Behav Ecol Sociobiol (2009) 63:605–615
DOI 10.1007/s00265-008-0695-0

ORIGINAL PAPER

Does interspecific competition influence relationships between heterozygosity and fitness-related behaviors in juvenile Atlantic salmon (*Salmo salar*)?

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Received: 2 June 2008 / Revised: 14 November 2008 / Accepted: 5 December 2008 / Published online: 24 December 2008

Abstract Very few studies have investigated the effect of genetic diversity on the behavioral and phenotypic traits linked to the competitive ability of individuals. In this study, we reared juvenile Atlantic salmon (*Salmo salar*) alone or with the competitive rainbow trout (*Oncorhynchus mykiss*) in order to: (1) to assess correlations between heterozygosity and traits related to individual competitive ability [i.e., heterozygosity–fitness correlations (HFCs)] in Atlantic salmon, and (2) to evaluate the effect of the competitive rainbow trout on any such HFCs. We also tested whether a few loci had a disproportionately large effect (i.e., the local effect hypothesis) or, on the contrary, if all loci contributed equally (i.e., the global effect hypothesis) in explaining the observed HFCs. We found significant HFCs for phenotypic traits related to the competitive ability of juvenile Atlantic salmon, i.e., the growth rate and the distance to the feeding source. Some HFCs were nonlinear, suggesting that individuals with intermediate levels of heterozygosity were favored. In addition, we found that the competition exerted by rainbow trout only weakly modified these HFCs as the relationships were highly consistent across treatments. We demonstrated that the local-effect hypothesis best explained both linear and nonlinear HFCs. Overall, our results illustrated the importance of genetic diversity in explaining the behavioral variability observed within populations. Moreover, we provide evidence that, even if a competitive species can have strong ecological effects, the relationships between genetic diversity and fitness-related traits in juvenile Atlantic salmon were not influenced by such effects.

Keywords Behavior · Additive genetic effects · Nonadditive genetic effects · Salmonids · Inbreeding · Genetic diversity · Aggression

Communicated by C. St. Mary

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Introduction

Among biotic interactions, competition is a selective force that can drive both intra- and interspecific phenotypic differentiation (Connell 1980; Bolnick et al. 2003). The ability of an individual to out-compete others influences its survival and the life-history strategies adopted during its lifetime (Connell 1980; Gurevitch et al. 1992). Some behavioral aptitudes (or "personality", Sih et al. 2004) are fitness-favoring in certain environments and are generally associated with a high competitive ability. For instance, in territorial animals, highly aggressive individuals are thought to be better at defending a territory and thus more successful in exploiting harsh environments (e.g., Nakano 1995; Côté and Festa-Bianchet 2001; Vollestad and Quinn 2003). Yet, the genetic and physiological traits that make an individual a better competitor than another are currently poorly understood (Meffert et al. 2002; Boake et al. 2002; Owens 2006; Välimäki et al. 2007).

Recently, several studies have highlighted strong links between genetic diversity and individual fitness (Keller and Waller 2002; DeSalle and Amato 2004). Classically, pedigree analyses are performed to evaluate the degree of inbreeding depression in a given population and used to establish genetic diversity–fitness relationships (e.g., Kruuk et al. 2002). Researchers have also used molecular markers to determine the degree of inbreeding (Zouros et al. 1980; David 1998; Hansson and Westerberg 2002), whereby

heterozygosity measured at a set of loci is expected to reflect genome-wide heterozygosity and thus the inbreeding level of a given individual. Significant correlations between heterozygosity and fitness (general HFCs) have been reported in a large number of taxa and based on a diversity of fitness traits such as lifetime reproductive success, parasitic load, and growth rate (see David 1998; Hansson and Westerberg 2002; Coltman and Slate 2003; Rijks et al. 2008). Hence, this latter approach offers a solid base to assess the genetic causality of interindividual variation in behaviors and phenotypic traits linked to the competitive ability of individuals (Tiira et al. 2003, 2006).

To date, the demonstration of HFCs has been limited to single populations observed in single environments. Indeed, few studies have attempted to infer the role of environmental variability in shaping the relationships between individual genetic diversity and fitness components (but see Tiira et al. 2006). However, there is strong theoretical evidence that, by changing population structure, changes in the environment could affect the strength and the shape of HFCs (Balloux et al. 2004). In the laboratory, Lesbarrères et al. (2005) demonstrated that for the common frog (*Rana temporaria*), the strength of HFCs was strongly dependent upon the evolutionary history of the population and upon the physical characteristics of the rearing environment. Individual competitive ability is also known to be highly context-dependent (Höjesjö et al. 2002; Blanchet et al. 2007). For instance, juveniles of Atlantic salmon (*Salmo salar*) that performed well in an intraspecific context were not necessarily good competitors when an exotic competitor (i.e., the rainbow trout, *Oncorhynchis mykiss*) was present (Blanchet et al. 2007; Roberge et al. 2008). Thus, the simultaneous analysis of several competitive contexts might be highly informative for assessing the generality of the effect of heterozygosity on traits related to the individual competitive ability.

Most studies reporting significant general HFCs have demonstrated a positive linear association between heterozygosity and a given fitness trait (e.g., Slate et al. 2000; MacDougall-Shackelton et al. 2005). This result supports the assertion that more inbred individuals (i.e., less heterozygous) are less fit. However, the assumption that heterozygosity calculated at a set of loci is a surrogate of the inbreeding coefficient has been challenged by several studies (Houle 1994; David 1997; Balloux et al. 2004; Markert et al. 2004; Slate et al. 2004). Using both theoretical and empirical approaches, these studies revealed a weak association between heterozygosity and inbreeding coefficient. It has been proposed that general HFCs may be better explained by associations at the level of individual markers (i.e., the direct effect hypothesis in the case of markers under selection) or of genes closely linked to them (i.e., the local effect hypothesis in the case of neutral markers) rather than global inbreeding depression (Balloux et al. 2004). Concomitantly, for neutral markers such as microsatellites, recent studies have reported strong and significant HFCs at the scale of a single locus (i.e., single-locus HFCs), thus, supporting the hypothesis that general HFCs were more prone to be explained by the local effect hypothesis rather than reflecting global inbreeding depression (e.g., Markert et al. 2004; Lieutenant-Gosselin and Bernatchez 2006). Mechanistically, for the local effect hypothesis, it has been proposed that overdominant effect (s) of allele(s) at the locus level improves the performance of heterozygous individuals (i.e., nonadditive genetic effects; Neff and Pitcher 2005; Lieutenant-Gosselin and Bernatchez 2006). However, the local (i.e. the overdominance hypothesis) vs. global (i.e. the inbreeding hypothesis) effect's debate remains open.

The primary objective of this study was to evaluate the relationship between individual heterozygosity and several phenotypic traits associated with the competitive ability of an individual. Specifically, we compared the shape and the significance of the general HFCs of a species reared alone or in the presence of an interspecific competitor. Further, we explored the occurrence and magnitude of single-locus HFCs to gain insight into the processes driving general HFCs. To address these issues, we reared juvenile Atlantic salmon in the laboratory in the absence or the presence of an exotic competitive species, the rainbow trout. In fish, as in many animals, the short-term competitive ability of an individual can be measured as its ability to acquire and defend nutritive resources (Hoffmann et al. 1999; Klemetsen et al. 2003). The successful defence of nutritive resources contributes to improving growth rate (along with physiological and genetic contributions) and hence individual fitness (Pujolar et al. 2005). Thus, behavior contributing to maximizing growth rate (e.g., territory defence, feeding rate; Klemetsen et al. 2003; Tiira et al. 2003) can be considered as important fitness-related traits indicative of the competitive ability of an individual. We calculated HFCs for Atlantic salmon growth rate and behaviors associated with competitive ability to test three hypotheses; (1) a positive linear association exists between heterozygosity and these traits in Atlantic salmon, (2) this relationship is explained by the local effect hypothesis, and (3) the heterozygosity–fitness relationship is affected by the presence of an interspecific competitor.

Materials and Methods

Biological materials

Wild young-of-the-year (YOY) Atlantic salmon and rainbow trout were caught by electrofishing in the Malbaie

River in September 2005 (Québec, Canada, 47°67′ N; 70° 16′ W). Rainbow trout have established a self-sustaining population in the downstream part of the Malbaie River since the early 1970s. The origin of this population is not certain, but rainbow trout appear to have emigrated from the Great Lakes to the Malbaie River through the Saint-Lawrence River corridor (I. Thibault, L. Bernatchez, and J.J. Dodson, personal communication). Juveniles of both species display high niche overlap in the Malbaie River, and it has recently been demonstrated that rainbow trout had strong effects on the growth rate and the social hierarchy of Atlantic salmon (Blanchet et al. 2007; Roberge et al. 2008).

YOY Atlantic salmon were sampled in two locations (125–140 fish per location) along a 2-km stretch of river located upstream of a fish ladder. Migrating rainbow trout are actively excluded from the fish ladder, and the sampled YOY salmon were thus considered to have had no previous experience with rainbow trout. There was no significant genetic differentiation between the two samples (Fisher's exact test, χ^2=44.72, *df*=30, p=0.10). YOY rainbow trout were sampled in several locations distributed along a river transect of 9 km. All the fish were transferred to the laboratory, where they were reared in three separate tanks and fed ad libitum with fish food pellets for 2 months before experiments began. Very few fish died during the rearing period (10 to 20 fish per species) so that roughly 250 and 150 salmon and trout, respectively, were available for the experiment. The two groups of Atlantic salmon were pooled during the experiment.

Laboratory experiments

Experiments were conducted using the 12 artificial channels described in Blanchet et al. (2007). Briefly, each channel was made of Plexiglas and was 1.90-m long, 0.30-m wide, and 0.30 m deep. The water depth in each channel was 12 cm, and average current velocity was 8 cm s^{-1}. A single layer of river cobbles covered the entire floor of the arena. Two half-bricks (12×5×0.8 cm) were added to serve as refuges. Light/dark cycle was 9:14 h plus 30 min of dawn and dusk. Water temperature was 14±1°C during the experiment. The values of the abiotic parameters controlled in this experiment were in the range of the habitat requirements of both species (see Blanchet et al. 2007). Daily food ration (a mix of frozen chironomid larvae and fish food pellets; in total 4% of the initial total wet fish biomass per channel) was dispensed in an unpredictable manner at the upstream end of the channel (see Volpe et al. 2001). The inner side of all channels was marked to define 15 equal zones to allow recording of horizontal distribution.

The experiment consisted of two treatments. In the first treatment, five salmon per channel were maintained in allopatry. In the second treatment, five salmon per channel were maintained together with five rainbow trout (i.e., additive design). Such densities were chosen according to a previous study on the same system (see Blanchet et al. 2007). Each treatment lasted 15 days and was replicated 18 times (n=90 salmon per treatment) for a total of 36 observations. As we were limited to 12 channels, the 36 observations were run over three consecutive trials. Each trial included six replicates of each treatment. The weight of the salmon [mean (±SD)=1.56 g. (±0.02)] did not vary among trials [nested analysis of variance (ANOVA), $F_{2,\ 141}$= 2.19, p=0.11] or between treatments (nested ANOVA, treatment nested within trials, $F_{33,\ 141}$=0.68, p=0.90). Rainbow trout [mean (±SD)=1.79 g (±0.05)] were bigger than Atlantic salmon in all the trials (nested ANOVA, species effect, $F_{1,\ 243}$=14.76, p<0.01; species within trials, $F_{2,\ 243}$=1.53, p=0.22).

Before experiments, salmon were anesthetized and individually marked (Visible Implant Elastomer tags, Northwest Marine Technology, Shaw Island, Washington). They were measured (±0.01 mm) and weighed (± 0.01 g) both before and after the experiments.

To ensure that the social hierarchy was stable in each tank, behavioral observation were carried out following 3 days of acclimatization. Each channel was observed for 15 min at four different occasions within the 15-day treatment period (i.e., an observation the morning of each 3 days experiment). We measured three behavioral variables related to the competitive ability of Atlantic salmon (Blanchet et al. 2007): (1) the position of each fish relative to the food source, (2) the number of feeding attempts, and (3) the number of aggressive interactions (chase, displays, and nips) initiated and received by each fish. We noted the donor and the recipient involved in each aggressive interaction to calculate the David's index of dominance for each fish (Gammell et al. 2003). This index allows ranking each individual along a social hierarchy while dealing with repeated interactions between group members (see Gammell et al. 2003 for details on the calculation).

At the end of the experiment, we calculated the growth rate (G) of salmon using the following formula:

$$G_{ij} = \frac{1\mathrm{n}(\mathrm{W}_{it2}) - 1\mathrm{n}(\mathrm{W}_{it1})}{(t_2 - t_1)}$$

Where G_{ij} is the daily growth rate of individual i in the channel j, W_{it1} is the mass of the individual at the beginning of the growth period, W_{it2} is the mass of the individual at the end of the experiment. (t_2-t_1) is the period of growth, i.e., 15 days in this experiment.

We also calculated a residual growth rate (resG) by extracting the residuals of the multiple regression linking G

Table 1 Description of the 15 microsatellites used to calculate the multilocus heterozygosity in individuals of Atlantic salmon

Locus	GeneBank accession	QTL/EST	Linkage groups	Hexp	Hobs	Fis	A	Rx volume	Rx buffer1	dNTPs 2 (μl)	Taq (U)	DNA (ng)	Cycle (temperature in °C)
SsaD 190	AF525206	–	NA	0.942	0.931	0.025	35	10	1	0.3	0.2	8	5 min at 94, 34×(45 s at 94, 45 s at 66, 120 s at 68), 10 min at 68
CA054978	CA054978	Unknown	NA	0.430	0.468	−0.031	5	10	1	1	0.5	8	2 min at 94, 35×(30 s at 94, 30 s at 55, 30 s at 72), 5 min at 72
CA 054565	CA054565	Unknown	NA	0.436	0.423	0.019	5	10	1	1	0.5	8	3 min at 94, 35×(50 s at 95, 50 s at 60, 50 s at 72), 3 min at 72
Ssa401UOS	AJ402718	Body weight	AS-8f	0.883	0.903	−0.02	18	10	1	1	1.5	8	2 min at 95, 35×(45 s at 95, 1 s at 59, 45 s at 72), 5 min at 72
Ssa417UOS	AJ402734	Body weight	AS-11f	0.91	0.885	0.029	23	10	1	0.5	0.5	8	3 min at 96, 32×(50 s at 95, 50 s at 60, 50 s at 72), 5 min at 72
SSAD 237	AF525207	–	NA	0.742	0.762	−0.007	21	10	1	0.3	0,4	8	5 min at 94, 34×(45 s at 94, 45 s at 60, 120 s at 68), 10 min at 68
Strutta-12	U60220	–	AS-16f	0.922	0.943	0,011	14	10	1	0.3	0.3	8	3 min at 94, 32×(45 s at 94, 45 s at 57, 60 s at 72), 5 min at 72
SSsp2215	AY081810	–	AS-7f	0.859	0.875	0.026	14	13.5	1.9	0.45	0.3	8	5 min at 94, 34×(45 s at 94, 30 s at 60, 120 s at 68), 10 min at 68
Ssa197	U43694	–	AS-8f	0.861	0.875	−0.011	15	10	1	0.3	0.2	8	3 min at 94, 32×(45 s at 94, 45 s at 57, 60 s at 72), 5 min at 72
Ssa 202	U43695	–	AS-1m	0.811	0.795	0.021	11	10	1	0.3	0.2	8	3 min at 94, 32×(45 s at 94, 45 s at 57, 60 s at 72), 5 min at 72
SSSOSl 417	Z48597	–	AS-2f	0.863	0.875	−0.02	15	10	1	0.3	0.2	8	3 min at 94, 32×(45 s at 94, 45 s at 57, 60 s at 72), 5 min at 72
SsaD 85	AF525213	–	AS-18f	0.927	0.971	−0.023	25	13.5	1.9	0.45	0.2	8	5 min at 94, 34×(45 s at 94, 30 s at 60, 120 s at 68), 10 min at 68
SSAD 144	AF525203	–	AS-11f	0.909	0.829	0.075[a]	21	13.5	1.9	0.45	0.2	8	5 min at 95, 34×(45 s at 95, 45 s at 60, 120 s at 68), 10 min at 68
SSAD 170	AF525205	–	NA	0.874	0.852	0.018	25	13.5	1.9	0.45	0.2	8	5 min at 95, 34×(45 s at 95, 45 s at 60, 120 s at 68), 10 min at 68
SSAD 71	AF525211	–	AS-4f	0.929	0.894	0.008	25	13.5	1.9	0.45	0.2	8	5 min at 94, 34×(45 s at 94, 30 s at 60, 120 s at 68), 10 min at 68

A Number of alleles, Fis, *Hobs* observed heterozygosity, *Hexp* expected, and the recipe are presented for each locus. The buffer comprised 10 mM Tris–HCl (pH=9.0), 1.5 mM $MgCl_2$, 0.1 % Triton X-100, 50 mM KCl. The concentration of dNTPs was 10 mM each. Linkage groups are defined by the GRASP (http://grasp.mbb.sfu.ca/), and QTL and EST have been defined by Reid et al. (2005) and Vasemagi et al. (2005)

[a] Indicates significant Fis after Bonferroni corrections

(dependent factor) and the three behaviors (the distance to the feeding source, the feeding rate, and the dominance score). The growth of an individual not only depends on its behavioral ability to defend a territory that provides enough energy, but also on its physiological ability to allocate resources to somatic growth. In our case, resG was used as a surrogate measure of this physiological ability. Moreover, some of the dependent variables showed significant dependency ($-0.44<r<0.37$); resG thus allowed us to take into account the possible associations between growth and behaviors.

Genetic analysis

Total DNA was extracted from white muscle tissue as described in Aljanabi and Martinez (1997). The DNA was used in polymerase chain reaction (PCR) to amplify 15 microsatellite loci (see Table 1 for more details on the loci). Out of these 15 microsatellites, ten were mapped to linkage groups in the Atlantic salmon genome using information available online by the GRASP (Genomic Research on Atlantic Salmon Project, http://grasp.mbb.sfu.ca/). These ten loci belonged to nine different linkage groups (see Table 1). Moreover, two out of these 15 loci (*Ssa 401UOS* and *Ssa 417UOS*, Table 1) are linked to quantitative traits loci (QTL) associated with body weight in Atlantic salmon (Reid et al. 2005), and two others (*CA 054978* and *CA 054565*, Table 1) are linked to expressed sequence tags (ESTs, Vasemagi et al. 2005). PCR products were run on an ABI™ 3100 automated capillary sequencer (Applied Biosystems). Allelic sizes were scored using GENESCAN™ analysis v.3.7 and GENOTYPER™ v.3.7 NT software. Fourteen out of the 15 loci were in Hardy–Weinberg equilibrium, whereas a slight deficit in heterozygotes was observed for one loci (*SsaD 144,* see Table 1). Such relatively small departures from HW expectations are unlikely to impact the outcomes of general HFC analyses (Lieutenant-Gosselin and Bernatchez 2006). After correction for multiple tests, no pair of loci was found to display significant linkage disequilibrium. Several metrics have been developed to evaluate heterozygosity [i.e., mean d^2, Coltman et al. 1998; standardized heterozygosity (Hs), Coltman et al. 1999; internal relatedness (IR), Amos et al. 2001; homozygosity by loci (HL), Aparicio et al. 2001). With the exception of the mean d^2 (see Coltman and Slate 2003), all these metrics were highly correlated in our dataset ($r>0.90$) and provided similar information. We preferred using the classical multilocus heterozygosity (MLH; calculated as the proportion of the 15 loci at which an individual was heterozygous, see Lieutenant-Gosselin and Bernatchez 2006) as it offered the advantage of being constrained between 0 and 1, which facilitates comparisons across taxa and studies.

Statistics

We assessed the general HFCs between the five traits (G, resG, distance to the food source, feeding attempts, and dominance score) and MLH calculated at the 15 loci using general linear mixed models (GLMMs). GLMMs allow analyses of data where the response variable is determined by both random and fixed effects. For each model, MLH and competitive treatments were the fixed factors. We also included a quadratic term (MLH^2) to test for the nonlinearity of the relationship (Neff 2004; Faraway 2006). The interaction terms between MLH and competitive treatments and between MLH^2 and competitive treatments were tested to quantify the effect of rainbow trout on the slope of the relationships. A significant interaction would indicate that the presence of the rainbow trout modified the slopes of HFCs. Initially, trial was included as a fixed effect, but it was excluded from the final models as it was not a significant source of variation. Channels were included as random factors, to control for potential non-independence of fish behavior and growth within a channel (Faraway 2006). We used the mean value of the four behavioral trials as an individual behavioral score to avoid pseudo-replication due to repeated measurements (i.e., the sample

Table 2 *P* values (and slope estimates under brackets for continuous factors) of generalized mixed linear models used to evaluate the effects of individual heterozygosity (MLH), its quadratic term (MLH^2) and competitive treatment on the growth rate, the residual growth rate (see the text), the distance to the food source, the feeding rate, and the David's index of dominance of Atlantic salmon reared alone or with the rainbow trout

	Response variables				
	Growth rate	Growth rate (residuals)	Distance to the feeding source	Feeding rate	Dominance Index
Source of variation					
MLH	**0.001 (0.12)**	**0.023 (0.17)**	**0.013 (−0.12)**	0.583 (−0.96)	0.638 (1.56)
MLH^2	**0.014 (−0.10)**	0.213 (−0.01)	**0.047 (0.15)**	0.462 (0.83)	0.066 (−1.59)
Treatment	**<0.001**	**0.002**	**<0.001**	**<0.001**	0.957
MLH × Treatment	0.741	0.802	0.826	0.141	0.391
MLH^2 × Treatment	0.579	0.203	0.619	0.254	0.915

Significant *p* values ($p<0.05$) are in bold

unit was a fish's identity). Quasi-Poisson error distribution was assumed for the number of feeding attempts, whereas Gaussian error distributions were assumed for the other traits. Shapiro tests for normality on the residuals of each model were all nonsignificant ($p>0.05$, results not detailed), thus justifying the choice of the error distributions (Sokal and Rohlf 1995). The explanatory power of each model was calculated as the percentage of explained deviance, which is an equivalent of the percentage of explained variance but applied to general linear models (Faraway 2006).

To verify whether the general HFCs were due to general or local effects, we followed the methods developed for microsatellites data by Balloux et al. (2004) and Lieutenant-Gosselin and Bernatchez (2006). We first tested for evidence of a global inbreeding effect by (1) subdividing our loci into two groups, (2) recalculating individual MLH for the two groups, and (3) measuring the correlation between those measures (this procedure was repeated 10,000 times; Balloux et al. 2004). Secondly, for the traits that showed significant general HFCs, and for the two treatments independently, we calculated single-locus HFCs for each locus individually using GLMMs (each trait was the dependent variable and the locus under consideration was the categorical predictor). In this case, heterozygosity at each locus was coded as 0 when an individual was homozygous and as 1 when it was heterozygous for the considered locus. We assessed the global occurrence of positive versus negative single-locus HFCs using the cumulative binomial exact probability test. We investigated the stability of the response (positive or negative) among traits and between treatments using GLMs with a binomial error distribution. Finally, we investigated whether some loci demonstrated significant associations with a given trait. For the traits that showed significant general HFCs, we computed GLMMs with a given locus and treatments as fixed factors. The interaction term allowed testing whether rainbow trout altered the slope of the association. We corrected for multiple tests by calculating the number of false-positive associations expected at $\alpha=0.05$ (see Lieutenant-Gosselin and Bernatchez 2006). We also tested whether the combination of loci falling into one of the two categories (positive or negative single-locus HFC) had a significant impact on each trait using the Fisher method combining probabilities of independent tests (i.e., loci were independent of each other, Sokal and Rohlf 1995; see also Lieutenant-Gosselin and Bernatchez 2006 for more details).

Results

For all dependent variables except the index of dominance, we found a significant effect of rainbow trout on Atlantic

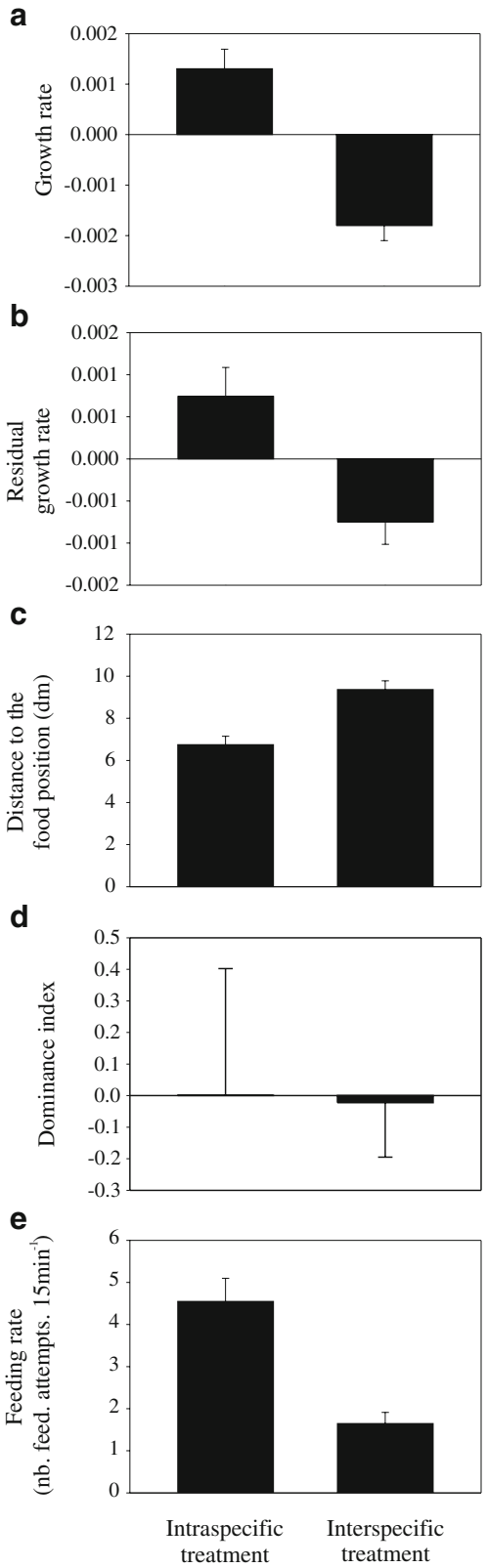

Fig. 1 Effects of the competitive treatment (intraspecific vs. interspecific competition) on **a** the growth rate, **b** the residual growth rate, **c** the distance to the food source, **d** the dominance index, and **e** the feeding rate of Atlantic salmon

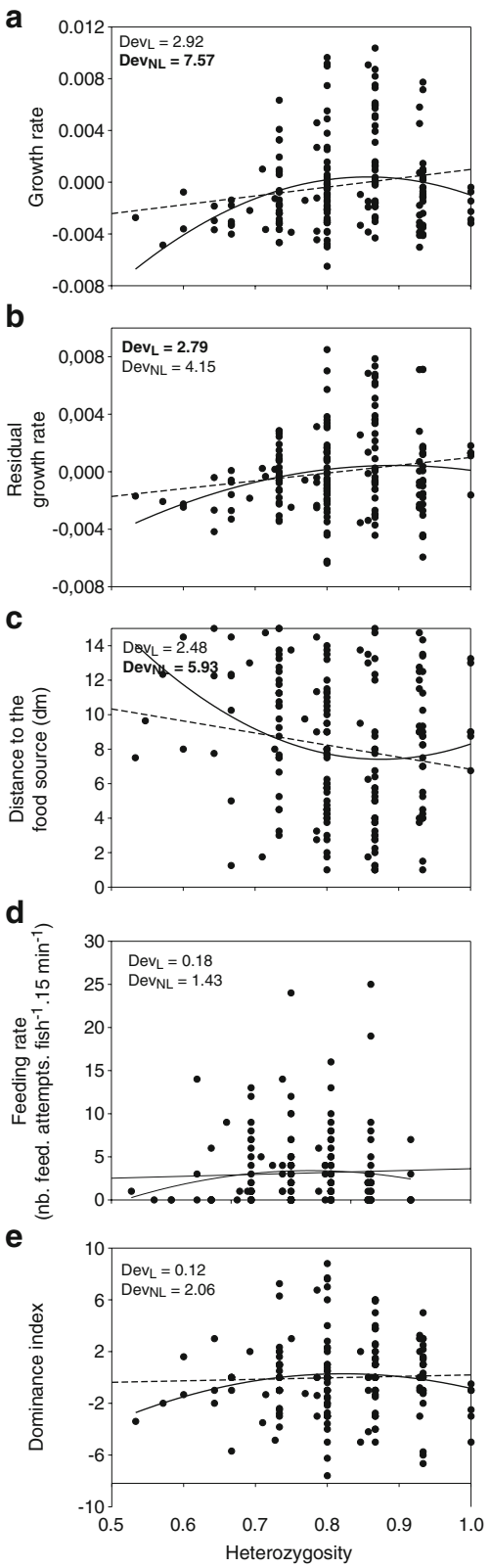

Fig. 2 Relationships between multilocus heterozygosity and **a** growth rate, **b** residual growth rate, **c** distance to the food source, **d** dominance index, and **e** feeding rate of Atlantic salmon. *Dotted lines* represent the relationship for linear models and full lines represent relationships for models including a quadratic term (i.e., nonlinear relationship). For each trait, "dev_l" is the percentage of deviance explained by the linear model and "dev_{nl}" is the percentage of deviance explained by the whole linear model that includes the quadratic term. The relationships that were significant ($p<0.05$) are in *bold*

salmon (Table 2, Fig. 1). In the presence of rainbow trout, the growth rate, the residual growth rate, as well as the feeding rate of Atlantic salmon was reduced (Fig. 1a, b and e). Also, Atlantic salmon were found farther from the food source when rainbow trout were present (Fig. 1c). Concerning the general HFCs, we found that for growth rate and the distance to the food source, both the MLH and MLH^2 were significant (Table 2). The individuals with intermediate MLH had higher growth and were closer to the food source (Fig. 2a and c). A significant positive linear relationship was also found between the residual growth rate (resG) and MLH (Table 2, Fig. 2b). The general HFCs were not significant for either the feeding rate or for the dominance index (Table 2, Fig. 2d and e). According to the trait we considered and the models we used, the percentage of deviance explained by MLH and MLH^2 varied between 0.12% and 7.57% (Fig. 2).

The correlation coefficient between MLH computed with the randomly created subset of loci was weak ($r^2=0.006\pm 0.028$), indicating that MLH was not a reliable indicator of individual inbreeding coefficient. Thus, the single-locus HFCs were considered for the traits that showed significant general HFCs (i.e., G, resG, and feeding position, see Table 2). When each trait and each treatment was considered separately, we found that 66.66 % of the single-locus HFCs were positive, which is significantly higher than the random expectation of 50% (cumulative binomial probability test, $p<0.001$, trials=90).

The proportion of positive versus negative single-locus HFCs did not significantly vary either among traits (binomial GLM, $p=0.29$) or between treatments (binomial GLM, $p=0.36$, interaction term, $p=0.64$). This result indicated that rainbow trout had little effect on the occurrence of positive or negative single-locus HFCs.

The number of significant single-locus HFCs varied from 1 to 3 according to the trait we considered (see Table 3) and was significantly different from the expected false discovery rate ($\chi^2=12.50$, $p<0.001$, $df=1$). We did not detect any significant interaction between treatments and a given locus, indicating that the rainbow trout had no effect on the slope of the relationships (result not shown). The overall effect of loci showing a positive association between heterozygosity and fitness was significant for the

three traits we considered (Fisher method on independent tests, G, df=20, p=0.004; resG, df=16, p<0.05; Feeding position, df=20, p<0.05). In contrast, we detected no overall significant effect between loci with a negative association and the fitness traits (Fisher method on independent tests, G, df=10, p>0.05; resG, df=14, p>0.05; Feeding position, df=10, p>0.05).

Discussion

A major finding of our experiment was that nonlinear general HFCs were detected for two out of the three significant relationships (i.e., for the growth rate and distance from the food source). This indicated that individuals with an intermediate level of heterozygosity were closer to the food source, had the higher growth performance, and might therefore be considered as having a higher competitive ability. This result was strengthened by the observation that it was consistent across two competitive environments. Similar results were obtained for others fitness traits in a wild bluegill sunfish population (Neff 2004) and in a wild bird population (Ortego et al. 2007). The third relationship (i.e., concerning the residual growth rate) was linear and positive, as observed for most reported general HFCs (Coltman and Slate 2003). For these significant relationships, we found that MLH explained on average 5.35% (range 2.63–7.57 %) of the total deviance of the traits we considered, similar values to those reported for several phenotypic traits in a recent meta-analysis (Coltman and Slate 2003). Finally, we did not detect any significant HFCs for the two other behaviors (i.e., the feeding rate and an index of dominance). The result concerning the index of dominance was unexpected since a significant association between the number of given aggressions and heterozygosity had previously been detected in Atlantic salmon, and in a related species, the brown trout *Salmo trutta* (Tiira et al. 2003, 2006). Here, we used a dominance index (Gammell et al. 2003) that integrated both the number of aggressions given and received by each individual. This may explain the discrepancy between our results and previous studies (Tiira et al. 2003, 2006). However, when computing similar analyses while using either the number of given or received aggressions as a dependent variable, we were unable to detect significant association with heterozygosity, even if each treatment was analyzed separately (results not shown). The populations studied by Tiira et al. (2003, 2006) had a long hatchery background and a specific history of inbreeding which may explain the discrepancies with our results. This therefore suggests that the relationship between dominance and genetic diversity may be constrained by the evolutionary and management history of populations (Lesbarrères et al. 2005).

We found little evidence that the presence of an interspecific competitor, i.e., the rainbow trout, modified the patterns of HFCs in our experimental population of Atlantic salmon. We detected strong effects of the rainbow trout on most of the traits we measured (see Fig. 1). These effects are unlikely to be due to an increase in the total fish density from the intraspecific treatment to the interspecific treatment. Blanchet et al. (2007) previously showed (using a more complete experimental design) that the effect of rainbow trout was highly species-specific and was independent of an increase in total density. In addition, Blanchet et al. (2007) demonstrated that rainbow trout strongly altered the within-hierarchical structure of groups of juvenile Atlantic salmon. Based on microarray experiments, Roberge et al. (2008) identified several candidate genes associated with this plastic loss of hierarchy. Despite such strong behavioral effects, HFCs remained remarkably

Table 3 p Values of linear models used to test the effect of single-locus HFCs on the growth rate, the residual growth rate (see the text), and the feeding position of Atlantic salmon

Locus	Growth rate	Residual growth rate	Distance to the food source
SSAD 190	0.711 (−)	0.393 (+)	0.711 (+)
CA 054978	0.915 (−)	0.357 (+)	0.989 (−)
CA 054565	0.202 (+)	0.398 (+)	0.379 (+)
Ssa 401	0.521 (+)	0.334 (−)	**0.025 (+)**
Ssa 417	0.260 (+)	0.558 (−)	0.320 (+)
St 12	**0.003 (+)**	**0.010 (+)**	0.120 (+)
SSAD 237	**0.013 (+)**	0.161 (+)	**0.045 (+)**
Sssp 2215	0.346 (+)	0.098 (+)	0.898 (−)
Ssa197	0.792 (−)	0.603 (−)	0.789 (−)
Ssa 202	**0.048 (−)**	0.321 (−)	0.326 (−)
Ssol 417	0.100 (−)	0.093 (−)	0.872 (−)
Ssa 85	0.669 (+)	0.638 (−)	0.923 (+)
SSAD 144	0.162 (+)	0.233 (+)	0.453 (+)
SSAD 170	0.042 (+)	0.245 (−)	0.079 (+)
D-71	0.281 (+)	0.365 (+)	0.279 (+)

Significant p values (p<0.05) are bolded. Symbol under bracket indicated whether the slope of the HFCs were either positive (+) or negative (−). See Table 1 for details of marker source and PCR conditions

consistent across the two competitive environments. This latter result therefore illustrates the important role that genetic diversity plays in the competitive ability of juvenile Atlantic salmon.

Although these relationships might appear relatively weak, they may nevertheless play a significant role in shaping the biological and behavioral evolution of a population. For example, we found that MLH contributes to 7.57% of the observed variance in growth rate, irrespective of the surrounding environment. This part of the variance can hence be considered as a highly predictable part of the phenotypic variation. Thus, by choosing an "optimal" mate (i.e., a partner having an optimal level of heterozygosity), an individual may increase the probability of producing progeny with a higher competitive ability. One should therefore expect a high selection differential for those advantageous genotypes. Accordingly, in studies involving several fitness traits of threespine sticklebacks (*Gasterosteus aculeatus*), Lieutenant-Gosselin and Bernatchez (2006) found high positive values of selection differentials for MLH (see also Bensch et al. 2006; Hoffman et al. 2007).

Our results support the hypothesis that the significant general HFCs (both linear and nonlinear) most likely arose from the effects of local associations between markers and fitness rather than from global inbreeding effects. Our 15 loci represented a non-negligible part of the whole genome since nine out of the 28 linkage groups were covered by at least one locus. However, we found no strong evidence that the markers we used reflected individual inbreeding level (Balloux et al. 2004). Additionally, significant single-locus HFCs and significant grouped positive effects were detected for all traits that demonstrated significant general HFCs. Such results are generally interpreted as providing strong support for the local effect hypothesis (Hansson and Westerberg 2002; Balloux et al. 2004; Lieutenant-Gosselin and Bernatchez 2006).

If we assume that the local hypothesis prevails in the present case, the nonlinear HFCs were apparently not the by-product of simultaneously occurring inbreeding and outbreeding depression as proposed by Neff (2004). In contrast, both positive (overdominant) and negative (dominant) local effects may interact to shape an individual's competitive ability (Lieutenant-Gosselin and Bernatchez 2006). This is consistent with the idea that behavioral traits might be influenced by nonadditive genetic effects (Meffert et al. 2002) and that both additive and nonadditive genetic effects can contribute to an individual's fitness (reviewed in Neff and Pitcher 2005). Moreover, when only considering positive local associations, the general HFCs were linear (and positive) for both the growth rate and the distance to the food source, thus further supporting the local-effect hypothesis. It was noteworthy that both measures of growth rate (*G* and resG) did not display similar relationships with genetic diversity, with one (*G*) being quadratic and the other (resG) being linear. The first measure (*G*) includes both the behavioral and the physiological components of the growth, while the second (resG) included only the physiological components. Thus, we hypothesize that behavioral components of growth may be more prone to be influenced by both additive and nonadditive genetic effects, while physiological components have been shown to be associated with overdominance effects of genes (Thelen and Allendorf 2001; Pujolar et al. 2005 see also Pujolar et al. 2006).

To summarize, behavioral and phenotypic traits related to the competitive ability of juvenile Atlantic salmon were partly explained by the internal genetic variability of individuals. This demonstrates the importance of genetic diversity (specifically at the level of the single locus) on the remarkable behavioral variability observed within animal species (Owens 2006). The shape of these relationships differed according to the trait that was considered, including linear and nonlinear relationships. These relationships were more likely explained by the local-effect hypothesis (Hansson and Westerberg 2002) and persisted even after introducing a competitor having strong effects on the traits linked to the competitive ability and the hierarchy of juvenile Atlantic salmon. We argue that simultaneously testing the two hypotheses that general HFCs may be linear or nonlinear, and of systematically verifying mechanisms that drive these relationships should enhance our ability to understand the evolutionary processes responsible for maintaining genetic diversity within populations. As such, our study provides new insight on how behavioral variability can be maintained within populations, and justifies further studies aimed at revealing the role of genetic factors in the transmission of behaviors across generations (Owens 2006)

Acknowledgments We are grateful to the LARSA team (Québec, Canada) for rearing fish. We thank V. Albert, G. Côté and L. Papillon for advices during genotyping. We sincerely thank M. Lieutenant-Gosselin, G. Loot, and anonymous referees for valuables comments on previous drafts. This research was financially supported by a Natural Sciences and Engineering Research Council of Canada grant (strategic program) to J.J.D. and L.B. The experiments conducted comply with current Canadian laws (license N° 2004-140).

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