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Differential patterns of spatial divergence in microsatellite and allozyme alleles: further evidence for locus-specific selection in the acorn barnacle, *Semibalanus balanoides*?

F. DUFRESNE,*† E. BOURGET‡ and L. BERNATCHEZ*
**Département de Biologie, Université Laval, Cité Universitaire, Sainte-Foy, Canada, †Département de Biologie, Université du Québec à Rimouski, 300 allée des ursulines, Rimouski, Québec, Canada, G5L 3A1, ‡Université de Sherbrooke, Sherbrooke, Canada*

Abstract

We compared patterns of genetic structure at potentially selected (two allozyme loci) and neutral molecular markers (six microsatellite loci) in the acorn barnacle, *Semibalanus balanoides* from the Gulf of St. Lawrence. Our results confirmed the presence of a geographical shift in alleles *MPI* and *GPI* near the Miramichi River. In contrast, no significant patterns of population differentiation among samples located north and south of the river mouth were detected for four of six microsatellite loci. However, analysis of molecular variance (AMOVA) at individual loci revealed that a significant proportion of the total variance in allele frequencies was partitioned among samples located north and south of the river for both the allozyme and the other two microsatellite loci. The two most common alleles at these microsatellites showed frequencies that were highly correlated ($r = 0.65–0.74$, $P < 0.05$) with those of the *MPI2 allele, perhaps because of either physical linkage or epistasis. The two allozyme loci were significantly correlated in barnacles located north of the Miramichi River ($r = 0.86$, $P < 0.05$). Overall, our results supported the hypothesis that the broad scale pattern of allozyme allelic shifts is maintained by selection. They also indicated that microsatellites may not always behave in a neutral way and must be used cautiously, especially when evidence for genetic structuring relies on only a few assayed loci.**

Keywords: allozyme, barnacles, gene flow, microsatellite, population structure, selection

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Introduction

The role of selection in maintaining genetic polymorphisms at allozyme loci has been reported in diverse marine species: temperature selection at the lactate dehydrogenase (*LDH*) locus in the killifish, *Fundulus heteroclitus* (Powers & Schulte 1998), osmoregulation selection at leucine aminopeptidase (*LAP*) in the blue mussel, *Mytilus edulis* (Koehn *et al*. 1980) and at glutamate-pyruvate transaminase (*GPT*) in the copepod, *Tigriopus californicus* (Burton & Feldman 1983). Despite more than 25 years of research, however, it is still not clear whether the allozyme loci themselves are the targets of selection or if other loci in close linkage with the allozymes are under selection (Mitton 1998).

Correspondence: F. Dufresne. Fax: +1 418 724 1849 E-mail: France_dufresne@uqar.uquebec.ca

One increasingly popular approach to assess the role of selection in determining allelic variation is to compare patterns of genetic structure at putative selected loci with those obtained from more neutral loci such as mitochondrial DNA (mtDNA) and microsatellite loci. Gene flow and drift should equally affect neutral loci, whereas selection is more likely to be locus specific (Lewontin & Krakauer 1973). When they are not linked to putatively selected genes, microsatellite markers are also potentially good markers to assess neutrality as they are located mostly in noncoding regions (Queller *et al*. 1993). For instance, a recent study on sea bass has revealed much higher F_{ST} values for allozymes (mean = 0.339) than microsatellites loci (mean = 0.017) (Lemaire *et al*. 2000). Fish from lagoons were more genetically similar than those from marine habitats at allozyme, but not microsatellite, loci thus confirming the selective role of allozymes in habitat differentiation.

The northern acorn barnacle, *Semibalanus balanoides* is a common inhabitant of intertidal rocky shores in the North Atlantic North of Cape Hatteras and northwest Spain, and on the Pacific coast of southern Alaska (Flowerdew 1983; Bourget *et al*. 1989). *S. balanoides* is a simultaneous hermaphrodite that presumably does not self-fertilize (Barnes 1957). Mating takes place in the fall and the larvae are brooded in the mantle cavity until the following spring (Bousfield 1954). The larvae then spend 3–5 weeks in the plankton during which time they go through six naupliar stages (Lucus *et al*. 1979). Temperature has been shown to be a very important selective agent in this sessile species (Bertness & Gaines 1993). High temperature tolerance experiments revealed that barnacles suffered 50% mortality after a 45-min exposure at 37 °C (Southward 1958). These temperatures are often encountered by barnacles in nature, thus confirming the potential role of temperature selection in shaping barnacles populations. A recent study by Schmidt & Rand (1999) showed that rock temperatures at a warm microhabitat site in Maine remained above the upper thermal limit of 37 °C on six consecutive dates in one month and that barnacles from warmer microhabitats had consistently higher frequencies of mannose-6-phosphate genotypes (*MPI-FF* and *MPI-SF*) than barnacles from colder microhabitats (separated by < 10 m).

In this study, we contrast patterns of genetic differentiation at allozyme (*MPI* and *GPI*) and microsatellite loci among 14 locations of *S. balanoides* to assess the possible role of selection and gene flow in the genetic structuring of barnacle populations at a large geographical scale. Previous genetic studies of *S. balanoides* along the western Atlantic coast of North America and the Gulf of St. Lawrence have revealed genetic discontinuity at both *MPI* and *GPI* over a distance of 50–100 km near the Miramichi River, one of the largest estuaries in Atlantic Canada (Holm & Bourget 1994). Furthermore, ecological studies have shown differences in settlement preferences between Gulf and Atlantic populations, which may potentially restrict gene flow between both regions (Chabot & Bourget 1988; Bourget *et al*. 1989). A lack of genetic structure at microsatellite loci in the presence of strong geographical discontinuity at allozyme loci would be consistent with selection on the latter ones. Alternatively, high levels of population structure at microsatellite loci suggests that historical separations and/or contemporary restriction to gene flow may have contributed importantly to the observed patterns of structuring at allozymes. Here, we report unexpected correlations between allele frequency variation at two microsatellite loci and that observed at the allozyme loci. Because significant genetic structure was found at the two potentially linked microsatellite loci and at none of the other four loci, we propose that selection is acting on the allozymes, and indirectly influenced the observed pattern of allelic distribution at these two microsatellites.

Materials and methods

Sampling

In July 1997, we sampled 14 sites in the St. Lawrence estuary and the Southern Gulf (Fig. 1). The sampling region spans a distance of over 1000 km, ranging from Métis on the south shore of the St. Lawrence Estuary to Grand Manan (GM), New Brunswick. Métis (ME) is the sole sample located in the St. Lawrence estuary, all other locations are in the Southern Gulf. Barnacles in these areas are found on rocky outcrops that occur primarily in the form of artifical jetties. Exposed barnacles (as opposed to those under algal cover) were randomly collected from multiple outcrops in the upper intertidal zone and immediately frozen in liquid nitrogen. Thus, the barnacles we sampled were those most exposed to selection by high temperature, as assessed by Schmidt & Rand (1999). Barnacles from all 14 sites were sampled in the high intertidal zone and hence all suffered similar heat exposure. Once in the laboratory, they were transferred to −80 °C until further analyses.

Allozymes

The barnacle's prosoma were homogenized in 10–30 μL of a grinding buffer (50 mM Tris–HCl, pH 8.0, 1 mM $MgCl_2$, 1 mM dithiothreitol, 50% v/v glycerol; Holm & Bourget 1994). All samples were analysed for variation at *GPI* and *MPI* using cellulose acetate according to Herbert & Beaton (1989). To ease comparison with the work of Holm & Bourget (1994), we identified alleles with numbers 1–4 based on relative mobility on the gel. We analysed only these two loci as they have previously been identified as loci potentially under selection (Holm & Bourget 1994). Other loci were tested (amino aspartate transferase, phosphoglucomutase, aldehyde oxidase) but revealed no or very low levels of variation.

Microsatellites

Total DNA was extracted using standard phenol–chloroform methods (Sambrook *et al*. 1989). Microsatellite analyses were not performed on the same individuals as those used for allozyme analyses because the small amount of available tissues would have made laboratory analyses unduly labour-intensive, given that we had no a priori reasons to expect linkage between allozyme and microsatellite markers. However, the individuals used for the microsatellite analyses came from the exact same sampling collections as those used for the allozyme studies. A total of 469 barnacles was screened for genetic variation at six microsatellite loci. Three loci (*SEBAL13*, *SEBAL14* and *SEBAL35*) were isolated for *Semibalanus balanoides*

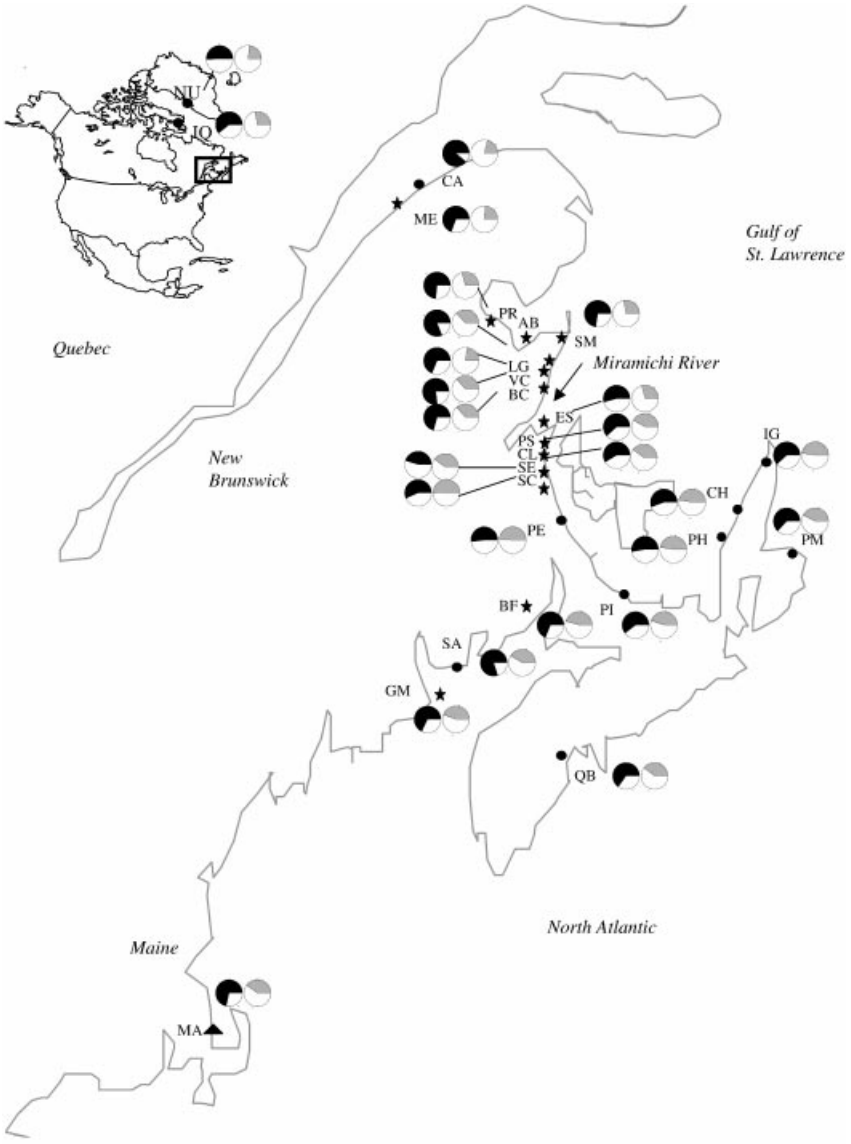

Fig. 1 Location map of 26 samples of *Semibalanus balanoides* from the Gulf of St. Lawrence and the North Atlantic. SA, Saint-Augustin; CA, Capucins; ME, Métis; PR, Petit-Rocher; AB, Anse-Bleue; SM, Sainte-Marie; LG, Le Goulet; VC, Val Comeau; BC, Burnt Church; ES, Escuminac; PS, Pointe-Sapin; CL, Cap Lumière; SE, St-Edouard-de-Kent; SC, Schédiac; PE, Port-Elgin; PI, Pictou; PH, Port Hood; CH, Chéticamp; IG, Ingonish; PM, Port Morien; QB, Queensland Beach; BF, Bay of Fundy; SA, St. Andrews; GM, Grand Manan; MA, Maine. Sites indicated by a point are samples analysed by Holm & Bourget (1994), those indicated with a star are sites sampled by us, and Schmidt and Rand's study site (MA) is indicated by a triangle. Pies represent allele frequencies at the *GPI* locus (pie to the left, black for *GPI*2* and white for *GPI*3*) and the *MPI* locus (pie to the right, grey for *MPI*1* and white for *MPI*2*).

by Dufresne *et al*. (1999), whereas the others (*1CA, 3GATA, 4GATA*) were kindly provided by Dr D. Rand (Brown University). *3GATA* and *4GATA* are tetranucleotide repeat loci, whereas the others are dinucleotides. All polymerase chain reactions (PCR) were run simultaneously as the six loci have the same annealing temperature (59 °C). *SEBAL13*, *SEBAL14* and *SEBAL35* were run in triplex, *1CA* and *3GATA* were performed in duplex, and *4GATA* in simplex. PCR conditions were performed as described in Dufresne *et al*. (1999). Two volumes of 0.7 µL of both the duplex and triplex and 1 vol. of 0.1 µL of the simplex along with 2 µL of blue formamide containing 10% of GS350 internal size standard (TAMRA 350 bp) were loaded on a ABI 377 automated DNA sequencer. Fragments were sized in reference to the standard using GENESCAN Version 2.1 and GENOTYPER Version 2.0. A reference sample from Bay of Fundy was included in each gel to ensure reproducibility of the results. The entire data set was scored twice.

Genetic data analyses

GENEPOP software (Version 3.1; Raymond & Rousset 1995) was used to measure intraspecific genetic variability (number of alleles, heterozygosity), heterozygote deficiencies and genotypic linkage disequilibria. F_{IS} was quantified by calculating estimates of *f* (Weir & Cockerham 1984) using GENETIX software, Version 3.3 (Belkhir *et al*. 1998). Pairwise F_{ST} were quantified by calculating θ estimates (Weir & Cockerham 1984) using ARLEQUIN software, Version 1.1 (Schneider *et al*. 1997) according to the method of Reynolds *et al*. (1983). One thousand permutations

were generated to assess significance values according to Excoffier *et al.* (1992). In all cases of multiple tests, significance levels were adjusted by using sequential Bonferroni corrections (Rice 1989). Microsatellite θ estimates were recalculated after pooling genotypes into three classes: homozygotes for the most common allele, heterozygotes involving the most common allele, and all other genotypes. The pooling was performed in order to reduce potential biases when comparing θ values between allozyme loci with only two major alleles and microsatellite loci which have numerous alleles (McDonald 1994). Confidence intervals were calculated by jackknifing over samples using FSTAT software (Goudet 1995). R_{ST} values (Slatkin 1995) were also estimated using RST-CALC (Goodman 1997).

Hierarchical analyses of molecular variance (AMOVA: Excoffier *et al*. 1992) were carried out using ARLEQUIN to assess the amount of variance imputable to genetic differences between both regions north and south of the Miramichi River, among samples within region, and within samples. This was first performed on combined loci for both allozymes and microsatellites, and also, locus by locus in order to assess the differential effect of specific loci on the observed patterns. Equidistance between alleles was considered in all cases. Mantel tests (Mantel 1967) were performed on matrices of F_{ST} values and geographical distances using GENETIX.

Results

Allozymes

The null hypothesis of Hardy–Weinberg equilibrium (HWE) was not rejected for any of the 14 samples (Table 1) and there was no significant genotypic linkage disequilibrium between *MPI* and *GPI* ($\chi^2 = 19$, df = 28, $P > 0.05$). Important geographical shifts in allele frequencies were observed at both *MPI* and *GPI*. Figure 1 shows allelic frequencies for *MPI* and *GPI* for 26 samples on a north–south gradient. We included 12 sites analysed by Holm & Bourget (1994) and one Maine sample (MA) from Schmidt *et al.* (2000). These authors identified their alleles as slow and fast at *GPI* and *MPI*. Because they found two rare alleles and two common ones, we deduced that their slow and fast running ones correspond to our alleles 1 and 2 at *MPI* and our alleles 2 and 3 at *GPI (*alleles 1 and 4 are the rare ones).

Holm & Bourget (1994) sampled sites in the same area in 1990–92. A two-way ANOVA using mean allele frequency (*MPI**2) as the dependent variable and sampling period (1990–92 vs. 1997) and sampling location (north of Miramichi vs. south of Miramichi) as independent variables showed a significant effect of location ($F = 36.7$, $P < 0.0001$), no significant effect of sampling period ($F = 0.3$, $P = 0.55$), and no significant interaction terms ($F = 2.9$, $P = 0.09$) on allele frequency. The same analysis was applied to the allele *GPI*3* and again a significant north vs. south effect was found ($F = 9.90$, $P < 0.004$) with no significant effect of sampling period ($F = 0.36$, $P = 0.55$) and no significant interaction between these two factors ($F = 0.95$, $P = 0.34$) on allele frequency. Allele frequencies shifted in the vicinity of the Miramichi River for both *MPI* and *GPI*. Allele frequencies increased between Burnt Church (BC) and Escuminac (ES) for *GPI**3, whereas a decrease in allele frequencies occurred one site down [ES and Pointe-Sapin (PS)] at *MPI**2. Frequencies of *MPI**2 and *GPI**3 were highly correlated for barnacles located north, from ME to BC (Pearson's $r = 0.95$, $P < 0.0001$) but not over their whole range (Pearson's $r = -0.27$, $P > 0.05$). Mean allele frequencies differed significantly for barnacles located immediately north (ME to BC) and south (ES to PS) of the Miramichi River (*MPI*: 0.73 ± 0.06 and 0.58 ± 0.09, $t = 4.55$, $P = 0.007$; *GPI*: 0.26 ± 0.04 and 0.41 ± 0.07, $t = -4.7$, $P = 0.005$).

Allelic shifts translated into a relatively important component of genetic variance imputable to regional groupings for *MPI* and *GPI* (Table 2). Whereas within population genetic variance accounted for 95.3% of total genetic variance, the remainder (4.7%) was partitioned exclusively among groups rather than among populations within group. These results are concordant with those of Holm & Bourget (1994). The AMOVA on individual loci revealed that the best north–south separation (as revealed by the highest percentage of variation intergroup and the lowest intragroup variation) differed between enzyme loci. For *MPI*, this was achieved when the northern group included samples from ME to ES and when the southern group included samples from PS to Grand Manan (GM) (among groups genetic variation = 7.5%, $P < 0.0001$; among populations within groups genetic variation = 0.70%, $P > 0.05$). In contrast, the best grouping for *GPI* included samples from ME to VC for the northern group and samples from BC to GM for the southern group (among groups genetic variation = 4.99%, $P < 0.0001$; among populations within groups genetic variation = 0%). Pairwise θ estimate comparisons revealed six significant comparisons (following sequential Bonferroni correction, $\alpha = 0.05$, $k = 0.002$), all of which included north–south comparisons.

Microsatellites

The microsatellite loci revealed higher levels of variability than the allozymes as the number of alleles ranged from 6 to 52 (Table 1). None of the 15 locus pairs exhibited significant linkage disequilibrium. Observed heterozygosity (H_O) values were, however, highly variable, ranging from 0.170 (*SEBAL13*) to 0.969 (*1CA*). GENEPOP exact tests assuming H1 = heterozygote deficit, revealed significant departures from the null hypothesis of HWE in several samples (Table 1). The deficits could not be ascribed to particular samples or loci, although the BF sample had

Table 1 Sample size (n), number of alleles (A), gene diversity (H_E, Nei 1987), observed heterozygosity (H_O: proportion of heterozygous individuals per sample), and f according to Weir & Cockerham (1984) Values in bold indicate samples which deviate significantly from Hardy–Weinberg's expectations after sequential Bonferroni corrections

	Samples													
Locus	ME	PR	AB	SM	LG	VC	BC	ES	PS	CL	SE	SCH	BF	GM
GPI														
n	45	42	39	47	26	41	41	22	38	35	28	29	26	15
A	3	3	2	2	2	2	2	2	2	2	3	2	2	2
H_E	0.450	0.403	0.294	0.400	0.426	0.356	0.414	0.495	0.472	0.485	0.541	0.494	0.440	0.464
H_O	0.422	0.261	0.359	0.383	0.461	0.414	0.341	0.545	0.342	0.428	0.464	0.275	0.576	0.733
f	0.073	0.362	0	0.054	0	0	0.187	0	0.287	0.131	0.160	0.456	0	0
MPI														
n	44	40	39	47	24	19	40	21	38	37	32	28	24	24
A	2	3	2	4	2	3	3	2	3	3	2	2	3	3
H_E	0.363	0.427	0.467	0.435	0.353	0.513	0.344	0.408	0.512	0.531	0.498	0.429	0.509	0.502
H_O	0.431	0.475	0.487	0.340	0.458	0.368	0.375	0.381	0.394	0.540	0.562	0.375	0.625	0.583
f	0	0	0	0.229	0	0.308	0	0.091	0.243	0	0	0.192	0	0
Allozymes														
A	2.5	3	2	3	2	2.5	2.5	2.5	2	2.5	2.5	2	2.5	2.5
H_E	0.406	0.415	0.385	0.418	0.389	0.435	0.379	0.453	0.492	0.508	0.519	0.462	0.475	0.483
H_O	0.427	0.368	0.423	0.361	0.460	0.391	0.388	0.462	0.368	0.484	0.513	0.325	0.601	0.658
Sebal13														
n	35	26	42	39	47	21	38	30	46	37	33	25	36	41
A	3	2	4	3	2	4	5	2	3	3	4	2	5	2
H_E	0.469	0.355	0.500	0.512	0.494	0.517	0.402	0.391	0.517	0.517	0.529	0.435	0.598	0.485
H_O	0.200	0.307	0.428	0.307	0.170	0.285	0.289	0.400	0.608	0.459	0.484	0.240	0.361	0.439
f	**0.583**	0.152	0.155	**0.410**	**0.661**	0.466	0.292	0	0	0.125	0.099	0.464	0.407	0.107
Sebal14														
n	39	26	44	41	47	23	40	34	47	41	36	26	42	47
A	9	6	8	9	10	6	7	8	10	8	9	9	11	8
H_E	0.668	0.772	0.714	0.740	0.719	0.778	0.738	0.756	0.830	0.810	0.783	0.749	0.817	0.762
H_O	0.717	0.615	0.727	0.731	0.574	0.739	0.725	0.794	0.638	0.682	0.666	0.538	0.666	0.872
f	0	0.222	0	0.024	0.211	0.073	0.030	0	**0.241**	0.169	0.162	0.299	0.196	0
Sebal35														
n	38	26	45	41	47	23	40	35	47	40	34	26	39	46
A	11	10	10	12	15	9	9	10	10	15	10	11	15	17
H_E	0.718	0.759	0.729	0.805	0.787	0.676	0.721	0.718	0.804	0.808	0.769	0.846	0.816	0.773
H_O	0.763	0.807	0.644	0.853	0.851	0.652	0.700	0.685	0.702	0.950	0.705	0.730	0.615	0.739
f	0	0	0.128	0	0	0.058	0.042	0.059	**0.137**	0	0.096	0.155	**0.258**	0.055
1CA														
n	37	26	41	38	46	19	38	33	47	38	34	26	45	45
A	33	27	34	30	31	21	30	27	32	32	25	28	35	27
H_E	0.955	0.955	0.959	0.953	0.954	0.940	0.951	0.950	0.950	0.952	0.942	0.948	0.954	0.944
H_O	0.864	0.961	0.829	0.763	0.891	0.842	0.789	0.969	0.957	0.947	0.911	0.884	0.911	0.755
f	0.108	0.013	0.147	0.212	0.076	0.131	**0.182**	0	0.003	0.018	0.047	0.086	**0.056**	0.210
3GATA														
n	37	26	44	41	47	23	38	34	47	41	33	26	42	46
A	11	8	8	11	6	5	10	8	17	13	6	6	15	8
H_E	0.761	0.656	0.644	0.765	0.701	0.670	0.761	0.676	0.791	0.756	0.671	0.680	0.837	0.687
H_O	0.432	0.576	0.613	0.609	0.574	0.565	0.684	0.264	0.702	0.536	0.515	0.269	0.714	0.565
f	**0.433**	0.139	0.059	0.215	0.191	**0.178**	**0.115**	0.618	0.123	0.301	0.247	**0.616**	**0.158**	0.188
4GATA														
n	33	19	43	36	44	22	37	31	40	34	31	27	43	47
A	14	13	14	15	12	13	15	12	13	10	14	11	18	16
H_E	0.850	0.865	0.905	0.869	0.804	0.886	0.884	0.691	0.812	0.798	0.843	0.861	0.898	0.895
H_O	0.757	0.842	0.790	0.694	0.659	0.772	0.810	0.774	0.825	0.735	0.774	0.963	0.744	0.446
f	0.124	0.054	0.138	0.214	**0.253**	0.151	0.096	0	0	0.094	0.098	0	**0.182**	**0.509**
Microsatellites														
A	13	11	13	13	13	10	13	11	14	13	11	11	16	13
H_E	0.737	0.727	0.742	0.774	0.754	0.745	0.743	0.697	0.784	0.774	0.756	0.753	0.820	0.758
H_O	0.622	0.685	0.672	0.660	0.620	0.642	0.666	0.648	0.739	0.719	0.676	0.604	0.668	0.636

Table 2 Analyses of molecular variance (AMOVA) among 14 samples of *Semibalanus balanoides* separated into two regional groups: North (Métis to Escuminac) and South (Pointe-Sapin to Grand Manan) of the mouth of the Miramichi River (see Fig. 1)

Loci	Source of variation variation	df	Variance components	% variation	Fixation indices	*P*
Allozymes	Among groups	1	0.020	4.77	CT = 0.047	< 0.001
	Among samples within groups	11	−0.003	0	SC = 0	> 0.05
	Within samples	909	0.404	95.91	ST = 0.040	< 0.001
	Total	921	0.42	100		
Microsatellites	Among groups	1	0.013	0.66	CT = 0.007	< 0.001
	Among samples within groups	12	0.016	0.81	SC = 0.008	< 0.05
	Within samples	1048	2.02	98.5	ST = 0.014	< 0.001
	Total	1061	2.04	100		
Microsatellites (without *SEBAL13* & *SEBAL14*)	Among groups	1	0.003	0.22	CT = 0.0008	> 0.05
	Among samples within groups	12	0.014	0.94	SC = 0.010	< 0.05
	Within samples	1066	1.508	98.84	ST = 0.011	< 0.001
	Total	1079	1.52	100		

significant *f* estimates for four of six loci. Ten comparisons over a total of 91 were significantly different at the 0.0005 level. These significant comparisons involved mostly PS with populations north of the Miramichi River.

The AMOVA for microsatellite loci revealed an overall weak but highly significant interregional (north vs. south of the Miramichi estuary) pattern of genetic structure (Table 2). When the AMOVA were performed on individual loci, two were found to be responsible for the weak but significant genetic structure among groups. *SEBAL13* and *SEBAL14* showed significant among group structuring (among groups genetic variation = 4.5%, $P = 0.005$ for *SEBAL13* and 0.56%, $P = 0.006$ for *SEBAL14*). The other four loci showed no evidence of regional structuring (among groups genetic variation = 0.11%, $P > 0.05$ for *SEBAL35*, 0.01%, $P > 0.05$, for *1CA*, 0.12%, $P > 0.05$ for *3GATA*, 0%, $P > 0.05$, for *4GATA*). The AMOVA performed using these four loci combined confirmed the absence of regional structuring among groups located north and south of the Miramichi River (Table 2). The overall weak population structuring at *SEBAL35*, *1CA*, *3GATA* and *4GATA* was also reflected by an absence of significant pairwise comparisons of θ estimates. In contrast, eight significant θ estimates involving north–south pairwise comparisons (25 before and eight following sequential Bonferroni corrections, $\alpha = 0.05$, $k = 0.0006$) were found when the analyses were performed on *SEBAL13* and *SEBAL14* combined. Mantel tests revealed no significant relationship between population divergence and geographical distance ($z = -1.75$, $P > 0.05$).

The two microsatellite loci (*SEBAL13* and *SEBAL14*) responsible for generating the significant pattern of genetic structuring north and south of the Miramichi River had their most frequent allele correlated with *MPI*2* (Fig. 2). *SEBAL13*171* and *SEBAL14*150* both showed significant

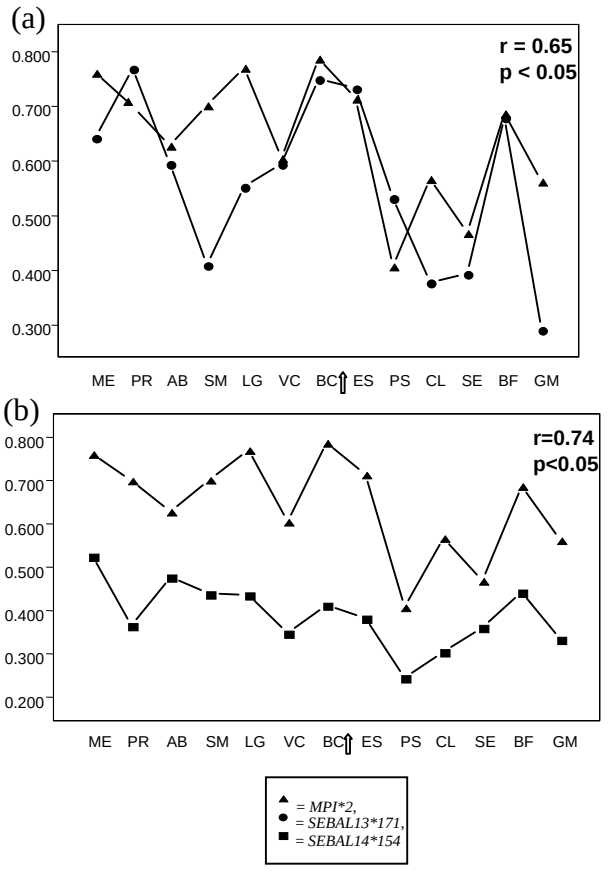

Fig. 2 Allele frequencies at microsatellite and allozyme loci among 13 sites along the Gulf of St. Lawrence and the Atlantic. (a) Frequencies of the allozyme allele *MPI*2* and the microsatellite allele *SEBAL13*171*, (b) frequencies of *MPI*2* and the microsatellite allele *SEBAL14*157*. The arrows indicates the mouth of the Miramichi River. Correlation coefficients and associated probabilities are shown for the alleles *MPI*2* and *SEBAL13*171* and *MPI*2* and *SEBAL14*154*.

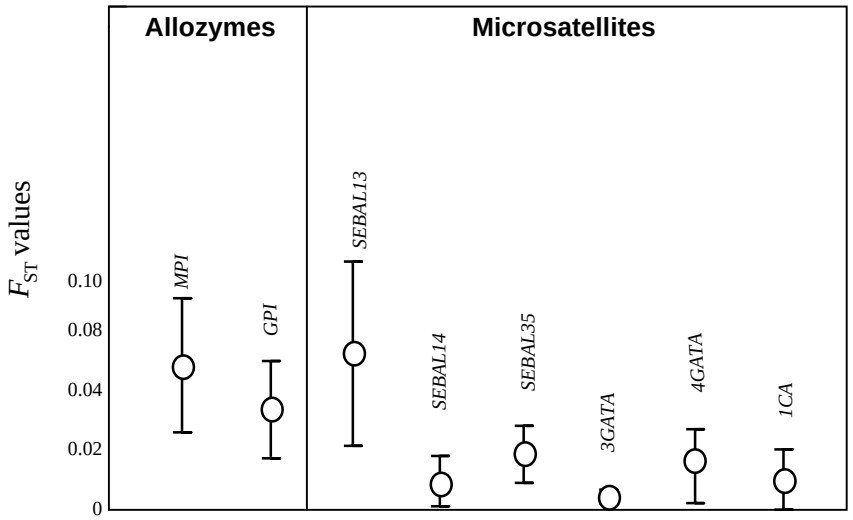

Fig. 3 F_{ST} estimates for each allozyme and microsatellite locus. Confidence intervals were obtained by jackknifing over populations.

correlations with *MPI*2* (Fig. 2a,b) (Pearson's $r = 0.65$, $P < 0.05$ for *SEBAL13*171* and $r = 0.74$, $P < 0.05$ for *SEBAL14*). *SEBAL13*171* and *SEBAL14*150* were not correlated with *GPI*3* (Pearson's $r = -0.17$ $P > 0.05$, and Pearson's $r = -0.30$, $P > 0.05$, respectively). *MPI*2* and *GPI*3* were significantly negatively correlated in barnacles located north of the Miramichi River ($r = -0.86$, $P < 0.05$).

Mean frequencies of the microsatellite alleles differed significantly between northern (ME to ES) and southern samples (PS to GM) for *SEBAL13*171* (0.67 ± 0.06 and 0.50 ± 0.06, $t = 3.2$, $P = 0.007$) and *SEBAL14*150* (0.43 ± 0.06 and 0.29 ± 0.06, $t = 4.06$, $P = 0.001$). The frequencies of the two microsatellite alleles *SEBAL13*171* and *SEBAL14*150* were also significantly correlated to one another (Pearson's $r = 0.47$, $P < 0.05$). We did not consider microsatellite alleles with very low frequencies (< 20%) as these typically showed null frequencies in several populations.

In contrast to the allozymes, a high number of pairwise comparisons exhibited significant F_{ST} values following sequential Bonferronni correction. Sixty-five comparisons of a total of 105 were significant. The sites BF and GM were significantly different from all other sites. PS also exhibited significant differences in allele frequencies with most northern sites. R_{ST} values were significantly different for only 13 of 105 comparisons, roughly the same proportion as was found for F_{ST} values estimated by allozyme data. Nearly all significant comparisons involved GM. Because F_{ST} estimates have been shown to be more reliable than R_{ST} estimates for recently diverged populations (Wenburg *et al*. 1998) such as *Semibalanus balanoides* from the Gulf of St. Lawrence, we therefore consider F_{ST} values for the remainder of the analyses. Allozyme loci showed higher overall F_{ST} values and confidence intervals than all but one microsatellite loci (*SEBAL13*) (Fig. 3). When microsatellite alleles were pooled according to McDonald (1994), F_{ST} values remained almost identical for all loci except for *1CA* which had a much higher differentiation value than was estimated with the unpooled data (pooled values: 0.055 for *SEBAL13*, 0.013 for *SEBAL14*, 0.012 for *SEBAL35*, 0.058 for *1CA*, 0.002 for *3GATA*, and 0.009 for *4GATA* and unpooled values 0.055 for *SEBAL13*, 0.008 for *SEBAL14*, 0.012 for *SEBAL35*, 0.019 for *1CA*, 0.003 for *3GATA*, and 0.012 for *4GATA*).

Discussion

Temporal stability at MPI and GPI

The main goal of this study was to contrast geographical patterns of allelic frequencies at allozyme and microsatellite loci in order to assess the possible role of selection and gene flow in structuring the barnacle populations at large geographical scale. We predicted that a lack of genetic structure at microsatellite loci in presence of strong geographical discontinuity at allozyme loci would be consistent with the effect of selection in shaping the broad scale pattern of variation at the latter ones. Significant patterns of regional structuring were found at both *MPI* and *GPI* in the vicinity of the Miramichi estuary. Allele frequencies at both *GPI* and *MPI* did not differ significantly from those found by Holm and Bourget in 1990–92, which suggests that the pattern is temporally stable. It is also noteworthy that Holm & Bourget (1994) sampled barnacles from the lower to the middle intertidal zone, and yet had allele frequencies at allozyme loci nearly identical to those found in our study. In contrast, four of six microsatellite loci failed to detect any significant regional genetic structuring, thus supporting the hypothesis of pronounced gene flow between northern and southern locations, possibly even implying that they belong to a single population. Overall, these results therefore tend to support the hypothesis that the broad scale pattern of the two allozyme loci is maintained by selection.

Alternative explanations for selection hypothesis

Before concluding that selection is responsible for the allozyme shifts near the Miramichi River, alternative hypotheses must be considered, namely that implying restricted gene flow at both allozyme and microsatellite loci *SEBAL13* and *SEBAL14*, combined with a lack of resolution due to high polymorphism at the other four microsatellites. Several lines of evidence argue against this hypothesis. First, it has been shown theoretically and by simulation studies that higher polymorphism may in fact increase the power of markers in detecting signals of differentiation and reduce variance around estimates of divergence (Goudet *et al*. 1996; Estoup & Angers 1998; Goldstein & Schlötterer 1999). Many studies on marine species have revealed that the high polymorphism at microsatellite loci allows a finer resolution of genetic structuring than less variable markers [e.g. European eel *Anguilla anguilla* (Wirth & Bernatchez 2001), eelgrass *Zostera marina* (Reusch *et al*. 2000), squid *Loligo forbesi* (Shaw *et al*. 1999), and Atlantic cod *Gadus morhua* (Ruzzante *et al*. 1999)]. Second, results obtained when pooling alleles according to McDonald (1994) gave almost identical F_{ST} values as those obtained with nonpooled data. Third, another study on *Semibalanus balanoides* populations from Maine detected as much genetic structure with *SR3* as with *SEBAL13*, these two loci showing the highest and the fewest number of alleles in the set of markers used in that study (D. Rand, personsal communication). A recent study on population genetic structure of a tropical tree, *Caryocar brasiliense* used 10 microsatellite loci which all had similar number of alleles (20–27) and yet F_{ST} values varied from 0.02 to 0.21 (Collevatti *et al*. 2001). In the common ash, *Fraxinus excelsior*, one microsatellite locus with 10 alleles had a F_{ST} of 0.075, whereas another locus with 59 alleles had a F_{ST} of 0.067 (Heurtz *et al*. 2001). In the plant, *Centaurea corymbosa*, there was no significant relationship between F_{ST} and within population expected heterozygosity (Freville *et al*. 2001). Lastly, differences in F_{ST} values could reflect different levels of homoplasy among loci but there is no relationship between genetic diversity and the level of homoplasy (Viard *et al*. 1998). Sequencing studies would have to be performed to test if the microsatellite loci that failed to show genetic structure have higher homoplasy levels.

Temperature as a selective agent

The possible role of selection in determining the broad scale patterns of allelic variation at both *MPI* and *GPI* has also been further supported by a recent transplantation study (Brindamour 2000). This showed that when larvae from locations north of the Miramichi River were transplanted to locations south of it, allelic frequency within these samples at *GPI* changed accordingly to that observed in untransplanted controls among southern locations because of high mortality. Temperature has been hypothesized to be a selective agent at both *MPI* (barnacles: Schmidt & Rand 1999) and *GPI* (amphipods: Patarnello *et al*. 1989, fish: Al-Hassan *et al*. 1987; sea anemones: Hoffmann 1981). Although there are no obvious temperature differences between sites located immediately north and south of the Miramichi River, the range of summer surface water temperatures can be as low as 4 °C in upwelling zones along the Northern Gulf and as high as 18 °C in the Southern Gulf (Koutitonsky & Budgen 1991).

Contrasts with Schmidt and Rand's studies

Although our results are supportive of the selective hypothesis at allozymes advanced by the studies of Schmidt & Rand (1999) and Schmidt *et al*. (2000), our findings contrast with theirs in several ways. First, the north–south pattern of differentiation found at *MPI* was opposite to that expected based on their findings. The frequency of *MPI**2 (their *MPI***F*) increased following transplantation in warmer microhabitats, whereas in our study, the frequency of *MPI**2 decreases in more southern and warmer locations. The larvae sampled by Schmidt & Rand (1999) (pretransplant populations) have similar frequencies at the *MPI* locus as our southern populations, further indicating that their *MPI***F* and our *MPI**2 alleles are the same ones. Second, our results suggest that both loci may be under the influence of selection, whereas Schmidt & Rand (1999) and Schmidt *et al*. (2000) observed that allele frequencies at the *GPI* locus remained unchanged following experimental treatments, therefore suggesting a lack of selective effect. More recently, Schmidt (2001) showed experimentally that barnacles cultured under a mannose-supplemented diet and thermal stress experienced genotypic differential growth rates and survivorship, whereas no such effect was observed when the barnacles were supplemented with fructose. The magnitude of changes in allele frequencies we observed for barnacles north (0.50 ± 0.07 for *MPI***FF* and 0.54 ± 0.06 for *GPI***FF*) and south (0.26 ± 0.06 for *MPI***FF* and 0.34 ± 0.06 for *GPI***FF*, $T = 6.15$, df = 11, $P = 0.0001$ for *MPI* and $T = 5.15$, df = 11, $P = 0.0003$ for *GPI*) of the Miramichi River is similar to changes in allele frequencies incurred by selection in Schmidt and Rand's system. Possible explanations for these apparent discrepancies can only be speculative at this time without further experimental investigations. We randomly sampled barnacles in the upper intertidal zone and only chose exposed barnacles. Therefore, our sampled barnacles likely experienced as much selective pressures as those in Schmidt and Rand's studies. Differences could perhaps be due to confounding factors such as algae with different mannose concentration north and south of the river.

Correlations of microsatellite alleles with MPI

Another salient and unexpected result of this study was that allelic frequencies at two of six microsatellite loci were highly correlated with those observed at allozymes. As argued above, the possibility that these two microsatellite loci, but not the other four, may reflect true regional population structuring appears unlikely. Alternatively, these two loci may reflect the selective effect on the allozyme loci, either because of physical linkage or epistatic interactions. In such a case, our allelic frequencies correlation results suggest that linkage and/or interaction between *SEBAL13* and *MPI* may be stronger than that between *MPI* and for *SEBAL14*. The F_{ST} estimate at *SEBAL13* was also higher and more variable than the other five microsatellite loci, and more similar to the pattern observed at *MPI*. Although *SEBAL14* showed significant north–south regional structuring, the percentage of among-group genetic variation was weaker than that observed at *SEBAL13*. A better understanding of the possible role of linkage and epistasis on the observed correlations must, however, await further breeding and experimental studies.

SEBAL13 and *SEBAL14* showed lower amounts of genetic polymorphism (6 and 12 alleles) than the other four (31, 52, 28 and 26 alleles). Reduction in polymorphism in proximity to selected genes can occur as a result of selective sweeps (Kreitman & Akashi 1995). Even though the high mutation rates of microsatellite should rapidly restore polymorphism and erase the signature of selection, the low genetic diversity found at several microsatellite loci in *Drosophila* has been attributed to such hitchhiking events (Schlötterer *et al*. 1997). The number of repeats is also significantly lower in *SEBAL13* and *SEBAL14* combined than in the four other loci (11 ± 2 and 33 ± 19, $t = -4.2$, $P = 0.0001$), suggesting that these two loci might be located in a coding region and selectively limited in their expansion rate (Metzgar *et al*. 2000). Although most models of microsatellite evolution assume selective neutrality, many microsatellites are thought to be functionally integrated in the genome so that changes in repeat lengths can exert regulatory effects on gene transcription (King & Soller 1999) whether they are associated with promoters or more distantly located in introns (Stallings *et al*. 1991). Microsatellite alleles were found to be associated with microhabitat differences in wild wheat (Li *et al*. 2000), suggesting that selection may act on these presumably regulatory noncoding tandem repeated regions. In the oyster, *Ostrea edulis*, microsatellite markers were found to cosegregate with fitness-associated genes (Bierne *et al*. 1998).

Conclusions

These results have obvious implications for the use of microsatellites in empirical population genetic studies. Our results showed that without a priori knowledge, or comparisons with potentially selected loci, using just a few microsatellite loci could lead to erroneous conclusions regarding patterns of population structuring. For instance, based solely on *MPI*, *GPI*, *SEBAL13* and *SEBAL14*, we would have most likely concluded that barnacles in this study were composed of two genetically distinct populations between which gene flow has been restricted. The correlation with a selected locus that we observed for two of six loci suggests that biased patterns of allelic variation at microsatellite loci may be more common than previously thought. As more studies comparing microsatellite with other markers are undertaken, it remains to be seen whether our results represent an exception. Until this is done, inferring population genetic structure from microsatellites should be made cautiously when evidence for structuring relies on one or a few of the assayed loci.

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