Field evidence for an association between growth and protein polymorphism in the acorn barnacle *Semibalanus balanoides*

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ABSTRACT: Organisms living in highly heterogeneous environments are useful for examining the effects of associations between phenotypes and ecologically relevant genes in the field. Here, we tested the null hypothesis of no association between the variation in 2 fitness-related traits (growth and fecundity) and polymorphism at 2 enzymatic loci, MPI^* and GPI^*, known to be subject to strong spatial selection. We also tested if such an association was related to the intensity of genotype selection observed for both allozymes at sampling locations in 2 barnacle cohorts. For both cohorts, individuals with the $GPI^*286/286$ genotype were larger in size than individuals with the $GPI^*100/100$ genotype, particularly in sites and microhabitats south of the Miramichi Estuary, Gulf of St. Lawrence, Canada. This coincided with a reduced $GPI^*100/100$ frequency in this region. In contrast, the growth–MPI^* genotype association showed no clear pattern. Overall, our results indicate that the phenotype–genotype association differed strikingly between the 2 genes, and that both the growth–GPI^* genotype association and genotype selection occur in localities south of the Miramichi Estuary only, and at different times during development, first a few days after settlement (genotype selection) and later through growth–genotype association.

KEY WORDS: Natural selection · Phenotype–genotype association · Allozyme · MPI^* · GPI^* · Marine invertebrates

INTRODUCTION

Adaptation of organisms to heterogeneous environments depends on genetic diversity and its potential to minimize negative effects on fitness. Thus, population persistence in heterogeneous environments is possible by maintaining stable variability of these genes and later by maximising phenotypic characteristics that confer more efficiency in each habitat (Feder & Watt 1992). Empirical studies on ecologically relevant genes corroborate this idea. For instance, enzymes utilising substrates from the external environment have been reported to be more polymorphic than those utilising internal metabolites (Gillespie & Kojima 1968, Kojima et al. 1970). Previous studies also demonstrated that allozyme genotypes which perform differently confer

different survivorship, behaviour and mating in some organisms (Watt et al. 1983, 1985, Dimichele & Powers 1991, Powers et al. 1991, Watt 1992, 1994). This is because enzymes control the rate of energy flow through the metabolic pathways, thereby controlling the rate of growth and development as well as influencing the proportion of energy allocated to various functions (Heinrich 1977, Mitton 1997). Thus, the link between genetic variability and phenotypic traits is relevant to the study of the adaptation of organisms to heterogeneous environments.

In marine invertebrates, most studies have either focussed on the effects of enzyme performance (metabolic rate) as a phenotypic trait (e.g. Koehn et al. 1983) or the heterozygosity-fitness correlation (global effects) and phenotypic traits such as fecundity and individual

growth (Diehl & Koehn 1985, Hansson & Westerberg 2002). However, few studies have tested for an association between the genotypic composition of specific candidate genes to fitness-related traits (e.g. Schmidt 2001). This is particularly true for studies conducted in the field (but see Planes & Romans 2004).

Recent studies on the acorn barnacle *Semibalanus balanoides* revealed that this species could be a model for studying phenotypic and genotypic adaptation in the natural environment. This species is a hermaphroditic and obligate cross-fertilised species (Barnes & Crisp 1956) with a planktonic larval development (Bassindale 1936). Sessile adult individuals inhabit the intertidal, a highly heterogeneous environment (fluctuating salinity, extreme temperatures, desiccation, wave action, solar radiation, etc.) (Raffaelli & Hawkins 1996). Several fitness-related traits, including survival, gametogenesis, fertilisation, and incubation time are affected by environmental variation encountered by the acorn barnacle (Bousfield 1953, 1955, Southward 1958, Barnes 1959, Barnes & Barnes 1965, 1976, Bergeron & Bourget 1984, 1986, Bertness & Gaines 1993, Brind'Amour et al. 2002). Moreover, previous studies supported the hypothesis that 2 allozymes (mannose phosphate isomerase [MPI; EC 5.1.3.8] and glucose phosphate isomerase [GPI; EC 5.1.3.9]) undergo pronounced directional selection along the Atlantic coast of North America (Holm & Bourget 1994, Rand et al. 2002, Véliz et al. 2004, 2006). Specifically, in the Gulf of St. Lawrence, New Brunswick, Canada, alleles from these 2 allozymes experience changes in frequency associated with environmental constraints in heterogeneous environments on regional scales (Holm & Bourget 1994, Dufresne et al. 2002). Thus, both *MPI** and *GPI** are possibly subject to strong directional selection south of the Miramichi Estuary, Gulf of St. Lawrence, whereas neutrality cannot be ruled out at sampling sites and microhabitats located north of the estuary (Véliz et al. 2004).

In this context, our main goal was to test the null hypothesis of no association between the variation in 2 fitness-related traits (growth and fecundity) (Ramirez 2002) and polymorphism of the *MPI** and *GPI** genotypes. We then tested if such an association, if present, was related to the genotype selection observed for both allozymes at sampling locations.

MATERIALS AND METHODS

Sampling sites. Samples were taken from intertidal sites on the Gulf of St. Lawrence, as described in Véliz et al. (2004). Two cohorts of barnacles (2000 and 2001) were sampled during 5 periods: early June (during larval settlement, labelled June 1), mid-June (2 wk after settlement, labelled June 2), August, October, and April–May (after ice scouring). For the Cohort 2000, 1 northern (Burnt Church) and 1 southern (Cap Lumière) site relative to the mouth of the Miramichi Estuary, were sampled whereas 2 northern (Le Goulet and Shippagan) and 2 southern (Pointe Sapin and Cap Lumière) sites were sampled for the Cohort 2001. Four categories of microhabitats were defined at each site according to the criteria shore level (high [H] and low [L] intertidal) and exposure to sun (exposed [E] and sheltered [S]). The statistical analysis of Véliz et al. (2004) has shown that microhabitats have different thermal and desiccation stress levels, with HE > LE > HS > LS.

Considering that all individuals have a similar size at settlement and that settlement occurs within 3 or 4 d (E. Bourget unpubl. data), one can assume that all individuals start the benthic stage at approximately the same date. Therefore, individual size was a valid proxy for an estimate of growth. In order to test for growth and genotype association, a total of 7214 barnacles were collected and placed in a liquid nitrogen container until storage at –80°C. The number of barnacles sampled for each microhabitat varied between 14 and 207 (mean = 80.93) (Table 1). The maximal length (rostro-carinal length) of each individual was measured to the nearest 0.01 mm using a stereomicroscope with an ocular micrometer. Because barnacles are easily broken during sampling, several individuals were collected incomplete. For this reason and in order to avoid possible error in length measurements, we used only complete individuals. This explains why sample size differs from that given in Véliz et al. (2004). Cap Lumière (October 2000) has a sample size larger than in Véliz et al. (2004), since additional analyses were carried out.

We used the number of larva from each larval mass as a measurement of fecundity. For this purpose, larval masses from 40 barnacles were taken at each microhabitat and site in May (before larval release) and stored in liquid nitrogen. Individuals were dissected and all larvae were isolated from the mantle cavity and counted. To avoid analysis error due to female mass larvae loss during sampling, the analysis was performed with data from barnacles with an apparently complete larvae mass only.

Allozyme electrophoresis. The genetic data used in this study to test for an association between genotypes and fitness-related traits were generated in Véliz et al. (2004). Briefly, for all samples, tissue from each individual was homogenised with a 5 ml homogenisation buffer (pH = 8.5) (Tremblay et al. 1998), and migration was performed on a cellulose acetate gel and stained according to Hebert & Beaton (1989). For both loci (*MPI** and *GPI**), 2 rare alleles with a low frequency

Table 1. *Semibalanus balanoides.* Number of barnacles analysed (*GPI**/*MPI**) for size–genotype association in different cohorts, on different dates and at different sites and microhabitats. HS: high sheltered; HE: high exposed; LS: low sheltered; LE: low exposed; –: no settlers; D: died

Site	Date	Microhabitats			
		HS	HE	LS	LE
Cohort 2000					
Burnt	Jun 1	14/14	–	26/26	–
Church	Jun 2	21/21	–	68/68	–
	Aug	32/32	–	38/38	–
	Oct	72/72	–	94/94	–
	May	88/90	–	94/94	–
Cap	Jun 1	69/69	58/58	67/67	67/67
Lumière	Jun 2	62/62	72/75	80/80	80/80
	Aug	81/81	40/40	83/83	83/83
	Oct	126/120	79/79	207/177	136/136
	May	41/41	36/36	75/75	42/42
Cohort 2001					
Shippagan	Jun 1	87/87	89/89	86/86	89/89
	Jun 2	87/87	D	89/89	89/89
	Aug	90/89	D	87/87	88/87
	Oct	89/89	D	88/88	90/78
	May	88/88	D	90/90	73/73
Le Goulet	Jun 1	84/83	–	83/83	–
	Jun 2	85/84	–	85/85	–
	Aug	75/70	–	89/88	–
	Oct	88/88	–	86/86	–
	May	88/88	–	89/89	–
Pointe	Jun 1	88/88	89/88	88/87	81/82
Sapin	Jun 2	83/84	D	80/79	88/86
	Aug	78/74	D	88/89	86/88
	Oct	90/90	D	88/88	D
	May	87/87	D	89/89	D
Cap	Jun 1	78/78	87/87	87/88	88/88
Lumière	Jun 2	67/67	69/67	84/79	84/84
	Aug	88/86	86/87	86/86	88/88
	Oct	88/90	90/90	90/90	90/90
	May	89/89	D	90/90	89/89

(<1%) were not considered in the analysis. Thus, 3 genotypes of *GPI** (*GPI**100/100, *GPI**100/286 and *GPI**286/286) and *MPI** (*MPI**85/85, *MPI**85/100 and *MPI**100/100) were used for all analyses.

Data analysis. We used factorial ANOVA to test the effect of Genotype, Microhabitat, Date and Site on barnacle growth. All factors were considered fixed, and a significance threshold of 0.05 was used. Data were log-transformed, according to the Box-Cox method to normalise variance, using the SAS statistical package (SAS Institute 1998). Normality and homoscedasticity was observed by graphical examination of residuals (Montgomery 2001). To analyze Cohort 2000, we performed a factorial ANOVA with Date (June 1, June 2, August, October and May), Site (Burnt Church and Cap Lumière), Microhabitat (HE, HS, LE and LS), and Genotype (2 homozygotes and 1 heterozygote) as factors. For Cohort 2001, a factorial nested ANOVA

was performed with Date (n = 5), Geographic region (n = 2), Microhabitat (n = 4), Genotype (n = 3) and Site (n = 4) nested within Geographic region (north: Le Goulet and Shippagan; south: Pointe Sapin and Cap Lumière). *MPI** and *GPI** genotypes were analysed separately. All data were analysed with the General Linear Model (GLM) procedures incorporated in the SAS statistical package, and when significant differences among treatments were detected, multiple comparisons were made. As the number of data varied among treatments, we applied the LS-means method (SAS Institute 1998) for the multiple comparisons. Results from the same ANOVAs were added to complement explanations of significant phenotype–genotype interactions. For example, when Site × Genotype interactions were significant, barnacle sizes from each site (pooling each genotype data from 1 locus) were also compared.

ANCOVA performed using the GLM procedure of SAS was used to test differences in fecundity at different scales (micro-habitat, sites and regions) and their association with genotypes. Due to incomplete egg masses obtained from several samples, separate analyses for each cohort were performed with n = 114 samples for Cohort 2000 and n = 260 for Cohort 2001. Maximal barnacle length (mm) was used as a covariate of fecundity. In Cohort 2000, factors included in the analysis were Sites (Burnt Church and Cap Lumière), Microhabitat (HE, HS, LE, LS) and Genotype (3 for each gene). For Cohort 2001, factors were Region (north and south), Site nested within Region (n = 4), Microhabitat (n = 4) and Genotype (n = 3).

RESULTS

Barnacle growth and *GPI** genotypes

There was a significant association between variation in barnacle growth and *GPI** genotypes for both cohorts. For Cohort 2000, the Date × Site × *GPI** genotype interaction was significant (Table 2), which was mainly due to 2 significant interactions showing that barnacles with a *GPI**286/286 genotype were significantly larger than those with the *GPI**100/100 genotype in Cap Lumière (southern region), both in October (p = 0.0158) and May (p = 0.0318) (Fig. 1a,b). For both comparisons, heterozygotes showed intermediate values between both homozygotes, but were not significantly different from either homozygote (p > 0.05). Comparisons among sites (all individuals pooled for each site) also revealed that barnacle growth at Cap Lumière (southern region) was overall lower than that observed at Burnt Church (northern region) (p < 0.001; Fig. 1c,d). Thus, genotype–growth association for *GPI**

Table 2. *Semibalanus balanoides.* Summary of factorial ANOVAs of size for Date, Microhabitat, Site and Region for both cohorts (2000 and 2001) and both Genotypes (*GPI** and *MPI**). Main effects and interactions not including genotype are not shown. Among the results not shown, the main factor Genotype was the only one that did not show significance (p > 0.05). Micro: microhabitat; Geno: genotype. *p < 0.05

| Source of variation	*GPI**				*MPI**			
	df	MS	*F*	p	df	MS	*F*	p
Cohort 2000								
Date × Site × Micro	4	0.1477	17.65	<0.0001*	4	0.1222	14.51	<0.0001*
Date × Geno	8	0.0123	1.47	0.1642	8	0.0069	0.82	0.5853
Site × Geno	2	0.0144	1.72	0.1788	2	0.0133	1.58	0.2064
Date × Site × Geno	8	0.0192	2.30	0.0188*	8	0.0084	1.00	0.4329
Micro × Geno	6	0.0066	0.78	0.5827	6	0.0014	0.17	0.9845
Date × Micro × Geno	24	0.0850	1.02	0.4348	24	0.0059	0.70	0.8548
Site × Micro × Geno	2	0.0091	1.09	0.3353	2	0.0078	0.93	0.3961
Date × Site × Micro × Geno	8	0.0129	1.55	0.1475	8	0.0138	1.63	0.1104
Error	2045	0.0084			2007	0.0084		
Corrected total	2133				2096			
Cohort 2001								
Date × Site(Region) × Micro	10	0.1303	14.20	<0.0001*	10	0.1527	16.91	<0.0001*
Date × Geno	8	0.0056	0.61	0.7743	8	0.0064	0.71	0.6815
Region × Geno	2	0.0051	0.55	0.5754	2	0.0230	2.55	0.0783
Date × Region × Geno	8	0.0148	1.61	0.1152	8	0.0092	1.02	0.4154
Site(Region) × Geno	4	0.0125	1.36	0.2455	4	0.0061	0.67	0.6108
Date × Site(Region) × Geno	16	0.0082	0.90	0.5731	16	0.0095	1.05	0.3953
Micro × Geno	6	0.0071	0.77	0.5927	6	0.0242	2.68	0.0133*
Date × Micro × Geno	22	0.0109	1.19	0.2469	22	0.0140	1.55	0.0495*
Region × Micro × Geno	6	0.0093	1.02	0.4118	6	0.0160	1.78	0.0997
Date × Region × Micro × Geno	16	0.0155	1.69	0.0407*	16	0.0069	0.77	0.7229
Site(region) × Micro × Geno	8	0.0087	0.95	0.4730	8	0.0163	1.81	0.0710
Date × Site(Region) × Micro × Geno	20	0.0164	1.78	0.0170*	20	0.0121	1.34	0.1432
Error	4896	0.0092			4863	0.0090		
Corrected total	5072				5039			
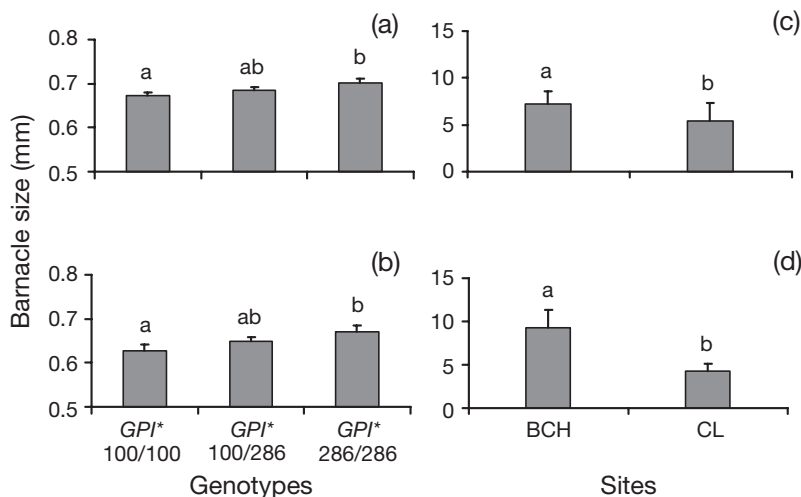
Fig. 1. *Semibalanus balanoides.* Barnacle size (log length) determined by LS-mean analysis. (a–c) Relationships between barnacle size and *GPI** genotype in Cohort 2000 for sites that contributed to the significant Date × Site × *GPI** genotype interaction. (a,b) Cap Lumière in (a) October, (b) May. (c,d) Mean barnacle size at different sites (all samples pooled) for Cohort 2000 in (c) October, (d) May. BCH: Burnt Church; CL: Cap Lumière. Bars sharing the same letter are not significantly different (p > 0.05). Error bars are +SE

was mainly observed in the southern region, where growth was most constrained.

For Cohort 2001, we observed a significant interaction for Date × Site (nested with region) × Microhabitat × *GPI** genotype (Table 2), which was due to 4 significant differences in growth associated with *GPI** genotypes, all observed in the southern region. As for Cohort 2000, individuals with the *GPI* *286/286 genotype were larger than those with the *GPI* *100/100 genotype (Fig. 2a–d; p < 0.05); heterozygotes (*GPI* *100/286) were intermediate in all cases, but were not significantly different from homozygotes (p > 0.05), except for the microhabitat HE on June 1 (p = 0.0210) (Fig. 2a). As was also the case for Cohort 2000, comparisons among sites (all individuals pooled for each site) revealed that the genotype–growth
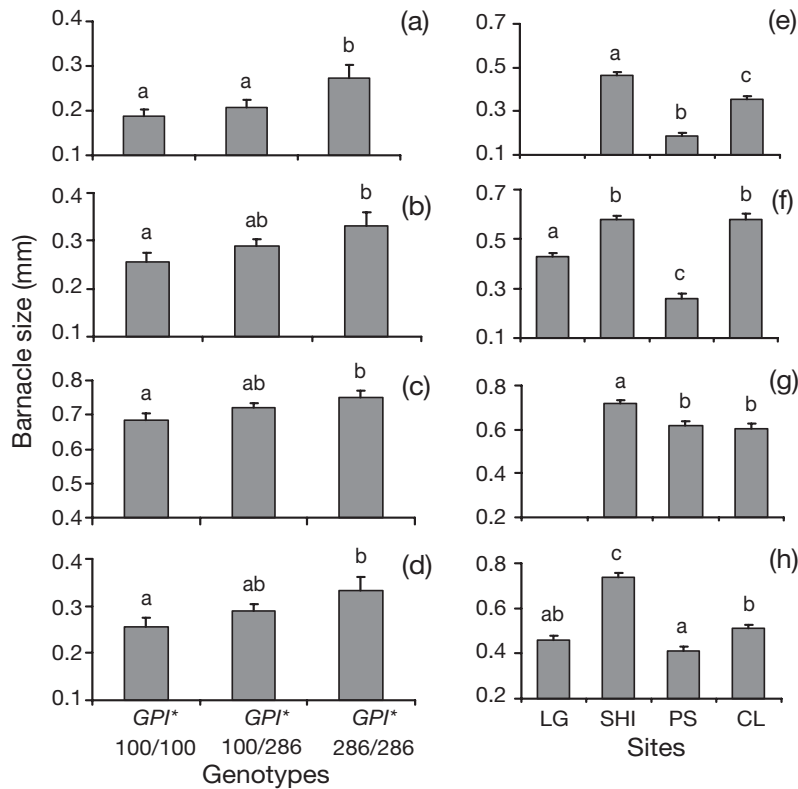
Fig. 2. *Semibalanus balanoides*. Barnacle size (log length) determined by LS-mean analysis. (a–d) Relationships between barnacle size and *GPI** genotype in Cohort 2001 for microhabitats that contributed to the significant Date × Site (Region) × Microhabitat × *GPI** genotype interaction. (a) High exposed (HE) microhabitat (June 1) at Pointe Sapin. (b) Low sheltered (LS) microhabitat (June 2) at Pointe Sapin. (c) Low exposed (LE) microhabitat (August) at Cap Lumière. (d) High sheltered (HS) microhabitat (August) at Pointe Sapin. (e–h) Mean barnacle size at different microhabitats (pooled samples from the different sites) for Cohort 2001: (e) HE microhabitat on June 1; (f) LS on June 2; (g) LE on August; (h) HS on August. LG: Le Goulet; SH: Shippagan; PS: Pointe Sapin; CL: Cap Lumière. Bars sharing the same letter are not significantly different (p > 0.05). Error bars are +SE

association for *GPI** was mainly observed at sites and times where growth was most constrained (Fig. 2e–h; p < 0.05).

Barnacle growth and *MPI** genotypes

For Cohort 2000, no significant individual or interaction effects on barnacle growth associated with the *MPI** genotypes were observed. However, for Cohort 2001, the analysis revealed a significant growth–*MPI** genotype association as seen by the Date × Microhabitat × *MPI** genotypes interaction (Table 2). For 3 out of 4 comparisons *MPI**100/100 homozygotes exhibited higher growth than *MPI**85/85 homozygotes. These significant interactions were observed in the LS microhabitat on June 1 (p = 0.0112), the LE microhabitat on June 2 (p = 0.0066) and HS in August (p = 0.0376) (Fig. 3a,b,d). How-

ever, the LS microhabitat on June 2 showed an opposite pattern, where individuals with the *MPI**85/85 genotype were significantly larger in size than individuals with the *MPI**100/100 genotype (p = 0.0062) (Fig. 3c). Heterozygotes did not show any clear tendency since intermediate growth was observed only in LE on June 2 (Fig. 3b, p = 0.1534 for *MPI**85/85 and p = 0.0668 for *MPI**100/100).

Comparisons among sites (pooled data) showed no clear pattern of association with the significant genotype–growth relationships (Fig. 3e–g); that is, there was no tendency for the genotype–growth association at sites where growth was differentially constrained. Overall then, a weaker pattern of association between genotype and growth emerged from the analysis of *MPI** in comparison to *GPI**.

Fecundity

The Size covariant revealed that barnacle size correlated positively with barnacle fecundity (p < 0.0001; Table 3). In contrast to what was observed for growth, no significant associations between genotypes and fecundity were observed for the 2 cohorts (2000 and 2001) and loci (*GPI** and *MPI**). Significant differences were only associated with site (Table 3). In Cohort 2000, barnacles from Burnt Church (northern region) contained more larvae (nauplii I) per individual than those from Cap Lumière (southern region) (mean 635.0 ± 656.3 and 114 ± 336.0 larvae ind.$^{-1}$, respectively; p = 0.0015). In Cohort 2001, barnacles from Le Goulet (northern region) exhibited higher fecundity than those from all other sites (mean: 1138.5 ± 679.7 larvae ind.$^{-1}$; Shippagan, p = 0.0071, Cap Lumière, p = 0.0114 and Pointe Sapin, p < 0.0001). In the same analysis, individuals from Shippagan (northern region) and Cap Lumière (southern region) showed similar fecundity (1044.7 ± 716.6 and 767.8 ± 623.9 larvae ind.$^{-1}$; respectively, p = 0.6486) with those from Pointe Sapin (southern region) exhibiting lower fecundity than those from other sites (287.2 ± 679.7 larvae ind.$^{-1}$; p < 0.0001 at Shippagan and Cap Lumière). Overall, for both cohorts, barnacles from the southern region tended to exhibit low fecundity compared to those from the northern region. However, fecundity was not associated with *MPI** or *GPI** genotypes.
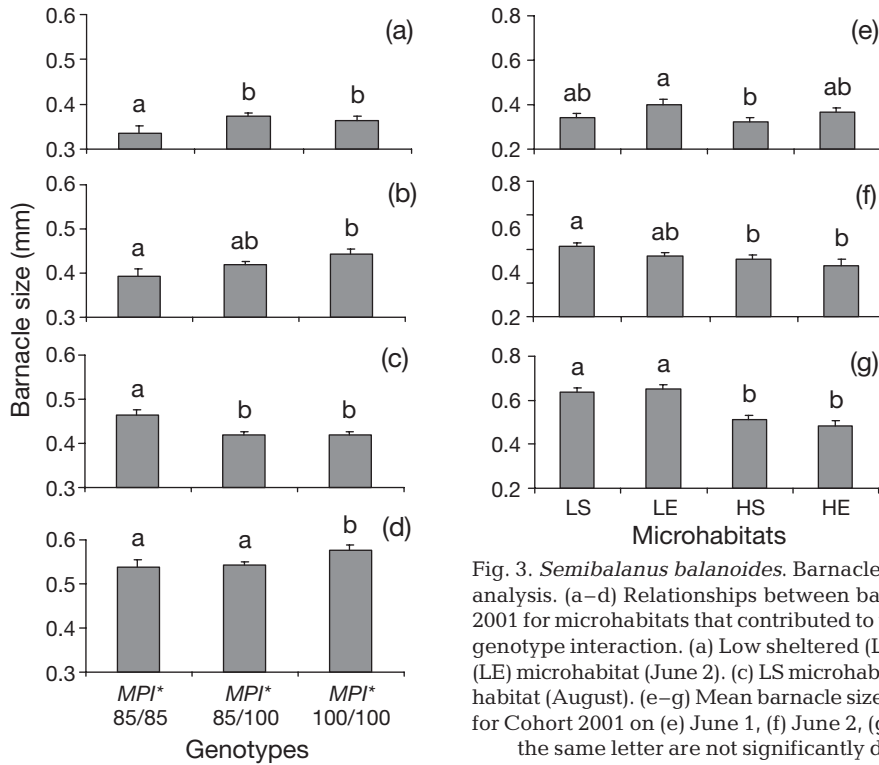
Fig. 3. *Semibalanus balanoides*. Barnacle size (log length) determined by LS-mean analysis. (a–d) Relationships between barnacle size and *MPI** genotype in Cohort 2001 for microhabitats that contributed to the significant Date × Microhabitat × *MPI** genotype interaction. (a) Low sheltered (LS) microhabitat (June 1). (b) Low exposed (LE) microhabitat (June 2). (c) LS microhabitat (June 2). (d) High sheltered (HS) microhabitat (August). (e–g) Mean barnacle size at different microhabitats (all sites pooled) for Cohort 2001 on (e) June 1, (f) June 2, (g) August. HE: high exposed. Bars sharing the same letter are not significantly different (p > 0.05). Error bars are +SE

Table 3. *Semibalanus balanoides*. Summary of ANCOVA analysis for fecundity variation at different scales (Microhabitat, Site and Region) and Genotypes (*GPI** and *MPI**) for both cohorts (2000 and 2001). Barnacle Size (mm) was used as a covariant. Micro: microhabitat; Geno: genotype. *p < 0.05

Source of variation	GPI*				MPI*			
	df	MS	F	p	df	MS	F	p
Cohort 2000								
Size	1	46.7270	86.14	<0.0001*	1	50.0680	95.36	<0.0001*
Site	1	3.0821	5.68	0.0191*	1	5.5852	10.64	0.0015*
Micro	3	0.5948	1.10	0.3545	3	1.3152	2.50	0.0637
Geno	2	0.0638	0.12	0.8891	2	0.6519	1.24	0.2935
Site × Micro	1	1.5342	2.83	0.0959	1	1.0779	2.05	0.1552
Site × Geno	2	0.4233	0.78	0.4612	2	0.9762	1.86	0.1514
Micro × Geno	6	0.1778	0.33	0.9208	6	0.2221	0.42	0.8621
Site × Micro × Geno	2	0.0396	0.07	0.9297	2	0.0208	0.04	0.9612
Error	96	0.5425			96	0.5251		
Corrected total	114				114			
Cohort 2001								
Size	1	87.1290	285.42	<0.0001*	1	86.7210	273.92	<0.0001*
Region	1	6.8829	22.50	<0.0001*	1	3.9159	12.37	0.0005*
Site(Region)	2	7.5694	24.80	<0.0001*	2	7.0611	22.30	<0.0001*
Micro	2	0.2293	0.75	0.4730	2	0.0668	0.21	0.8100
Geno	2	0.2556	0.84	0.4342	2	0.0172	0.05	0.9470
Region × Micro	2	0.2649	0.87	0.4213	2	0.3294	1.04	0.3550
Site(Region) × Micro	2	0.0228	0.07	0.9281	2	0.1243	0.39	0.6757
Region × Geno	2	0.5803	1.90	0.1518	2	0.1762	0.56	0.5739
Site(Region) × Geno	4	0.2326	0.76	0.5510	4	0.2174	0.69	0.6018
Micro × Geno	4	0.1130	0.37	0.8299	4	0.1621	0.51	0.7270
Region × Micro × Geno	4	0.3792	1.24	0.2938	4	0.3201	1.01	0.4024
Site(Region) × Micro × Geno	4	0.5460	1.79	0.1319	4	0.2551	0.81	0.5227
Error	230	0.3053			230	0.3166		
Corrected total	260				260			

DISCUSSION

The main goal of this study was to test the null hypothesis of no association between the variation in 2 fitness-related traits (growth and fecundity) and polymorphism for both the *MPI** and *GPI** genotypes. We then tested whether or not such an association, if present, was related to the genotype selection observed for both allozymes at our sampling locations. Our results rejected the null hypothesis for *GPI**, which provided evidence that this enzyme may influence growth under natural conditions. However, this association was expressed only in specific environments where growth was apparently constrained relative to other habitats. In contrast, no clear pattern of an association between growth and genotype emerged for *MPI**, nor for a relationship between fecundity and genotypes for either locus.

Growth–*GPI** genotype association

Several authors have previously suggested that specific *GPI** genotypes have an adaptive nature (Watt et al. 1985, Hawkins et al. 1989, Patarnello & Battaglia 1992), based on the metabolic role of the GPI enzyme. GPI occupies an important regulatory region between glycogen storage and glycolysis, and for some organisms minor differences in habitat are sufficient for the selection of specific alleles with different kinetic properties (Hawkins 1996). Our study provides several lines of evidence for the association between the intensity of genotype selection and growth–*GPI** in the acorn barnacle. (1) Both the growth–genotype association and genotype selection apparently occur in the southern region only (Véliz et al. 2004, present study). Thus, when individuals with the *GPI**100/100 genotype were smaller than individuals with the *GPI**100/286 or *GPI**286/286 genotype, this genotype also occurred less frequently in this region (see Véliz et al. 2004). This phenomenon was confirmed by data from 2 independent cohorts and occurred in the southern region only. (2) Both genotype selection and a growth–*GPI** genotype association confirmed the same type of selection acting on genotypes characterized by additive effects. That is to say, heterozygote *GPI**100/286 individuals showed intermediate growth, ranging between that of *GPI**100/100 and *GPI**286/286. Similarly, fitness estimates in terms of differential survival at time of settlement were significantly smaller for *GPI**100/100 than for *GPI**286/286, with heterozygotes having an intermediate value (Véliz et al. 2004, 2006). (3) When a growth–*GPI** genotype association was detected, barnacles were generally smaller than

barnacles from other sites at the same date, suggesting that selection is more intense when conditions for growth are more limiting.

In *Semibalanus balanoides*, selection of *GPI** genotypes thus seems to act on 2 different levels in the southern region. (1) Selection reduces the number of individuals with the genotype, resulting in lower performance in a given habitat ('lethal phase'). Thus, within a few days after settlement in the southern region, the frequency of individuals with the *GPI**100/100 genotype decreased drastically from 0.53 to 0.34 (Cohort 2001) (Véliz et al. 2004). Evidence from *ADH** in *Drosophila* (Freriksen et al. 1994) and *LAP** in *Mytilus edulis* (Koehn et al. 1980) also corroborate the idea that lethal selection on genotypes acts early in the development of the organism. For example, *ADH** alleles may be subject to selective and/or neutral processes of varying strengths during different fruit fly life stages, processes which have strong effects on the larval but not the adult stages (Heinstra et al. 1987, Freriksen et al. 1994). (2) Selection may further reduce the performance of a genotype which already has a low performance ('sublethal effect'). In the southern region and following reduction in the frequency of the *GPI**100/100 genotype, individuals with this genotype were smaller than individuals with *GPI**100/286 or *GPI**286/286. Susceptibility to stress (e.g. desiccation) may decrease in adult organisms relative to younger life stages, because there is a decrease in the surface area to volume ratio (Foster 1971). Thus, it is plausible that selection changes from lethal to sub-lethal, because growing individuals show more resistance to stress than early juveniles.

This scenario assumes that the selective factors acting in the southern region persist throughout barnacle life, reducing genotype performance (e.g. growth) when lethal selection occurs a few days after settlement. As we recently proposed (Véliz et al. 2004), differences in the food concentration (microalgae) between the northern and southern region might cause differences in the nutritional condition of premetamorphic larvae due to different genotype performance. Thus, differences in the microalgae concentration could be manifested at all benthic stages, maintaining differences in individual growth due to different genotypes as low microalgae concentrations persist during summer in the southern region (Steven 1974). However, data on the microalgae concentration for the years 2000 and 2001, which would have allowed us to more firmly establish a direct effect of food concentration on genotype–phenotype association, were not available.

Because microalgae concentration displays intra- and inter-annual variation in the Gulf of St. Lawrence

(de Lafontaine et al. 1991), this variation could also explain differences in the growth–genotype associations observed between cohorts. Briefly, the associations observed in October and May at the site level in Cohort 2000 and between June and August at the microhabitat level in Cohort 2001 could be due to such intra- and inter-annual variation. 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). For example, spatial and temporal variation (intra- and inter-annual) in directional selection was found for hatching date in both first- and late-clutch hatchlings in the lizard *Uta stansburiana* (Svensson & Sinervo 2004).

Growth–*MPI** genotype association

Evidence is accumulating for several marine invertebrates, including *Semibalanus balanoides*, that *MPI** genotype frequency changes among habitats (Hedgecock 1986, McDonald 1990, Schmidt & Rand 1999, Véliz et al. 2004, 2006), which suggests that an interaction with phenotypes is generally more prevalent than reported for *GPI**. Yet, this was not observed in our study. Whereas strong genotype selection was observed for both cohorts (Véliz et al. 2004) in the southern region of the Gulf of St. Lawrence, a growth–*MPI** genotype association was observed only for Cohort 2001 on the microhabitat scale with an inverse relationship in 1 case (LS microhabitat; June 1 vs. June 2, Fig. 3a,c). In contrast, a laboratory study conducted with barnacles from Maine (USA) showed a clear association between growth and *MPI** genotypes (Schmidt 2001). Thus, in treatments under high stress conditions (high temperature and the presence of mannose), barnacles with the *MPI**100/100 genotype grew faster than *MPI**85/85 homozygotes. However, it has also been reported that this pattern was reversed in more southern populations (Rhode Island, USA), resulting in changes in genotype frequencies due to selection similar to what we observed in the Gulf of St. Lawrence but on a larger geographic scale (Rand et al. 2002). Thus, all evidence (Maine, Rhode Island and Gulf of St. Lawrence) suggests that *MPI** genotype selection and its association with growth may be more difficult to disentangle than *GPI** genotype selection or that it is possibly less associated with the growth of individuals. Clearly, further analyses will be necessary to elucidate the real pattern of selection acting on *MPI** in barnacles and its possible association with variations in fitness-related traits.

Fecundity

Association between fecundity and genotype was expected to be high, since fecundity is associated with the heterozygosity of allozymes in several organisms, including *Mytilus* (Rodhouse et al. 1986), *Drosophila* (Serradilla & Ayala 1983) and *Pinus* 'ponderosa pine' (Linhart & Mitton 1985). Moreover, the correlation between size and fecundity in barnacles and the association between growth and *GPI** genotype association in the present study further reinforced this expectation. Whereas no phenotype–genotype association was detected for fecundity, we cannot rule out the possibility that our study lacked the statistical power to produce evidence for such an association. Thus, while our analysis confirms an association between fecundity and size, the difficulty of obtaining a complete larvae mass from many individuals sampled directly from the intertidal may have limited our ability to detect a fecundity–genotype association.

CONCLUSIONS

This study provided evidence that genotypic variation in enzymatic loci may influence the growth of barnacles under natural conditions. Since mortality rates generally decrease with increasing body size (Peterson & Wroblewski 1984, Sogard 1997, Schmidt 2001), individuals that grow more rapidly should spend less time in the more vulnerable size classes. Larger individuals should produce more progeny, making a more important genetic contribution (in terms of genotype frequency) to the next generation compared to smaller individuals. Our study has also demonstrated the importance of temporal and spatial sampling to determine the scale at which natural selection acts. It shows that selection may be a dynamic process that works in different ways, first by affecting genotype frequencies and later by changing fitness, at least in barnacle growth. Overall, this study exemplifies the usefulness of testing for associations between genotypic variation and fitness-related traits in order to elucidate the importance of balanced polymorphism for the persistence of species striving to exist in heterogeneous environments.

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