetically substructured owing both to isolation and to habitat heterogeneity over a spatial scale of kilometres. What was surprising was that over this scale, habitat heterogeneity has an effect on population substructure of the same magnitude as island isolation; however island isolation is quite effective owing to the lack of a pelagic spreading stage.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Swedish Science Research Council and by the EC contract EVK3-2001–00048.

REFERENCES

Allendorf FW, Seeb LW. 2000. Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. *Evolution* **54:** 640–651.

Casgrain P, Legendre P. 1999. *The R Package for multivariate and spatial analysis.* Version 4.0d1. Montréal: Université de Montréal.

Eanes WF. 1987. Allozymes and fitness: evolution of a problem. *Trends in Ecology and Evolution* **2:** 44–48.

Eanes WF. 1999. Analysis of selection on enzyme polymorphisms. *Annual Review of Ecology and Systematics* **30:** 301–326.

Erlandsson J, Rolán-Alvarez E, Johannesson K. 1998. Migratory differences between ecotypes of the snail *Littorina saxatilis* on Galician rocky shores. *Evolutionary Ecology* **12:** 913–924.

Felsenstein J. 1995. *PHYLIP (Phylogeny inference package).* Version 3.57c. Washington, DC: University of Washington.

Fortin M-J, Gurevitch J. 1993. Mantel tests: spatial structure in field experiments. In: Scheiner SM, Gurevitch J, eds. *Design and analysis of ecological experiments*. New York: Chapman & Hall.

Grosberg RK, Levitan DR, Cameron BB. 1996. Characterization of genetic structure and genealogies using RAPD-PCR markers: a random primer for the novice and nervous. In: Ferraris JD, Palumbi SR, eds. *Molecular zoology: advances, strategies, and protocols*. New York: Wiley-Liss Inc.

Grundmann HJ, Towner KJ, Dijkshoorn L, Gerner-Smidt P, Maher M, Seifert H, Vaneechoutte M. 1997. Multicenter study using standardized protocols and reagents for evaluation of reproducibility of PCR-based fingerprinting of *Acinetobacter* spp. *Journal of Clinical Microbiology* **35:** 3071–3077.

Hadrys H, Balick M, Schierwater B. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecology* **1:** 55–63.

Hedrick PW. 1986. Genetic polymorphism in heterogeneous environments: a decade later. *Annual Review of Ecology and Systematics* **17:** 535–566.

Hey J. 1999. The neutralist, the fly and the selectionist. *Trends in Ecology and Evolution* **14:** 35–38.

Janson K. 1982. Genetic and environmental effects on the growth rate of *Littorina saxatilis* Olivi. *Marine Biology* **69:** 73–78.

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 characters 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, 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 B, Johannesson K. 1996. Population differences in behaviour and morphology in *Littorina saxatilis*: Phenotypic plasticity or genetic differentiation? *Journal of Zoology* **240:** 475–493.

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, USA* **92:** 2602–2606.

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

Kashi Y, King D, Soller M. 1997. Simple sequence repeats as a source of quantitative genetic variation. *Trends in Genetics* **13:** 74–78.

Kassen R. 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. *Journal of Evolutionary Biology* **15:** 173–190.

Kluge AG, Farris JS. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* **18:** 1–32.

Koehn RK, Newell RIE, Immermann F. 1980. Maintenance of an aminopeptidase allele frequency cline by natural selection. *Proceedings of the National Academy of Sciences, USA* **77:** 5385–5389.

Lönn M, Prentice HC, Bengtsson K. 1996. Genetic structure, allozyme–habitat associations and reproductive fitness in *Gypsophila fastigiata* (Caryophyllaceae). *Oecologia* **106:** 308–316.

Lynch M, Milligan BG. 1994. Analysis of population genetic structure with RAPD markers. *Molecular Ecology* **3:** 91–99.

Maddison WP, Maddison DR. 1992. MacClade, Version 3.1 Computer Program. Distributed by Sunderland, Massachusetts: Sinauer Associates, Inc.

Manly BFJ. 1986. *Multivariate statistical methods*. London: Chapman & Hall.

McKay JK, Latta RG. 2002. Adaptive population divergence: markers, QTL and traits. *Trends in Ecology and Evolution* **17:** 285–291.

Merilä J, Cronokrak P. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology* **14:** 892–903.

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

Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* **43:** 223–225.

Riddoch BJ. 1993. The adaptive significance of electrophoretic mobility in phosphoglucose isomerase (PGI). *Biological Journal of the Linnean Society* **50:** 1–17.

Rolán-Alvarez E, Johannesson K, Ekendahl A. 1997. The maintenance of a cline in the marine snail *Littorina saxatilis*: the role of home site advantage and hybrid fitness. *Evolution* **51:** 1838–1847.

Schmidt PS, Bertness MD, Rand DM. 2000. Environmental heterogeneity and balancing selection in the acorn barnacle *Semibalanus balanoides*. *Proceedings of the Royal Society of London, Series B* **267:** 379–384.

Schneider S, Roessli D, Excoffier L. 2000. *Arlequin Ver 2.000: a software for population genetic data analysis.* Geneva, Switzerland: Genetics and Biometry Laboratory, University of Geneva.

Sokolova IM, Granovitch AI, Berger VJ, Johannesson K. 2000. Intraspecific physiological variability of the gastropod *Littorina saxatilis* related to the vertical shore gradient in the White and North Seas. *Marine Biology* **137:** 297–308.

Stewart CN Jr, Excoffier L. 1996. Assessing population genetic structure and variability with RAPD data: Application to *Vaccinium macrocarpon* (American Cranberry). *Journal of Evolutionary Biology* **9:** 153–171.

Sullivan DT, Atkinson PW, Starmer WT. 1990. Molecular evolution of the alcohol dehydrogenase genes in the genus *Drosophila*. *Evolutionary Biology* **24:** 107–147.

Sundell K. 1985. Adaptability of two phenotypes of *Littorina saxatilis* (Olivi) to different salinities. *Journal of Experimental Marine Biology and Ecology* **92:** 115–123.

Swofford DL. 1993. *PAUP: phylogenetic analysis using parsimony*, Version 3.1. Champaign, Illinois: Illinois Natural History Survey.

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.

Thorpe JP, Solecava AM. 1994. The use of allozyme electrophoresis in invertebrate systematics. *Zoologica Scripta* **23:** 3–18.

Vaudor A. 1990. *'R' Package, Mantel 3.0*. Montréal: Université de Montréal.

Watt WB. 1985. Bioenergetics and evolutionary genetics: opportunities for new synthesis. *American Naturalist* **125:** 118–143.

Watt WB. 1992. Eggs, enzymes, and evolution – natural genetic variants change insect fecundity. *Proceedings of the National Academy of Sciences, USA* **89:** 10608–10612.

Wilding CS, Butlin RK, Grahame J. 2001. Differential gene exchange between parapatric morphs of *Littorina saxatilis* detected using AFLP markers. *Journal of Evolutionary Biology* **14:** 611–619.

Williams JGW, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18:** 6531–6535.