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Correlations between fitness and heterozygosity at allozyme and microsatellite loci in the Atlantic salmon, *Salmo salar* L.

YJ Borrell[1,3], H Pineda[1], I McCarthy[2], E Vázquez[1], JA Sánchez[1] and GB Lizana[1]
[1]*Departamento de Biología Funcional, Área de Genética, Universidad de Oviedo, Julián Clavería s/n 33071 Oviedo, Asturias, Spain;* [2]*School of Ocean Sciences, University of Wales – Bangor, Menai Bridge Anglesey, Gwynedd LL59 5EY, UK;* [3]*Departamento de Bioquímica, Universidad de la Habana, Calle 25 entre J e I. No. 455, Vedado, Ciudad de la Habana, Cuba*

The relationship between heterozygosity at genetic markers (six allozyme and eight microsatellite loci), and fluctuating asymmetry (FA), length and weight was investigated in two samples of Atlantic salmon (*Salmo salar* L.) with different timings of first active feeding (early (EA) and late (LA) salmon). This trait had previously been related to fitness. EA fish show smaller values of FA, are longer, heavier and are more heterozygous at allozyme loci than are conspecific LA fish. Also within both samples, heterozygosity at allozyme loci was inversely related to FA and was positively related to weight and length. However, no significant differences in microsatellite diversity (heterozygosity and mean d^2 measurements) were observed between samples (EA *vs* LA). Furthermore, no association was observed between the variability at microsatellite loci and FA, weight or length within each sample. These results suggest that allozyme loci, in themselves, influence fitness components, rather than associations arising from associative overdominance.
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Introduction

Two interesting debates have concerned population biologists in the last decade. Firstly, they have studied the genetics basis of the positive correlations observed between multilocus heterozygosity at allozyme loci and fitness-related traits such as growth rate, viability, feeding rate or fecundity reported in several organisms (reviewed by Zouros and Pogson, 1994; Mitton, 1998). Secondly, there has been intense debate about what kinds experimental evidence might confirm a role for fluctuating asymmetry (FA) as an indicator of the ability of an individual to resist environmental challenges during growth and development, and therefore be a way of assessing fitness (Møller, 1997; Møller and Swaddle, 1997; Clarke, 1995, 1998; Houle, 1998; Møller, 1999; Palmer, 2000).

It had been suggested that enzyme loci, through the control of metabolic reactions, have a direct influence on fitness components and that associations exist between fitness and allozyme heterozygosity because enzyme heterozygosity enhances physiological efficiency, by decreasing the energetic cost of metabolism. If so, then individuals with multiple allozyme heterozygosity will have a higher proportion of energy available to prevent them from being affected during development by stress and environmental variations. As a result, they will exhibit greater developmental stability, and will also be able to use this available energy on growth, reproduction or other activities (Mitton and Koehn, 1985; Koehn *et al*, 1988; Blanco *et al*, 1990, 1998; Pogson, 1991; Mitton, 1993; Zouros and Pogson, 1994; Mitton, 1998; Myrand *et al*, 2002). Nevertheless, other hypotheses have been proposed to explain these associations. One is the associative overdominance hypothesis, which assumes that allozymes are neutral markers and that the associations are caused because allozyme loci are in linkage disequilibria with other, deleterious loci. Inbreeding may be a cause of such associations, and, although it is expected that some disequilibria may remain for a limited number of generations in a population, disequilibria are also continuously being generated. Pooling individuals into heterozygote classes (irrespective of loci or alleles) will thus be expected to produce a positive association with fitness (Zouros and Pogson, 1994; Mitton, 1998). Under this hypothesis, a correlation should not be restricted to any particular type of genetic marker whether they be (allozyme or other DNA markers). All markers are likely to have the same potential to assess inbreeding levels or to be linked to deleterious recessive genes.

A number of studies comparing the effects of heterozygosity at allozyme and DNA markers on fitness-related traits have been developed to test the associative overdominance hypotheses, but these have produced inconsistent results. Sometimes associations between fitness and allozyme heterozygosity, but not DNA heterozygosity, were found, and these results have been put forward as evidence that the allozymes have a role by themselves, as causes of these correlations (Zouros and Pogson, 1994; Coltman *et al*, 1998; Coulson *et al*, 1998, 1999; Thelen and Allendorf, 2001). However, this

Correspondence: GB Lizana, Departamento de Biología Funcional, Area de Genética, Universidad de Oviedo, Julián Clavería s/n 33071 Oviedo, Asturias, Spain. E-mail: gbl@correo.uniovi.es
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phenomena is not generally observed (Coltman *et al*, 1999; Rowe *et al*, 1999; Tsitrone *et al*, 2001; Slate and Pemberton, 2002).

However, several studies have been published showing that morphological variability and FA decrease as heterozygosity at enzyme loci increases (Eanes, 1978; Mitton, 1978, 1993; Knowles and Mitton, 1980; Leary *et al*, 1984; Mitton and Grant, 1984; Blanco and Sánchez 1986; Zouros and Foltz, 1987; Blanco *et al*, 1988, 1990; Vázquez *et al*, 1988). Although there are a number of examples of studies in which this kind of associations has not been found (Handford, 1980; McAndrew *et al*, 1982; Houle, 1989), in general, the data seem to support Lerner's hypothesis, according to which individuals or populations with higher heterozygosity levels possess a higher homeostasis or developmental stability, shown by a lower degree of bilateral FA and a lower phenotypic variance in biometric traits (Lerner, 1954). However, whether FA is indeed correlated with fitness is still the focus of intense debate (Møller, 1997, 1999; Clarke, 1995, 1998; Palmer, 2000). Houle (1998) had argued that, in addition to the low r^2 found in most of regression analyses between asymmetry and fitness, the lack of positive correlations between degrees of asymmetry of different traits, the erroneous inclusion of traits exhibiting directional asymmetry or antisymmetry, and finally the incorrect analyses of FA data in some meta-analyses have led to false conclusions.

In Atlantic salmon (*Salmo salar* L.), significant correlations have been demonstrated between allozyme heterozygosity and length, weight, growth rates and FA (Blanco *et al*, 1990, 1998), but no data exist about relationships between these traits and variation at DNA markers. In this study, we test whether the heterozygosities at six allozyme and eight microsatellite loci show the same relationship with the level of FA in three meristic characteristics, and with length and weight, in two samples of Atlantic salmon reared in a common hatchery environment but with different timings of first active feeding (early and late salmon), a trait that is related to different life-history strategies in salmon (McCarthy *et al*, 2003). Furthermore, since, in some studies (Coltman *et al*, 1998; Coulson *et al*, 1998, 1999), it has been found that some fitness components increase with mean d^2 (the squared difference in allele size – number of repeats – in a heterozygote), but not with individual microsatellite heterozygosity, we also tested the relationship between mean d^2 and the other traits.

Materials and methods

Samples

A 400 family cross of eggs (20 males × 20 females) were obtained from Atlantic salmon adult returning to spawn in the River Shin (Scotland). Approximately 1000 eggs were randomly sampled, transported to the Department of Zoology at Aberdeen University and reared in egg incubators. The fish were examined daily in order to check the rate of yolk sac absorption and for the removal of any dead individuals. On 25 May 1994, the fish were divided by eye into three groups, early ($n = 243$), intermediate ($n = 543$) and late ($n = 160$) first feeding fish according to the amount of yolk remaining, using the classification of Metcalfe and Thorpe (1992). On 25th May 1994, 150 early first feeding fish (EA), which had almost exhausted their yolk reserves, were randomly selected, placed in a 1 m diameter circular tank supplied with aerated freshwater and offered food (Hatch to first feed = 290 degree days). The late first feeding fish (LA) were placed in a separate tank and reared until they were assessed as ready to be offered food on 4 June 1994 (Hatch to first feed = 395 degree days) when 150 late first feeding fish were randomly selected, anaesthetised (MS 222, 0.1 g l^{-1}), their anal fins carefully removed, and added to the tank containing the early first feeding fish. No mortalities occurred as a result of the tagging process. The fish were reared together in freshwater until 19 April 1995 (320 days), when all fish were killed and measures of length (cm) and weight (g) were registered.

Early (EA) and Late (LA) fish were considered to be different samples following the criteria of the demonstrated biological differences between groups in order of weight, length, the subsequent life history strategies (proportions of different physiological classes inside groups) (McCarthy *et al*, 2003).

Meristic traits

Counts of the following three bilateral meristic traits were taken on the left and right side of each fish: gill rakers on the first branchial arch, and rays in the pectoral and in the pelvic fins. Measurements of the samples were conducted on three different days by the same observer. Nonconcordant data were eliminated (five cases). For each character, we calculated

- The average FA (A) estimated as the mean absolute value of |number on right side−number of left side|
- The magnitude of FA (D) reflecting the minimum and maximum values of deviation from bilateral symmetry.
- The proportion of asymmetrical characters per individual, as the number of asymmetric characters divide by the total number of characters analysed (PACh).
- Global means of absolute FA per individual (GMA), computed as the sum of the FA values of the three characters, corrected for scaling effect by the use of data normalization (GMA = (A_1/population mean + A_2/population mean + A_3/population mean)/3) (Blanco *et al*, 1990).

Genetic analyses

Tissue samples of liver and muscle were analysed by horizontal starch gel electrophoresis. Six polymorphic loci were scored: *MDH-3,4**, *mMEP-2**, *IDHP-3**, *sAAT-4**, *IDDH-1** and *IDDH-2** using the procedures outlined in Sánchez *et al* (1991). Individual allozyme heterozygosity was defined as the number of heterozygous loci from the total number of loci for which an individual was scored.

Genomic DNA was purified from skeletal muscle tissue using Chelex procedures (Estoup *et al*, 1996). Eight microsatellite loci were analysed by specific PCR: SsaF43, SsaD30, Ssa20.19, Ssa13.37 (Sánchez *et al*, 1996), SSOSL311, SSOSL417 (Slettan *et al*, 1995) and Str543, Str15 (Presa and Guyomard, 1996). Aliquots of amplification products were resolved on 6% denaturing polyacrylamide-sequencing gels. The products were visualized by silver staining (Promega Silver Sequence™ DNA Staining). The following genetic variables were defined

for microsatellite loci: (1) Individual microsatellite heterozygosity, defined as the number of heterozygous loci from the total number of loci for which an individual was scored. (2) Following Coulson *et al* (1999), two d^2 values were calculated: (a) mean d^2: as the average of squared differences in the number of unit repeats of heterozygotes for the eight loci under study, and (b) outbreeding d^2 (d^2out): as a mean d^2 score, excluding homozygous loci, which is expected to correlate with the degree of outbreeding (Coulson *et al*, 1999).

Statistical analyses

The one-sample *t*-test was used to detect directional asymmetry in any of the three meristic characters evaluated within samples. Regression analyses were used to assess the relationships among FA reflected by each of the meristic characters and to assess the relationships between FA and other traits such as length and weight.

To compare groups in order of heterozygosity, d^2 values, FA, length or weight; ANOVA and generalized linear model (GLM) analyses were used. To assess relationships between genetic parameters (allozyme and microsatellits heterozygosity, and d^2 values) and FA, length or weight, regression analyses were also used.

All these tests are available in the SPSS10.0 statistical software programme. In all cases when multiple comparisons were made, Bonferroni adjustment was applied (Rice, 1989).

Results

In each sample, the departure of the mean difference ($R-L$) for each character was tested against the expected value of zero, and, in all cases, mean values were not different from zero ($P>0.01$), indicating that no directional asymmetry was present for the traits used in this study.

Differences in mean values of FA between samples (early (EA) *vs* late (LA)) were found in this study (Table 1). The LA group showed higher values of FA than did the EA group for all the characters under study, as well as for the global magnitude of fluctuating asymmetry (GMA), but the only significant difference was found in the mean proportion of asymmetric character per individual (PACh) ($F=3.97$; $P=0.047$) (Table 1). In addition, EA fish were significantly larger ($F=39.94$; $P<0.001$) and heavier ($F=34.18$; $P<0.001$) than the LA fish (Table 1). There was also found to be a negative correlation among GMA and length and weight. Within samples, fish with smaller GMA values were longer and heavier than those with bigger values of GMA (Figure 1).

Average heterozygosities for allozyme and microsatellite loci, and d^2 values for microsatellite loci in each group are shown in Table 2. At allozyme loci, the EA group had a significantly higher average of heterozygosity ($He=0.189$) than did the LA group ($He=0.145$) ($F=6.10$; $P=0.018$) (Table 2), but no differences were found for any particular locus (data not shown). For microsatellite loci, no significant differences between groups were found in the comparisons of mean heterozygosity, mean d^2 and outbreeding d^2 values (Table 2). Allozyme heterozygosity was not related to microsatellite heterozygosity within the EA ($r^2=0.001$; $F=0.07$; $P=0.791$) or LA groups ($r^2=0.000$; $F=0.03$; $P=0.864$). However, a positive correlation between heterozygosity at microsatellite loci and mean d^2 values was found ($r^2=0.311$; $F=60.15$; $P<0.001$ in the EA group, and $r^2=0.570$; $F=144.55$; $P<0.001$ in the LA group), but not between microsatellite heterozygosity and outbreeding d^2 ($r^2=0.020$; $F=0.30$; $P=0.582$ in the EA, and $r^2=0.006$; $F=0.63$; $P=0.430$ in LA group).

The averages of FA (A), the magnitude of FA (D), GMA and PACh for each heterozygous group at allozyme loci, are shown in Table 3. For the LA group, significant differences were found between groups for all traits, and for gill rakers in the EA group (Table 3). The allozyme homozygous groups (Hom in Table 3) showed highest values of A for the three characters under study, reflecting a pattern in which the level of FA decreases inversely with the number of heterozygous enzyme loci. The level of GMA ($r^2=0.106$; $F=35.74$; $P=0.001$ in the EA, and $r^2=0.242$; $F=34.82$; $P<0.001$ in LA group) also were inversely related with the number of heterozygous allozymes (Table 3).

To investigate the effect of each individual locus, the GMAs for homozygous and heterozygous individuals were calculated for all loci at which at least five or more heterozygous individuals were available (data not shown). In all cases (11), heterozygotes had smaller values of GMA than did homozygotes ones, but significant differences were observed only between homozygotes and heterozygotes at *mMEP-2** (EA group: $F=2.765$; $P=0.006$ and LA group: $F=3.831$; $P<0.001$) and at the *sAAT-4** locus (EA group: $F=2.610$; $P=0.011$ and LA group: $F=4.356$; $P<0.001$). When these two loci

Table 1 Mean values of fluctuating asymmetry (±SD), global magnitude of asymmetry(GMA), mean proportion of asymmetric characters per individuals (PACh), length (cm) and weight (g) in two samples from Atlantic salmon

Character	*EA*	*LA*	*F*	P
Pectoral fins	0.207 (0.49)	0.272 (0.57)	0.863	0.354
Pelvis fins	0.097 (0.35)	0.168 (0.48)	1.600	0.207
Gill rakers	0.504 (0.79)	0.640 (0.80)	1.791	0.182
GMA	0.848 (1.38)	1.185 (1.68)	3.011	0.084
PACh	0.215 (0.02)	0.282 (0.02)	3.971	0.047*
Length	41.588 (18.0)	26.523 (15.1)	39.949	<0.001***
Weight	15.218 (2.3)	12.507 (2.1)	34.185	<0.001***

*$P<0.05$; ***$P<0.001$.
EA = early fish; LA = late fish; GMA = global magnitude of asymmetry; PACh = mean proportion of asymmetric characters per individual.

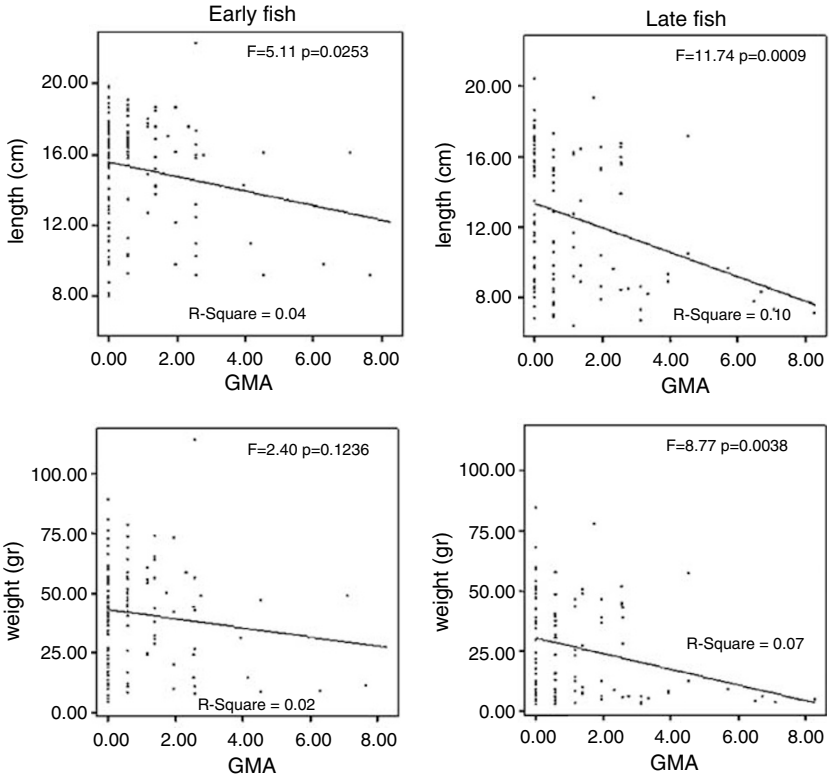

Figure 1 Relationship between FA and length and weight in two samples from Atlantic salmon. GMA = global magnitude of asymmetry.

Table 2 Observed heterozygosity at allozyme (Henz) and microsatellite (Hμ) loci and different d^2 values in two samples from Atlantic salmon

	EA	*LA*	*F*	P
Henz	0.189	0.145	6.107	0.018*
Hμ	0.488	0.497	0.142	0.706
Mean d^2	0.979	1.033	0.570	0.451
Outbreeding d^2	41.07	49.13	2.049	0.154

*$P<0.05$.
EA = early fish; LA = late fish.

were removed from the analysis, the relationship between GMA and allozyme heterozygosity is still negative in both groups but only significant in the LA group ($r^2=0.022$; F = 2.96; $P=0.088$ in the EA, and $r^2=0.125$; F = 15.62; $P<0.001$ in LA group).

When multilocus heterozygosity at allozyme loci (grouping the individuals into classes according to the number of heterozygous loci) was related with length and weight, a positive correlation was observed, with three of the four correlation coefficients (τ, Kendall coefficient of rank correlation) significantly different from zero (Table 4).

Values of A, D, GMA and PACh for different microsatellite heterozygotic groups are shown in Table 5. Five out of six regression analyses performed showed no relationship between microsatellite heterozygosity and average of FA (A) of the trait. Only in the case of the pectoral fin ray trait, in the LA group, was a positive and significant relationship between A and microsatellite heterozygosity found ($r^2=0.037$; F = 4.20; $P=0.042$) (Table 5). No significant relationships between GMA or PACh and microsatellite heterozygosity per individual were found (Table 5).

When multilocus heterozygosity for microsatellite loci (grouping the individuals in classes according to the number of heterozygous loci) was related with length and weight, different results were found in the two groups of samples analysed (Table 6). While, in the EA group, a positive, although not significant, correlation is observed (Kendall coefficient of rank correlation, $\tau=0.2$ for length and $\tau=0.467$ for weight), in the LA group, the correlation found was negative and significantly different from zero ($\tau=0.810$; $P<0.05$ for length and $\tau=0.905$; $P<0.01$ for weight).

When values of A of each meristic character, GMA and PACh were related with mean d^2 and outbreeding d^2 values, no significant associations were observed in either of the two samples analysed (Table 7) and the same each of significance was found when each of d^2 values was related to either length or weight (Table 7).

Discussion

In this study, the relationship between the level of variability of two different genetic markers (enzyme-encoding and microsatellite loci) and the level of FA, weight and length were evaluated in two large samples (EA and LA fish, each with $n>100$) of Atlantic salmon (*Salmo salar*). Although all fish analysed in this study came from the same broodstock, the samples were not obtained at random. Instead, the timing of first feeding was used; individuals in the LA sample began their

Table 3 Relationships between fluctuating asymmetry and level of heterozygosity at allozyme loci in two samples from Atlantic salmon

Character		*Groups of heterozygotes*				*Regression*		
		Hom(31)	*Het-1(62)*	*Het-2(35)*	*Het-3(7)*	r^2	*F*	P
(A) Early (EA) fish group								
Pectoral fin ray	D	0–1	0–2	0–3	0–0			
	A	0.35	0.18	0.17	0.00	0.026	3.531	0.062
Pelvic fin ray	D	0–2	0–1	0–1	0–0			
	A	0.19	0.09	0.05	0.00	0.023	3.090	0.081
Gill rakers	D	0–4	0–4	0–1	0–0			
	A	1.19	0.39	0.20	0.00	0.199	33.030	<0.001***
GMA		1.68	0.72	0.50	0.00	0.110	35.740	0.001**
PACh		1.26	0.56	0.37	0.00	0.169	27.140	<0.001***

Character		*Groups of heterozygotes*			*Regression*		
		Hom(42)	*Het-1(42)*	*Het-2+3(27)*	r^2	*F*	P
(B) Late (LA) fish group							
Pectoral fin ray	D	0–3	0–1	0–0			
	A	0.60	0.12	0.00	0.174	23.130	<0.001***
Pelvic fin ray	D	0–3	0–1	0–1			
	A	0.38	0.02	0.07	0.072	8.620	<0.001***
Gill rakers	D	0–4	0–2	0–1			
	A	1.02	0.48	0.30	0.125	16.50	<0.001***
GMA		2.40	0.50	0.36	0.243	34.82	<0.001***
PACh		1.45	0.52	0.41	0.232	32.84	<0.001***

Sample size is given in parentheses. Hom = individuals homozygous for all loci; Het = individuals heterozygous for 1, 2 or 3 loci; D = magnitude of fluctuating asymmetry; A = average of fluctuating asymmetry; GMA = global magnitude of asymmetry; PACh = mean proportion of asymmetric characters per individual.
Minimum number of individuals by group: 5.

Table 4 Relationship between number of heterozygous allozyme loci and length and weight in the Atlantic salmon samples analysed

No. of het. loci	*EA*		*LA*	
	Length (cm)	*Weight (g)*	*Length (cm)*	*Weight (g)*
0	15.029	41.123	11.867	23.752
1	15.138	41.259	12.844	27.837
2 (+3 in LA)	15.314	40.949	12.985	28.827
3	16.286	49.776		
τ	1.000***	0.333	1.000***	1.000***

τ(Tau): Kendall coefficient of rank correlation. ***$P<0.001$.
EA = early fish; LA = late fish.

active feeding 10 days after the individuals of EA samples (see McCarthy *et al*, 2003). It is known, in salmonids, that the earliest fish to emerge and to colonise the stream have an advantage in subsequent competition for profitable feeding sites over those emerging later (Metcalfe and Thorpe, 1992; Brannas, 1995). Experimental studies have also demonstrated that EA Atlantic salmon adopt alternative life history strategies when compared to LA fish (Metcalfe *et al*, 1995; Cutts *et al*, 1999; McCarthy *et al*, 2003). In a previous study of the samples assayed in this work, McCarthy *et al* (2003) found that the relative date of first active feeding and the subsequent life history strategy are significantly related. Thus, individuals for the EA samples grew faster at ambient water temperature, and a significantly higher proportion of them adopted early freshwater maturation (age 0+, male fish) or early migrant (age 1+, mainly female fish) strategies compared to individuals of the LA sample, the proportions of early migrants in the EA and LA groups being 89 and 57%, respectively (McCarthy *et al*, 2003).

In this work, EA fishes are found to be larger and heavier (Table 1).

The existence of a general negative relationship between FA and various fitness components such as growth, fecundity, longevity, and the idea of their being a higher competitive ability and a lesser risk of predation and parasitism of symmetrical individuals compared with more asymmetrical conspecifics is what emerges from the reviews of Møller (1997) and Møller and Swaddle (1997). However, Clarke (1998), Houle (1998) and Palmer (2000) indicate that it is necessary to be cautious about accepting negative associations between fitness and FA. The problems arise from the weak, heterogeneous, somewhat equivocal relationship between stability and fitness (Clarke, 1998) and the absence

Table 5 Relationships between fluctuating asymmetry and level of heterozygosity at microsatellite loci in two samples from Atlantic salmon

Characters		*Groups of heterozygotes*						*Regression*		
		Hom +Het1 (6)	*Het-2 (12)*	*Het-3 (37)*	*Het-4 (36)*	*Het-5 (22)*	*Het-6+7+8 (22)*	r^2	*F*	P
(A) Early (EA) fish group										
Pectoral fin rays	D	0–0	0–1	0–2	0–1	0–1	0–3			
	A	0.00	0.08	0.19	0.25	0.14	0.36	0.018	2.459	0.119
Pelvic fin rays	D	0–0	0–0	0–2	0–1	0–0	0–2			
	A	0.00	0.00	0.16	0.11	0.00	0.18	0.002	0.318	0.574
Gill Rakers	D	0–1	0–1	0–2	0–3	0–4	0–4			
	A	0.33	0.17	0.49	0.58	0.55	0.59	0.012	1.549	0.215
GMA		0.19	0.21	0.96	0.97	0.50	1.31	0.020	2.410	0.122
PACh		0.33	0.25	0.68	0.81	0.50	0.77	0.015	2.030	0.156

Characters		*Groups of heterozygotes*							*Regression*		
		Hom+ Het-1 (7)	*Het-2 (11)*	*Het-3 (24)*	*Het-4 (27)*	*Het-5 (27)*	*Het-6 (8)*	*Het7+8 (7)*	R^2	*F*	P
(B) Late(LA) fish group											
Pectoral fin rays	D	0–0	0–1	0–1	0–2	0–2	0–3	0–0			
	A	0.00	0.09	0.13	0.37	0.33	0.87	0.00	0.037	4.202	0.042*
Pelvic fin Rays	D	0–1	0–1	0–1	0–3	0–2	0–1	0–1			
	A	0.14	0.09	0.04	0.19	0.30	0.25	0.14	0.013	1.4513	0.231
Gill Rakers	D	0–1	0–3	0–2	0–4	0–2	0–2	0–1			
	A	0.43	0.82	0.33	1.10	0.56	0.63	0.14	0.002	0.1991	0.656
GMA		0.61	0.83	0.47	1.63	1.54	2.22	0.44	0.031	2.990	0.086
PACh		0.57	0.73	0.50	1.11	1.07	0.25	1.29	0.026	2.910	0.091

Sample size is given in parentheses. Hom = individuals homozygous for all loci; Het = individuals heterozygous for 1, 2 or more loci; D = magnitude of fluctuating asymmetry; A = average of fluctuating asymmetry; GMA = global magnitude of asymmetry; PACh = mean proportion of asymmetric characters per individual.
Minimum number of individuals by group: 5.

Table 6 Relationship between number of heterozygous microsatellite loci and length and weight in the Atlantic salmon samples analysed

No. of het. loci	*EA*		*LA*	
	Length (cm)	*Weight (g)*	*Length (cm)*	*Weight (g)*
0+1	14.791	37.788	13.641	30.743
2	15.542	45.169	13.018	29.200
3	15.492	42.041	13.187	29.179
4	14.708	39.139	12.663	27.007
5	15.677	45.641	12.204	26.230
6 (or more in EA)	15.833	45.417	9.671	12.557
7+8	—	—	11.725	22.763
τ	0.200	0.467	−0.810*	−0.905**

τ(Tau): Kendall coefficient of rank correlation. *$P<0.05$; **$P<0.01$.
EA = early fish; LA = late fish.

of parallel asymmetry variation among individuals, the limited statistical power of test due to sampling and measurement errors, and the obvious evidences of selective reporting (Palmer, 2000).

Our results point to a negative relationship between fitness and FA in Atlantic salmon. Within both samples, general parameters of asymmetry, GMA (Figure 1) and also PACh (although not shown) were negatively associated with length and weight, although none of the three characters has a large effect in this correlation (Table 1). On the other hand, the necessity of finding 'the asymmetry parameter' (an asymmetry in one trait predicting asymmetries in other traits) (Houle, 1998; Palmer 2000) remains unsolved since the relationship between levels of FA of different traits in this work were low ($r^2 = 0.03$ as maximum) or absent.

In this study, both at the population (EA *vs* LA) and the individual level (within each sample) relationships between heterozygosity at allozyme loci and FA, length and weight were observed. In the sample, EA individuals more heterozygous for allozyme loci exhibited lower values of FA, and were longer and heavier than individuals in the LA sample, and individuals less heterozygous in the EA sample (Tables 1–4). Similar correlations between heterozygosity at allozyme loci and fitness-related traits, including developmental stability, growth rate, etc, have been reported for various organisms (Eanes, 1978; Mitton, 1978; Knowles and Mitton, 1980; Leary *et al*, 1984; Mitton and Grant, 1984; Zouros and Foltz, 1987; Blanco *et al*, 1988, 1990, 1998; Vázquez *et al*, 1988). The genetic basis that rules these relationships remains

Table 7 Relationships between different d^2 values and asymmetry. Length and weight in two samples from Atlantic salmon

		Mean d²			*Outbreeding* d²		
		R^2	*F*	P	r^2	*F*	P
Pectoral fin rays	EA	0.020	2.68	0.104	0.001	0.27	0.862
	LA	0.020	2.22	0.139	0.002	0.18	0.667
Pelvic fin rays	EA	0.001	0.09	0.759	0.002	0.29	0.588
	LA	0.004	0.45	0.500	0.002	0.23	0.626
Gill rakers	EA	0.004	0.49	0.480	0.002	0.24	0.612
	LA	0.001	0.12	0.727	0.034	3.84	0.052
GMA	EA	0.011	1.52	0.218	0.000	0.01	0.917
	LA	0.015	1.68	0.197	0.004	0.47	0.499
PACh	EA	0.013	1.74	0.189	0.001	0.04	0.833
	LA	0.014	1.59	0.209	0.012	1.39	0.235
Length (cm)	EA	0.005	0.68	0.409	0.003	0.42	0.514
	LA	0.004	0.42	0.515	0.004	0.39	0.535
Weight (g)	EA	0.004	0.47	0.490	0.012	1.63	0.205
	LA	0.001	0.06	0.804	0.010	1.06	0.306

EA = early fish; LA = late fish; GMA = global magnitude of asymmetry; PACh = mean proportion of asymmetric characters per individual.

unclear and different hypotheses had been proposed to explain that association.

However, our results indicate that the two types of genetic markers are related differently to the traits under study. Although both samples differ in mean values of FA for each trait, GMA, PACh, weight and length (Table 1), differences in mean value of microsatellite heterozygosity were not observed between them (Table 2). Also, within each sample (EA and LA), individual multilocus heterozygosity at microsatellite loci did not correlate with the level of FA (Table 5), and different associations were observed the two samples when the level of heterozygosity at microsatellite loci was compared with length and weight (Table 6). While in the EA sample a positive (but not significant) correlation was observed, the opposite (a negative and significant correlation) was observed in the LA sample (Table 6). These results obtained with microsatellite loci may be indicating, at least in part, that microsatellite heterozygosity is not associated with the expression of fitness-related traits, and that the relationships (positive in EA sample and negative in LA) found in this study could simply be due to chance.

Our results agree with those obtained in other previous studies, such as Zouros and Pogson (1994), on *Placopecten magellanicus,* where the authors did not find any correlation between RFLPs or VNTR markers and growth rate, while a significant positive correlation between this trait and heterozygosity at seven allozyme loci was found, and with the study of Thelen and Allendorf (2001) on *Oncorhynchus mykiss,* another salmonid species, where more heterozygous individuals at allozyme loci had a significantly higher condition factor, whereas no evidence was found that increased heterozygosity at microsatellite loci was associated with this fitness related trait.

These results (including our work) do not fit with associative overdominance predictions, and suggest that allozymes and microsatellites are differentially affected by natural selection.

Nonetheless, positive heterozygosity–fitness correlations have recently been reported using RFLPs (Pogson and Fevolden, 1998) and microsatellite markers (Coltman *et al*, 1999; Rowe *et al*, 1999). These association between noncoding DNA markers and fitness traits are interpreted as showing that at least some correlations are not due to the direct effects of the marker genes on the phenotype, and they thus support the associative overdominance hypothesis. In addition, Slate and Pemberton (2002) argue that, in many cases, correlations between microsatellite heterozygosity and fitness-related traits are not observed due to the experimental design, since obtaining sufficient power for the detection of significant correlations requires, at least, the analysis of 20 loci in large samples of more than 100 individuals.

However, this last point does not seem to be able to explain the results obtained in our study since we analysed large samples ($n = 135$ in EA and $n = 111$ in LA sample), and it seems difficult to assume that linkage disequilibria exist between some deleterious genes and the six protein-coding loci analysed in this study, but not with the eight microsatellite loci given that the data available on microsatellites describe them as having a ubiquitous chromosomal distribution in most species investigated (Dib *et al*, 1996; Dietrich *et al*, 1996). Thus, our data seem to confirm that if there were indeed deleterious genes, the same probability would exist of finding them in linkage disequilibria with microsatellite markers as with allozyme loci.

Another possibility for explaining positive heterozygosity–fitness correlations is inbreeding (Weir and Cockerhan, 1969). In this case, individuals with the lowest level of heterozygosity will be the most inbred, and inbreeding depression will result in a positive correlation between heterozygosity and fitness (Mitton, 1998). Coulson *et al* (1999) and Pemberton *et al* (1999) propose that, perhaps, for microsatellite loci, other measures, better than heterozygosity, may reflect the degree of inbreeding. Assuming that microsatellites mutate mainly by stepwise changes in the number of repeat units, they

propose a new index, d^2, the squared difference in repeat units between the two alleles of an individual, as a useful indicator of inbreeding and outbreeding. This measurement, mean d^2, is related to more distant events in population lineages, whereas heterozygosity mainly measures the consequences of recent breeding patterns. In this way, Coulson *et al* (1998, 1999) in the red deer, *Cervus elaphus*, and Coltman *et al* (1998) in the harbour seal, *Phoca vitulina*, found positive correlations between some fitness components (like birth weigh, neonatal survival) and mean d^2, although not between these traits and microsatellite heterozygosity. In our study, differences in d^2 values between the two samples analysed were not found (Table 2) and neither was it possible to establish any correlation between d^2 values and the different parameters analysed at the individual levels within each sample (Table 7). Neither, Rowe and Beebee (2001), in *Bufo calamita* and *Rana temporaria* nor Shikano and Taniguchi (2002), in *Poecilia reticulata*, found any association between quantitative traits relevant to fitness and d^2 measurements.

Tsitrone *et al* (2001) and Slate and Pemberton (2002) indicate that heterozygosity usually provides higher correlations than d^2 under most biologically plausible conditions and conclude that if correlations exist, they will be better detected by individual heterozygosity than by d^2 values.

In summary, our results do not fit the associative overdominance predictions and suggest that allozymes and microsatellites are differentially affected by natural selection and that allozyme loci, possibly through the control of metabolism, influence fitness components.

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