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Stable genetic polymorphism in heterogeneous environments: balance between asymmetrical dispersal and selection in the acorn barnacle

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Abstract

Elucidating the processes responsible for maintaining polymorphism at ecologically relevant genes is intimately related to understanding the interplay between selection imposed by habitat heterogeneity and a species' capacity for dispersal in the face of environmental constraints. In this paper, we used a model-based approach to solve equilibria of balanced polymorphism, given values of fitness and larval dispersal among different habitats in the acorn barnacle *Semibalanus balanoides* from the Gulf of St Lawrence. Our results showed that allele frequencies observed at both *MPI** and *GPI** loci represented stable equilibria, given empirical estimates of fitness values, and that considerably more larvae dispersed from one region (north) to the other (south) than *vice versa*. Dispersal conditions were predicted to be similar for the maintenance of polymorphism at both loci. Moreover, the values of asymmetrical dispersal required by the model to reach stable equilibria were compatible with empirical estimates of larval dispersal and oceanic circulation documented in this system. Overall, this study illustrated the usefulness of a modified and computable version of Bulmer's model (1972) in order to test hypotheses of balanced polymorphism resulting from interactions between spatial selection and asymmetrical dispersal.

Introduction

Elucidating the processes responsible for maintaining polymorphism at ecologically relevant genes remains a fundamental question in evolutionary biology. This issue is intimately related to understanding the interplay between selection and a species' capacity for dispersal in the face of habitat heterogeneity (Linhart & Grant, 1996; Kawecki & Holt, 2002; Svensson & Sinervo, 2004). Species occupying habitats with few physical barriers to dispersal at early life stages, and with limited mobility and/or potential for phylopatry at the adult stage must cope with heterogeneous environments that vary in time and space (Holt & Gaines, 1992; Lenormand, 2002). In such a case, theory predicts that genetic polymorphism at genes differentially affecting fitness in contrasting environments will be maintained by a balance between selection and dispersal across habitats (Kreitman & Akashi, 1995; Hedrick, 1998).

Levene (1953) and Dempster (1955) were the first to propose models for evaluating the general conditions allowing the maintenance of balanced genetic polymorphism in heterogeneous environments. However, the usefulness of these first models has been hampered by their biological simplifications, such as random mating within a common pool, as well as fixed contribution of each habitat to the next generation (Maynard Smith & Hoekstra, 1980; Spichtig & Kawecki, 2004). In this context, Bulmer's (1972) generalization of Levene's model offers several attributes that make it of particular interest. First, Bulmer's model accommodates the fact that for many organisms inhabiting heterogeneous environments and displaying reduced adult mobility such as in sessile species (e.g. benthic aquatic invertebrates, macroalgae, plants, etc.), reproduction occurs within

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habitats and propagule dispersal is the underlying mechanism promoting gene flow among groups inhabiting different habitats (Kinlan & Gaines, 2003; Levin *et al.*, 2003). Second, this model allows asymmetrical dispersal among habitats that could result from the directional influence of environmental agents (e.g. gravity, river currents, oceanic circulation and dominant wind direction) (Bertness *et al.*, 1996; Wares *et al.*, 2001; Kawecki & Holt, 2002; Tackenberg *et al.*, 2003; Spichtig & Kawecki, 2004). Despite its obvious interest, and the paucity of more recent model development for the specific purpose of solving equilibrium conditions for balanced polymorphism at individual loci, Bulmer's model has remained almost unused, possibly due to the unavailability of a computable formulation. Moreover, very few studies have coupled empirical data with deterministic models to explain the maintenance of balance polymorphism in heterogeneous environments.

The acorn barnacle is an abundant intertidal species throughout the Northeast Atlantic coast of North America (Barnes & Barnes, 1976). It is a sessile, hermaphroditic, obligate cross-fertilized species with a dispersive planktonic larva that produce one cohort per year (Barnes & Crisp, 1956). In the Gulf of St Lawrence, mating occurs in October, and embryos incubate until larvae are released in April of the following spring. Planktonic larval development (six nauplii and one cypris) lasts 4–5 weeks before larval settlement occurs in early June (Bousfield, 1953). Because of logistical constraints, larval displacement has not been measured directly. However, by considering them as passive particles within a unidirectional current of water flowing 0.01 ms^{-1}, Drouin *et al.* (2002) estimated that larvae can potentially disperse up to 300 km.

Previous studies conducted over 1800 km of the Atlantic Coast have provided compelling evidence that two nonlinked genes that are part of parallel feeder pathways in glycolytic carbohydrate metabolism: *MPI** (Mannose Phosphate Isomerase; EC 5.3.1.8) and *GPI** (Glucose Phosphate Isomerase; EC 5.3.1.9) are under strong directional selection in this species. In adult barnacles, both *GPI** and *MPI** exhibit a strong break in allele frequencies over a distance of 50–100 km in the Gulf of St Lawrence (Canada) that has remained stable over at least 15 generations (Holm & Bourget, 1994). This break contrast with a total lack of regional population structuring or isolation by distance deduced from microsatellites (Dufresne *et al.*, 2002). More recently, Véliz *et al.* (2004) showed that both *MPI** and *GPI** were subjected to strong directional selection soon after settlement in early June south of the Miramichi estuary, whereas neutrality could not be ruled out at sampling sites located north of the estuary. However, whether or not the observed polymorphism at both loci represent quasi-stable equilibria that have been maintained by a balance between differential spatial selection and dispersal remains to be tested.

In this paper, we developed and applied a computable version of Bulmer's model in order to solve equilibria of balanced polymorphism and associated conditions of asymmetrical dispersal in the acorn barnacle *Semibalanus balanoides* from the Gulf of St Lawrence. More specifically, we addressed two specific questions: (i) Do allele frequencies observed at both *MPI** and *GPI** in this population represent stable equilibria, given empirical fitness values measured in the system? (ii) Are the values of asymmetrical dispersal required by the model to reach stable equilibria compatible with observed patterns of larval dispersal and oceanic circulation in the system?

Materials and methods

Empirical estimates of allele frequencies

Empirical estimates of allele frequencies were obtained from the analysis of 2523 individual barnacles in a previous study (Véliz *et al.*, 2004). Two cohorts of two different years (2000 and 2001) were sampled from the intertidal zone at different sites along the mid-western part of the Gulf of St Lawrence (Fig. 1a). Whole individual tissues were put in 5 μL homogenization buffer (pH = 8.5) (Tremblay *et al.*, 1998) before performing migration on cellulose acetate gels and staining according to Hebert & Beaton (1989). For both *MPI** and *GPI** loci, two alleles were scored resulting in three genotypes for both *GPI** (*GPI**100/100; *GPI**100/286; *GPI**286/286) and *MPI** (*MPI**85/85; *MPI**85/100; *MPI**100/100).

Estimates of fitness values

Since spatial homogeneity in genotype frequencies was observed at time of larval settlement (early June) among all sites for a given year ($P > 0.05$, Véliz *et al.*, 2004), a single initial frequency value was estimated by pooling all data for each locus (see Table 1). Single-locus fitness values (w) for each genotype within both regions defined as north and south of the Miramichi River were then calculated based on the ratio between two frequencies values of a genotype at two different time periods (Hartl & Clark, 1997; see details in Véliz *et al.*, 2004). For each cohort and sampling site, we considered the frequencies of each genotype during settlement (June 1) and 11 months later (May) that is just, prior to the release of larvae by surviving adults. Fitness averages and standard deviations were calculated using genotype frequency from different sampling sites in May as independent values (see details in Véliz *et al.*, 2004). Fitness values from genotypes of the same locus and cohort were compared using one-way ANOVAS with a Tukey *a posteriori* test performed using the GLM procedure of SAS software (SAS Inc., 1998). Bonferroni corrections for multiple independent tests

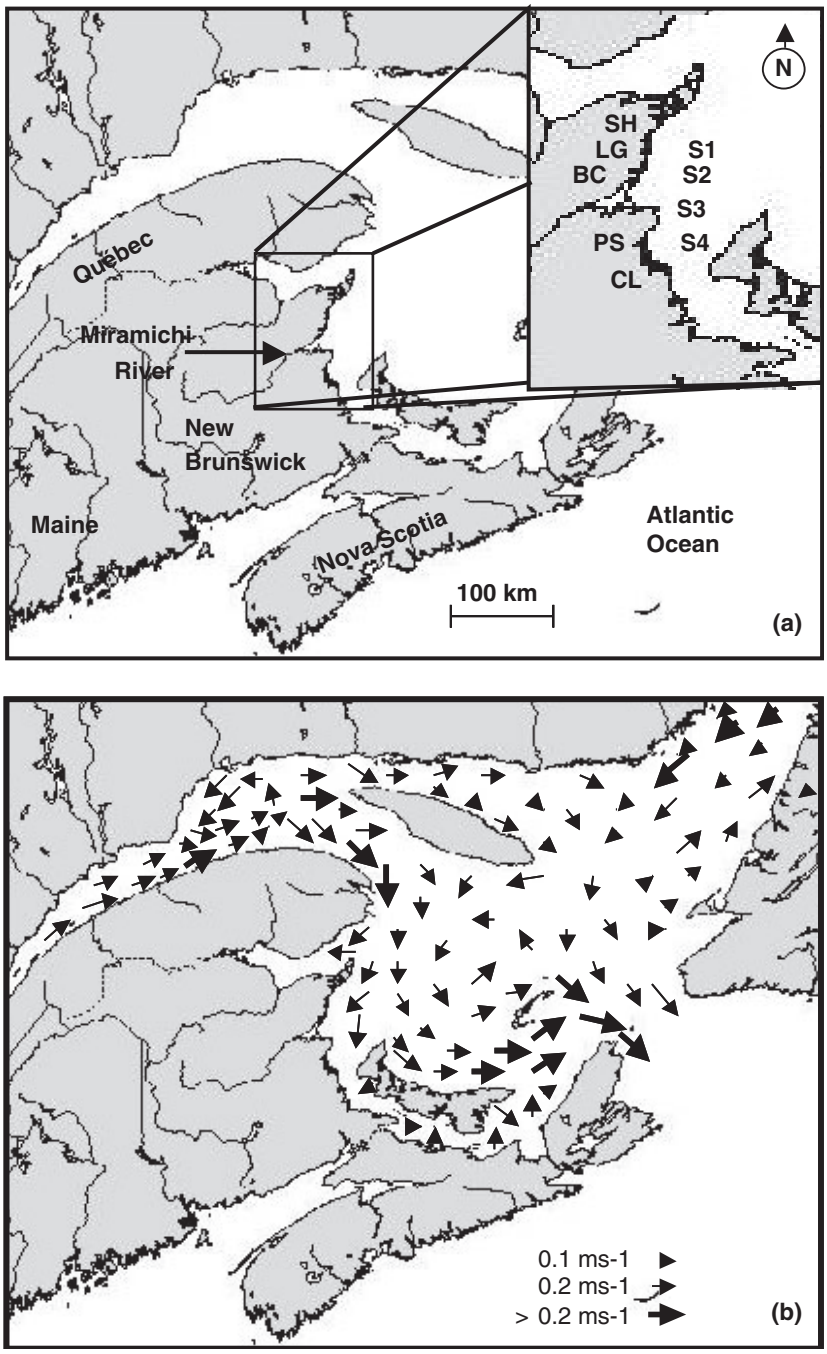

Fig. 1 (a) Location of sample sites of *S. balanoides* located north and south of the Miramichi R. estuary in the Gulf of St Lawrence. SH, Shippagan; LG, Le Goulet; BC, Burnt Church; PS, Pointe Sapin; CL, Cap Lumière. S1–S4 represent planktonic larval sampling station, accordingly to Drouin *et al.* (2002). (b) Near-surface currents (0–30 m zone) in the Gulf of St Lawrence during April and June 1997. Sizes of the arrows are proportional to velocity of horizontal currents. Modified with permission of the authors (Saucier *et al.*, 2003).

were applied in order to reduce type-I error (Rice, 1989).

Estimation of asymmetrical dispersal

Given these estimated fitness values, we then searched for proportions of dispersers (*K*-values) contributing to reproduction in the two regions such that the model would output a stable equilibrium with the same allelic frequencies as the observed ones (see Appendix 1, Fig. 2). We defined K_{N_S} as the proportion of dispersers coming from the south region and reproducing in the north region, and K_{S_N} as the proportion of dispersers coming from the north region and reproducing in the south region. K_{N_S} and K_{S_N} were taken as estimates of the real dispersal between the two regions (north and south regions of the Miramichi River).

Table 1 Observed genotype frequency at settlement (June) and following differential selection on genotypes (following May, same cohort) both north and south of the Miramichi River.

	Frequency at settlement (pA)			Frequency after settlement (PA)	
	North region	South region	(Both regions)	North region	South region
Cohort 2001					
*GPI**					
*GPI**100/100	0.540 ± 0.054	0.500 ± 0.024	0.530 ± 0.045	0.528 ± 0.064	0.340 ± 0.029
*GPI**100/286	0.390 ± 0.077	0.400 ± 0.012	0.390 ± 0.058	0.402 ± 0.057	0.498 ± 0.043
*GPI**286/286	0.070 ± 0.032	0.100 ± 0.018	0.080 ± 0.029	0.058 ± 0.037	0.168 ± 0.020
n	532	349	881	428	444
*MPI**					
*MPI**85/85	0.080 ± 0.024	0.100 ± 0.030	0.090 ± 0.029	0.086 ± 0.018	0.266 ± 0.071
*MPI**85/100	0.440 ± 0.024	0.460 ± 0.011	0.447 ± 0.021	0.462 ± 0.013	0.464 ± 0.053
*MPI**100/100	0.470 ± 0.024	0.440 ± 0.024	0.460 ± 0.029	0.448 ± 0.036	0.272 ± 0.042
n	531	346	877	428	444
Cohort 2000					
*GPI**					
*GPI**100/100	0.410 ± 0.031	0.420 ± 0.072	0.416 ± 0.0645	0.465 ± 0.049	0.232 ± 0.059
*GPI**100/286	0.370 ± 0.124	0.460 ± 0.034	0.455 ± 0.029	0.460 ± 0.071	0.543 ± 0.057
*GPI**286/286	0.220 ± 0.093	0.140 ± 0.076	0.146 ± 0.064	0.075 ± 0.021	0.225 ± 0.017
n	40	261	301	184	196
*MPI**					
*MPI**85/85	0.130 ± 0.085	0.080 ± 0.021	0.100 ± 0.048	0.105 ± 0.007	0.230 ± 0.057
*MPI**85/100	0.300 ± 0.225	0.540 ± 0.044	0.461 ± 0.163	0.445 ± 0.021	0.508 ± 0.038
*MPI**100/100	0.570 ± 0.311	0.370 ± 0.037	0.439 ± 0.175	0.455 ± 0.021	0.258 ± 0.057
n	40	261	301	166	196

n = sample size.
Since genotype frequencies were not statistically different between north and south regions at settlement, we calculate fitness with an average value of both regions (see details in Véliz *et al.*, 2004).

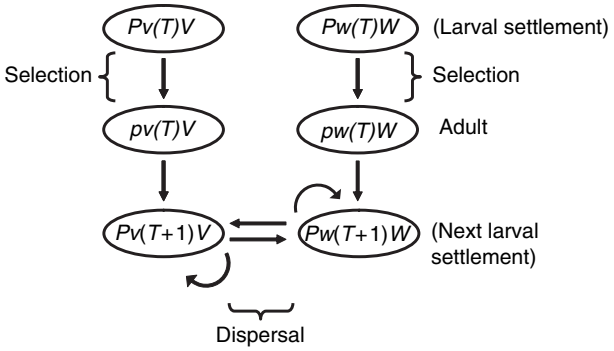

Fig. 2 Graphical representation of the analytical model. Settlement is produced [$P(T)$] in different environmental habitats or patches with different selection regimes (V and W). After selection and mating within a patch or environmental habitat, propagules can settle in the same patch or disperse to another patch [$P(T + 1)$]. This parameter is expressed in percent of dispersers into a given habitat (K-value).

Patterns of larval dispersal

Having found a set of dispersal parameter values K_{N_S} and K_{S_N} such that the model produced the observed stable equilibrium (see Results), we subsequently assessed whether the computed asymmetrical dispersal was compatible with patterns of larval dispersal and oceanic circulation empirically documented in the system. Evidence of asymmetrical dispersal was indirectly obtained by comparing densities at two larval stages, as well as recruits densities between both regions. Thus, a net temporal increase of the relative density in one region vs. the other was interpreted as evidence for a prevailing asymmetrical dispersal from the other region (Drouin *et al.*, 2002).

Density estimates for nauplii (first planktonic larvae) and cypris larvae (last larval stage) were obtained by re-analysing the data from Drouin *et al.* (2002). Briefly, weekly planktonic samples were obtained from four stations (two north and two of the south Miramichi River) at three distances from the coast (2, 6 and 10 km) between May and July 1998. For nauplii larvae, a Mixed-Model nested ANOVA was performed using the MIXED procedure of the SAS package (SAS Inc., 1998), with sites nested within regions (north and south of Miramichi River) as independent variables and Ln transformation (Sokal & Rohlf, 1996) of nauplii density (number per m^3) as the dependent variable. For this analysis only early nauplii density from the first sampling was used. For cypris larvae, a Mixed-Model

nested ANOVA was performed in the same way, except that the model considered date (4 weekly samples), sites (two sites per region) and regions (north and south) as the independent variables. Regions and dates were fixed factors and sites were a random factor nested within region. For both nauplii and cypris analyses, effects of random factors and their interactions were calculated contrasting −2 REML (restricted maximum likelihood) log likelihood values from the models with and without the random statement, according to Littell *et al*. (1996).

In order to estimate differences in density of recruits, between 10 and 40 quadrats (0.25×0.25 m) were randomly placed in each microhabitat (see detail of microhabitats in Véliz *et al*., 2004) during larval settlement (early June). The total number of recruits was counted and Ln transformed to normalize for analysis (Sokal & Rohlf, 1996). A Mixed-Model nested ANOVA was performed using the MIXED procedure of SAS software (SAS Inc., 1998), using juvenile density as dependent variable and regions, sites and microhabitats as independent variables. For the analysis, sites were considered a random factor and regions and microhabitats as fixed factors. Effects of random factors and their interactions where calculated as above. Finally, we also compared the congruence between solutions of asymmetrical dispersal obtained by the analytical model with the patterns of surface currents that prevail in the system when barnacle larvae are present in the water, which is between April and June (Trites, 1972; Drouin *et al*., 2002; Saucier *et al*., 2003).

Results

Spatial pattern in genotype frequencies

Estimates of initial allele frequencies at time of larval settlement and prior to selection were very similar between both years for both loci (Table 1). North of the Miramichi, the mean genotype frequencies at time of settlement (early June) and 11 months later (next May) for the same cohort were identical. In contrast, south of the Miramichi, a significant decrease of the 100/100 homozygote genotype (and significant increase of the alternate homozygote) was observed at both loci and in both years.

Fitness estimates from genotype frequencies

For the sampling sites located north of the Miramichi River, and in both cohorts 2000 and 2001, no significant difference in mean fitness estimates was observed between genotypes at both loci ($P > 0.05$), although the absolute value observed for *GPI**286/286 in 2000 was quite lower than 1.00 (Table 2). In contrast, fitness values observed south of the Miramichi River differed significantly among genotypes at both loci and in a consistent manner in both

Table 2 Fitness values and conditions for maintenance of polymorphism at both *GPI** and *MPI**.

	Fitness (*w*)		Observed equilibrium			
	North	South	Allele 100	K_{S_N}	K_{N_S}	Model equilibrium
Cohort 2001						
*GPI**						
*GPI**100/100	0.99 ± 0.13[a]	0.65 ± 0.06[a]	0.72 ± 0.02	0.98	0.05	Stable = 0.72
*GPI**100/286	1.06 ± 0.14[a]	1.26 ± 0.11[b]				Unstable = 0, 1
*GPI**286/286	0.75 ± 0.46[a]	2.10 ± 0.26[c]				
*MPI**						
*MPI**85/85	0.94 ± 0.07[a]	2.94 ± 0.54[a]	0.68 ± 0.02	0.99	0.001	Stable = 0.68
*MPI**85/100	1.03 ± 0.04[a]	1.03 ± 0.07[b]				Unstable = 0, 1
*MPI**100/100	0.99 ± 0.06[a]	0.59 ± 0.14[b]				
Cohort 2000						
*GPI**						
*GPI**100/100	1.12 ± 0.12[a]	0.56 ± 0.14[a]	0.65 ± 0.08	0.72	0.31	Stable = 0.65
*GPI**100/286	1.01 ± 0.16[a]	1.19 ± 0.12[b]				Unstable = 0, 1
*GPI**286/286	0.52 ± 0.15[a]	1.56 ± 0.12[c]				
*MPI**						
*MPI**85/85	1.02 ± 0.07[a]	2.31 ± 0.55[a]	0.67 ± 0.10	0.97	0.045	Stable = 0, 1
*MPI**85/100	0.96 ± 0.04[a]	1.10 ± 0.07[b]				Unstable = 0.67
*MPI**100/100	1.04 ± 0.06[a]	0.59 ± 0.15[b]				

Values sharing the same letter are not significantly different, one-way ANOVA and Tukey test *a posteriori* ($P > 0.05$).
K_{S_N} = percent of dispersers arriving at reproductive age in the southern region and originating from the northern region.
K_{N_S} = percent of dispersers arriving at reproductive age in the northern region originating from the southern region.
Model equilibrium represents stable or unstable conditions.

years (Table 2). Fitness estimates of the 100/100 homozygote were significantly lower than that of the alternate homozygote at both loci, whereas the fitness value of the heterozygote genotype was intermediate.

Equilibrium solutions

Using these empirical estimates of fitness values and equilibrium frequencies, numerical solutions for disperser proportions K_{N_S} and K_{S_N} were computed. For cohort 2001, the observed equilibrium allele frequencies (*GPI**100 = 0.72; *MPI**100 = 0.68) corresponded to stable equilibria for both *MPI** and *GPI** (Table 2; Fig. 3). For both loci, the model also produced very similar solutions of dispersal under the estimated fitness and equilibrium frequencies. This implicated a strong pattern of asymmetrical dispersal, whereby the proportion of disperser arriving at reproductive age in the southern region and originating from the northern region (K_{S_N}) was much larger (nearly 100-fold) than the reciprocal case (K_{N_S}).

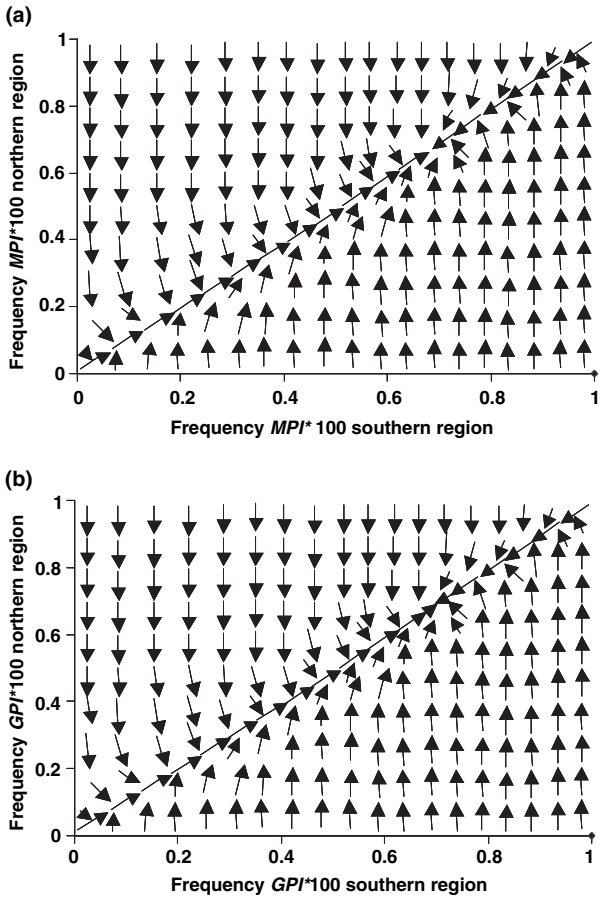

Fig. 3 Example of vector field that represent genotype equilibrium for (a) *GPI** cohort 2001 and (b) *MPI** cohort 2001. For both loci, a stable point was observed at similar allele frequency values at settlement.

Since larval dispersal may fluctuate in time, we then asked whether such fluctuations are likely to push the system away from a polymorphic stable equilibrium towards allelic fixation. If dispersal fluctuations are such that the current allelic frequencies are well within the neighbourhood of the also fluctuating stable polymorphic equilibrium, then the allelic frequencies will remain at intermediate levels. A thorough investigation of this question is well beyond the scope of this study. We therefore partially addressed the issue by searching for stable polymorphisms under many combinations (K_{S_N}, K_{N_S}) of the disperser proportions, based on the same fitness estimates as above (Fig. 4). Clearly, the observed equilibrium values stands well inside the polymorphic zone, thus indicating the resilience of balanced polymorphism against sizable dispersal fluctuations.

In contrast to cohort 2001, cohort 2000 lead to dispersal proportions that differed between loci (Table 2). For *GPI**,

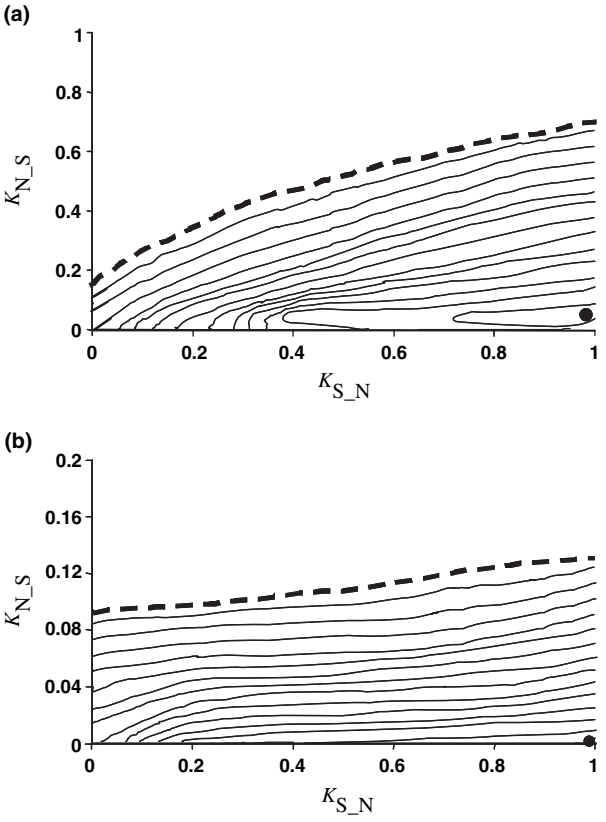

Fig. 4 Allele frequency iso-lines plotted against (K_{S_N}, K_{N_S}) pairs for (a) *GPI** and (b) *MPI**. Each iso-line stands for a narrow band (width = ±0.05) of stable equilibrium frequencies at settlement for the region north and south of the Miramichi. Each iso-line therefore corresponds to a specific difference from the observed allelic equilibrium (filled point). The filled points represent the (K_{S_N}, K_{N_S}) pairs associated with the observed equilibria. The dashed line sets apart the dispersal conditions wherein stable polymorphism is no more maintained, thus dividing the dispersal parameter space into two regions (balanced polymorphism vs. allele fixation).

results were consistent with 2001 since the dispersal solution computed via the model corresponded to a stable polymorphic equilibrium. The difference between K_{S_N} and K_{N_S} was also in the same direction as with cohort 2001, but was less pronounced. In contrast, the allelic frequency (0.67) observed at *MPI** could not be solved as a stable equilibrium, given fitness estimates for that year.

Larval dispersal and oceanic circulation

The analysis of barnacle densities revealed a contrasting regional pattern among life stages (nauplii, cyprids and settlers) that was largely congruent with the model solutions of asymmetrical dispersal from north to south. Soon following larval release, there was no significant differences in nauplii density neither between sites within region ($\chi^2_1 = 0.01$; $P = 0.46$) nor between regions (north vs. south Miramichi) ($F_{1,2} = 0.64$, $P = 0.06$) (Fig. 5a). At the older cypris stage, however, estimates of larval density were much greater at sites located south of the Miramichi River (between 8 and 16 cyprids per m^3) compared to those observed north of it (generally <1 cypris per m^3) (Fig. 5b). Indeed, when pooling data from all sampling-collecting sites in each region, larvae were two orders of magnitude greater in the south than in the north. Nevertheless, because of high variance in larval density observed at some sampling stations [e.g. station 3 (from 0 to 83 cyprids per m^3) and station 4 (0–60 cyprids per m^3)], statistical analyses did not reveal significant differences at fixed factors (region: $F_{1,2} = 4.74$, $P = 0.1613$; date: $F_{3,86} = 2.58$, $P = 0.056$; region × date: $F_{3,86} = 1.20$, $P = 0.31$), but showed a significant effect of site (region) random factor ($\chi^2_1 = 5.6$; $P < 0.01$). Overall, regional similarity in larval density at the early nauplii stage and a pronounced trend towards higher density at the older cypris stage south of the Miramichi indicated a strong pattern of asymmetrical dispersal from north to south, as inferred by the model.

This pattern was further supported by differences in the density of newly settled recruits north and south of the Miramichi River (Fig. 5c). The regional density values were nearly 60 times higher in the south than in the north (401.5 ind/0.125 m^2 vs. 6.5 ind/0.125 m^2). Here again, however, the effect of sites (site (region); $\chi^2_1 = 358.5$; $P < 0.001$) was statistically more important than the regional effect ($F_{1,4.03} = 7.56$, $P = 0.05$) because of large variance in recruit density within each site (microhabitat effect; Véliz, unpublished data).

The analytical model solutions also corroborated patterns of oceanic circulation that have been documented in the study area. Trites (1972), Drouin *et al.* (2002), and Saucier *et al.* (2003), all described similar oceanographic conditions for the April–June period, which are characterized by a cyclonic circulation of near surface current (0–30 m depth) around the Gulf of St Lawrence with a predominantly north–south pattern of net circulation in the vicinity of the Miramichi River (Fig. 1b).

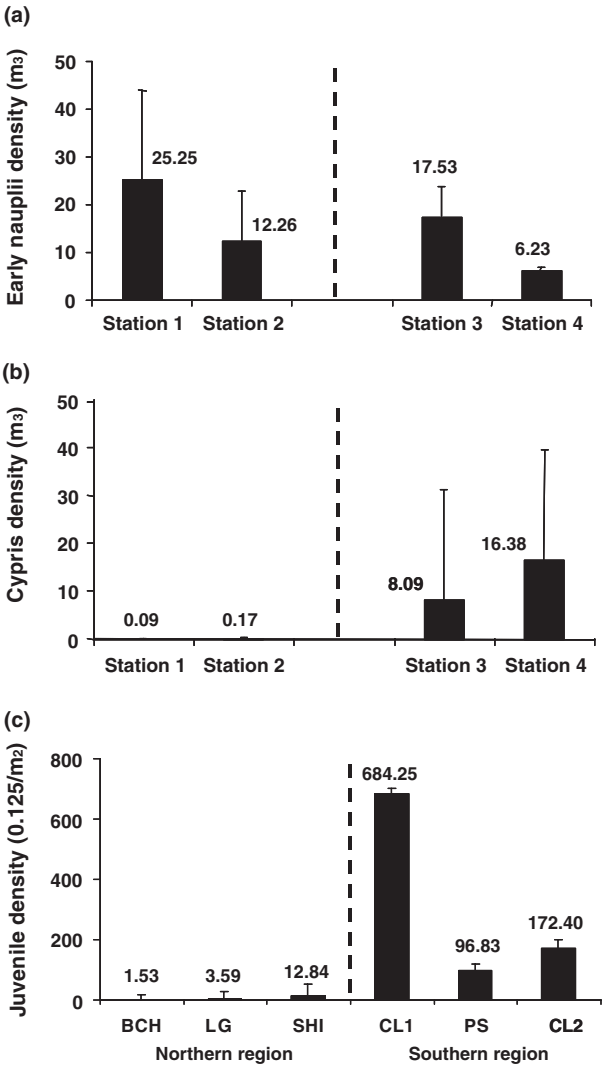

Fig. 5 (a) Early nauplii density (±SD) from different sites sampled 4 May 1998. Stations 1 and 2 are located north whereas stations 3 and 4 are located south of the Miramichi River, respectively. (b) Cypris density (±SD) from different stations and regions. (c) Juvenile barnacle densities (±SE) from different sites and regions. 0 = no settlement. Site: BCH, Burnt Church; LG, Le Goulet; SHI, Shippagan; CL1, Cap Lumière cohort 2000; PS, Pointe Sapin; CL2, Cap Lumière cohort 2001.

Discussion

The main objective of this study was to develop and apply a computable version of Bulmer's model in order to solve equilibria of balanced polymorphism and associated conditions of asymmetrical dispersal in the acorn barnacle *S. balanoides* from the Gulf of St Lawrence. Our results showed that allele frequencies at both *MPI** and *GPI** loci may represent stable equilibria, given empirical estimates of fitness values and strong asymmetrical dispersal from one region to the other. Moreover, the values of

asymmetrical dispersal required by the model to reach stable equilibria were similar for both loci and compatible with empirical estimates of larval dispersal and oceanic circulation documented in this system. Therefore, modelling genotype equilibrium and larval – juvenile distribution in both regions clearly revealed that a highly asymmetric dispersal from the northern to the southern region was the most likely explanation for the observed balanced polymorphism at both *MPI** and *GPI** loci. In fact, directional intra-niche selection regimes would otherwise lead to allelic fixation.

Sensitivity of the model to parameter estimations

In contrast to the 2001 cohort, the results obtained for the 2000 cohort differed from expectations, especially so for *MPI**. Thus, the model revealed that the allele frequencies observed at this locus prior to selection did not represent a stable polymorphic equilibrium, given allele frequencies and fitness estimates in that year. Furthermore, this differed greatly with *GPI** for which similar levels of asymmetrical dispersal for maintaining stable equilibrium at observed allele frequencies were resolved for both 2000 and 2001. The most likely explanation for the discrepancies between both years relates in the sensitivity of the model to the accuracy of genotype proportions from which fitness values were estimated. Namely, model predictions should be less reliable in 2000 than 2001 since sample sizes that were collected in 2000 were substantially lower and variance around estimates of genotypic proportion generally larger than those collected in 2001 (Table 1). Indeed, the sensitivity of the model is illustrated by the fact that a similar solution to that found for cohort 2001 can be resolved with slight changes in the observed fitness within estimated variance (see Table 2). For instance, after increasing fitness value of the heterozygote (*MPI**85/100; from 0.96 to 1.00) and decreasing one homozygote (*MPI**85/85; from 1.02 to 0.95) in northern region, the model predicted a stable equilibrium for *MPI* (frequency observed for *MPI**100 = 0.67), with $K_{S\_N} = 0.97$ and $K_{N\_S} = 0.056$. The observed fitness average for 2000 would then give values of K similar to those of cohort 2001, providing evidence that similar conditions maintained stable polymorphic equilibrium in both cohorts. These observations emphasize the need to obtain estimates of genotype frequencies (and fitness calculations) as precise as possible in order to obtain reliable assessments of equilibrium conditions.

Temporal variation in selective regime?

Alternatively, we cannot rule out the possibility that discrepancies observed at *MPI* between 2000 and 2001 reflected temporal variation in selective regime, leading to inter-annual differences in fitness values, and thereby different predicted solutions for dispersal conditions to maintain equilibrium. Empirical evidence for temporal variation in selective regimes caused by temporal variation in environmental conditions has been documented in invertebrates (e.g. Borash *et al.*, 1998; Mateus & Sene, 2003), fishes (e.g. Reimchen & Nosil, 2002; Aubin-Horth & Dodson, 2004) and plants (e.g. Weinig *et al.*, 2003). Temporal fluctuations in the direction and intensity of selection may also contribute to maintain stable polymorphism within populations (Kirkpatrick, 1996). For example, spatial and temporal variation (intra- and inter-annual) in selection on egg mass in the lizard *Uta stansburiana* act jointly in promoting the maintenance of variance (Svensson & Sinervo, 2004). In this case, temporal variation in directional selection was found for hatching date in both first- and late-clutch hatchlings, where selection for quadratic selection egg mass (stabilizing) and hatching date (disruptive) in the late-clutch hatchlings appeared to explain the maintenance of lizard hatching date variability.

In the Gulf of St Lawrence, inter-annual changes in water circulation speed could potentially produce intra-annual differences in patterns of larval dispersal. For instance, Saucier *et al.* (2003) showed that the inter-annual variability is greater than 0.1 ms^{-1}, and during barnacle planktonic larval life (April–June) the average velocity of ocean currents may vary among years, from 0.1 ms^{-1} (1997; Saucier *et al.*, 2003) to 0.01 ms^{-1} (1998; Drouin *et al.*, 2002) in the studied area. Unfortunately, current speeds were not available for 2000 and 2001. If these conditions promote changes in larval dispersal between years, conditions for maintaining polymorphism would change accordingly, possibly providing an inter-annual mechanism that could permit polymorphism maintenance over time. In this context, it is particularly relevant to understand how changes in dispersal may influence balanced polymorphism. Figure 4 shows that dispersal conditions for maintaining polymorphism seems to be resilient. Thus, the maintenance of polymorphic equilibria seems possible over a wide range of asymmetrical dispersal conditions. In contrast, allele fixation (stable monomorphic equilibria) occurs over a much more restricted range of dispersal conditions, and may be unlikely to occur in the area we studied, given the long-term trends of the north-to-south currents that prevail in the region (Fig. 1b). Under such conditions, polymorphism may be maintained even if dispersal fluctuates substantially among years, as suggested by the stable allele frequencies observed in this system over 15 generations.

Comparison with other studies

Few studies have coupled empirical data with deterministic models to explain the maintenance of balanced polymorphism in heterogeneous environments. In one of the most rigorous empirical tests of Levine's model (1953), Schmidt *et al.* (2000) could not resolve a stable

equilibrium for allele frequency at *MPI** in their study of *S. balanoides* from Maine (US) despite habitat-specific variation in selection at this locus. In a second study, Schmidt & Rand (2001), again using Levene's model, predicted both stable and unstable frequency equilibria, but the observed frequency coincided only with the unstable equilibrium. The limitations of finding a solution for observed genotypes in these studies could be due to the biological simplifications of Levene's model (e.g. Maynard Smith & Hoekstra, 1980; Spichtig & Kawecki, 2004). Alternatively, failure to solve equilibria could be due to different spatial scale of selection in Maine relative to our study site. Thus, Schmidt & Rand (1999) clearly showed that selection was acting on *MPI* at the microhabitat scale, which was clearly not the case in the Gulf of St Lawrence (Véliz *et al*., 2004). In such a situation, the relative importance of each microhabitat (habitat size, number of individuals, reproductive output, etc.) may render the task of explaining variability over a large geographic scale extremely difficult. In fact, using the data of Schmidt & Rand (2000) from Maine, we were unable to find solutions for stable equilibria using Bulmer's model (data not shown).

Clearly, further research aiming at elucidating the causes of these discrepancies will be required for a general understanding of the ecological mechanisms that may realistically explain the maintenance of balanced polymorphism at larger geographical scales in the acorn barnacle.

Conclusions

Overall, this study illustrated the usefulness of a modified and computable version of Bulmer's model (1972) in order to test hypotheses of balanced polymorphism resulting from interactions between spatial selection and asymmetrical dispersal. By formulating Bulmer's mathematical development that outlined the conditions to maintain equilibrium in a stationary system within an algebraic computing environment (Maple v7.0 2001), we were able to determine the dynamics of a system based on empirical evidence. Thus, accurate numerical solving of the model equations could produce equilibrium points for any set of fitness and dispersal parameter values. Once empirical values of its parameters are specified, the model can be turned into a very informative global graphical representation of the dynamics of the corresponding system. This model could also be modified to accommodate more complex situations characterized by multiple habitats (rather than only two) under spatially distinct selective pressures and asymmetrical dispersal rates.

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Appendix 1: general outline of the model

Given the absence of isolation by distance as well as the evidence of regional selection acting between two habitats in barnacles from the Gulf of St Lawrence, we developed an analytical model that considers a system with two habitats (patches) between which dispersal of propagules can be asymmetrical (Fig. 2), and that produces numerical and graphical solutions of the resulting dynamics. Genotypes of a given locus are under differential selective pressure between the two habitats, say V and W. Reproduction takes place in each habitat, but each is contributing breeders to the other in the next generation through dispersal of propagules (e.g. pelagic larvae). Because adult barnacle densities measured at different sites are generally large (up to 1200 adult individual per m^2; Véliz, unpublished data) and variance around estimates of effective population sizes based on microsatellites and using the temporal method (Waples, 1989) includes infinite values, we considered that the population size was sufficiently large for genetic drift to be negligible relative to selection. The state of the system at generation T is described as $P_V(T)$, $P_W(T)$, i.e. the frequency of allele a within V and W, respectively. Note that each habitat may present a distinct allele frequency at T as expressed by $P_V(T)$ and $P_W(T)$. While moving from generation T to generation $T + 1$, the system first undergoes a selection phase followed by a dispersal phase resulting in allelic states $P_V(T + 1)$, $P_W(T + 1)$. We express the state modification at time $T + 1$ in terms of the current state T after a selection phase, after a dispersal phase and a sequence of the two phases. This leads to a system of differential equations and then to the resolution of any conditions of spatial selection and dispersal between the two habitats, as represented by specific fitness and dispersal parameter values.

Selection phase

The intensity of selection in both habitats is reflected by differential fitness values for different genotypes. Symbols *Vaa*, *Vab* and *Vbb* represent fitness in habitat *V* of homozygote *aa*, heterozygote *ab* and homozygote *bb*, respectively. Similarly, *Waa*, *Wab* and *Wbb* represent fitness in *W*. For the sake of simplicity we define the average fitness in *V*, *W* at time *T*:

$$V(T) = P_V(T)^2 Vaa + 2P_V(T)(1 - P_V(T))Vab + (1 - P_V(T))^2 Vbb \quad (1)$$

$$W(T) = P_W(T)^2 Waa + 2P_W(T)(1 - P_W(T))Wab + (1 - P_W(T))^2 Wbb \quad (2)$$

Following standard calculations (Hartl & Clark, 1997) and expressing the post-selection states as $p_V(T)$, $p_W(T)$ we obtain the relationships:

$$p_V(T) = \frac{P_V(T)(P_V(T)Vaa + (1 - P_V(T))Vab)}{V(T)} \quad (3)$$

$$p_W(T) = \frac{P_W(T)(P_W(T)Waa + (1 - P_W(T))Wab)}{W(T)} \quad (4)$$

Dispersal phase

The proportion of habitat *V* genitors from *V* is denoted *Kv* whereas the *V* genitors originating from *W* are in proportion $(1 - Kv)$. Similarly, *Kw* and $(1 - Kw)$ represent the proportions of locals and immigrants among genitors of *W*. Hence, the state at generation $T + 1$ expressed in terms of post-selection frequencies at *T* is:

$$P_V(T + 1) = K_V p_V(T) + (1 - K_V)p_W(T) \quad (5)$$

$$P_W(T + 1) = K_W p_W(T) + (1 - K_W)p_V(T) \quad (6)$$

To obtain allele frequencies at generation $T + 1$, $P_V(T + 1)$, $P_W(T + 1)$, in terms of previous frequencies $P_V(T)$, $P_W(T)$, the expressions of post-selection frequencies (eqns 3 and 4) are substituted into the post-migration eqns 5 and 6. After these substitutions and also substituting $V(T)$, $W(T)$ for expressions in eqns 1 and 2, a system of recurrence equations is obtained:

$$P_V(T+1) = \frac{K_V(P_V(T)(P_V(T)Vaa+(1-P_V(T))Vab))}{P_V(T)^2 Vaa+2P_V(T)(1-P_V(T))Vab+(1-P_V(T))^2 Vbb} + \frac{(1-K_V)(P_W(T)(P_W(T)Waa+(1-P_W(T))Wab))}{P_W(T)^2 Waa+2P_W(T)(1-P_W(T))Wab+(1-P_W(T))^2 Wbb} \quad (7)$$

$$P_W(T+1) = \frac{K_W(P_W(T)(P_W(T)Waa+(1-P_W(T))Wab))}{P_W(T)^2 Waa+2P_W(T)(1-P_W(T))Wab+(1-P_W(T))^2 Wbb} + \frac{(1-K_W)(P_V(T)(P_V(T)Vaa+(1-P_V(T))Vab))}{P_V(T)^2 Vaa+2P_V(T)(1-P_V(T))Vab+(1-P_V(T))^2 Vbb} \quad (8)$$

Once given specific fitness and dispersal parameter values, eqns 7 and 8 express the frequencies of state $T + 1$ solely in terms of the frequencies of the previous state *T*. Thus, a complete description of the dynamics of the system is obtained.

Numerical solutions of equilibrium states

The system is considered to be in equilibrium when its state at time *T* equals its state at time $T + 1$, that is:

$$P_V(T + 1) = P_V(T)$$

$$P_W(T + 1) = P_W(T)$$

Equilibrium states can be either stable or unstable, whereby a stable equilibrium has a neighbourhood of states such that once the system has entered that neighbourhood; it is attracted to the corresponding stable state. As for unstable equilibrium states, the system will tend to move away from such states. Allelic fixation is considered a trivial equilibrium state and in stable nontrivial equilibrium states, the allele frequency is maintained at an intermediate level.

Graphical representations

The above system of recurrence equations was translated into the following system of differential equations:

$$\frac{\partial P_V}{\partial T} = P_V(T + 1) - P_V(T)$$

$$\frac{\partial P_W}{\partial T} = P_W(T + 1) - P_W(T)$$

A plotting command of the algebraic calculator Maple v7.0 (2001) accepts the above system together with a domain specification to output a vector field representation of its dynamics. Such a representation shows all equilibrium states as well as the attraction neighbourhood of each stable equilibrium state. The source code of the model in Maple format is available at http://bio.ula-val.ca/louisbernatchez/download.htm.