Effects of rearing environment and strain combination on heterosis in brook trout

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Short title: Heterosis in brook trout

Abstract

In this study, three strains (domestic [D], Laval [L], and Rupert [R]) of brook trout (*Salvelinus fontinalis*) and their reciprocal hybrids were reared from 7 to 21 months of age in three different environments (indoor, constant temperature conditions; indoor, seasonal temperature variations; outdoor, seasonal temperature variations) to test for the occurrence of heterosis of important life history traits also of interest for production (body mass, length, condition factor, absence of early sexual maturation, survival). For each cross, body mass, length, and mortality were measured at regular intervals and sexual maturity was assessed in 1+ animals (21 months of age). We found evidence for heterosis in mass and length that varied according to strain, cross direction in reciprocal hybrids, developmental stage, or environment; no significant outbreeding depression was detected for these traits. Heterosis expression for weight varied from 4.9% to 23.8% depending on hybrids and environments. We found that one out of five reciprocal hybrids tested ($L_{♀}R_{♂}$) expressed heterosis at each age stage throughout the experiment in the three environments while the other four had mixed results. No evidence for heterosis was observed for sexual maturity and survival. These results provide one of the first clear pieces of evidence for the occurrence of heterosis in salmonids but also illustrate the complex nature and the unpredictability of this phenomenon.

Keywords: heterosis; outbreeding depression; environment; performance; hybrids; brook trout, *Salvelinus fontinalis*

Introduction

Heterosis, or hybrid vigor, refers to the increased performance and fitness of first generation progeny when compared to parental lines (Falconer and Mackay 1996; Birchler et al. 2003). The main explanation supporting the occurrence of heterosis is based on non-additive genetic components: the dominance effect seen in hybrids, which is based on the replacement or complementation of deleterious alleles accumulated in one parental line by superior alleles from the other parent; over-dominance, which suggests that heterozygotes perform better than homozygotes; and epistasis, which refers to allelic position and interactions in the hybrid (Birchler et al. 2003; Hochholdinger and Hoecker 2007; Lippman and Zamir 2007). The relative contribution of each of these processes in the expression of heterosis is still a matter of debate (Lippman and Zamir 2007).

The intensity of heterosis is usually higher when parental lines are highly inbred or genetically distant from each other (Shikano et al. 2000; Wang and Xia 2002; Hochholdinger and Hoecker 2007). However, the opposite phenomenon that results from genome admixture—outbreeding depression—could also affect crosses involving genetically distant strains. Outbreeding depression may arise from a disruption of the linkage arrangement of co-adapted gene complexes in the presence of a divergence in the genetic architecture of populations (based on epistasis components and referred to as intrinsic outbreeding depression) or from a loss of favorable allelic interactions (based on additive and dominance components and referred to as extrinsic outbreeding depression) (Edmands 2007; McClelland and Naish 2007; Tymchuk et al. 2007; Wang et al. 2007). When a cross is made, it is difficult to predict which phenomenon might appear since both heterosis and outbreeding depression, result from outbreeding crosses between distant parental lines and are controlled, at least in part, by similar non-additive effects.

Breeding programs in plants and animals commonly use heterosis to improve traits of interest for production as an alternative to the use of additive genetic components (Falconer and Mackay 1996; Comings and MacMurray 2000; Hochholdinger and Hoecker 2007). While such practice has been more limited in fish production, it has been used to improve aquaculture in carp (*Cyprinus carpio*; Wohlfarth 1993; Hulata 1995; Nielsen et al. 2010), tilapia (*Oreochromis niloticus*; Marengoni et al. 1998), and also experimentally explored in guppy (*Poecilia reticulate*; Shikano and Taniguchi 2002a). Previous studies have also investigated heterosis for various traits, including growth, survival, salinity and temperature tolerance (Moav and Wohlfarth 1976; Bentsen et al. 1998; Nakadate et al. 2003), and more recently for patterns of gene expression (Bougas et al. 2010).

In salmonids, it is still unclear if heterosis occurs. Heterosis for growth and survival in intra-specific hybrid crosses have been reported (Ayles and Baker 1983; Gjerde and Refstie 1984; Bryden et al. 2004) while other authors only observed additive interactions for these same traits (Cheng et al. 1987; Einum and Fleming 1997; Glover et al. 2006) and even outbreeding depression (Gharrett et al. 1999). From these studies, it has been hypothesized that heterosis may be generally rare in salmonids (Gjerde and Refstie 1984; Gharrett et al. 1999; Bryden et al. 2004). More specifically, Tymchuk et al. (2007) suggested that salmonid populations may be too genetically distant and locally adapted to produce heterosis. However, in brook trout (genus *Salvelinus*) in particular, previous studies on hybrid crosses between wild and domestic populations have suggested a potential for heterosis expression for growth and survival (Fraser 1981; Webster and Flick 1981) in this species although it has not been investigated in details.

96 The choice of the strain used as dam or sire in the cross may also be determinant on heterosis
97 expression (Bentsen et al. 1998). A strain can perform better when used as dam or sire, improving
98 specific capacities in hybrids (Bentsen et al. 1998; Perry et al. 2004; Wang et al. 2006b). The
99 environment may also modify genetic expression and influence the additive and non-additive genetic
100 components. A decrease in the additive variance and an increase in the epistasis variance are usually
101 expected under unfavorable environmental conditions (Wohlfarth 1993; Hoffmann and Merilä 1999).
102 In addition, heterosis seems to be more sensitive to environmental variations than additive components
103 (Bentsen et al. 1998). Different strains could also express different sensitivities to environmental
104 variations involving possible genotype – environment interactions relative to heterosis expression
105 (Falconer and Mackay 1996; Bentsen et al. 1998).
106
107 In this context, the aim of this study was to investigate the effects of rearing environment and strain
108 combination on the occurrence of heterosis for growth in the brook trout (*Salvelinus fontinalis*). In
109 teleost fishes, body mass and size at the juvenile stage can be considered as fitness-related traits since
110 they are correlated with different components of fitness such as survival, life history tactic, or
111 reproductive success (Sogard 1997; Wilson et al. 2003; Garcia de Leaniz et al. 2007; Thériault et al.
112 2007). Our specific objectives were therefore to evaluate (1) the occurrence of intra-specific heterosis
113 on important life history traits also of interest for production (body mass, length, condition factor,
114 absence of early sexual maturation, survival), (2) the presence of dam or sire effects on the hybrid
115 performance and heterosis for the traits considered, and (3) the effects of environment on heterosis
116 expression.
117
118

Materials and methods

Brook trout strains

Three strains of brook trout were used as parental stock. The Laval strain originates from a wild population of anadromous brook trout from the Laval River (48°44'N; 69°05'W) on the north shore of the St. Lawrence estuary (Quebec). The fish used as breeders were third generation individuals produced in captivity at the Station aquicole of ISMER/UQAR (Rimouski, Quebec). The Rupert strain originates from a freshwater resident wild population inhabiting the Rupert River system (51°05'N; 73°41'W) draining Mistassini Lake (Quebec). The breeders were again third generation fish produced in captivity at the Laboratoire régional en sciences aquatiques (LARSA, Université Laval, Quebec). The domestic strain is widely used by the Québec fish farming industry. It originates from two strains (Nashua and Baldwin), and breeders were obtained from the Pisciculture de la Jacques Cartier (Cap-Santé, Quebec). The two strains recently domesticated from wild populations were selected for breed improvement because adults exhibit late sexual maturation and large adult size in the wild. The Laval and Rupert strains were shown to be genetically distant from the domestic strain. Thus 76.2% of alleles from the wild strains were not found in the domestic strain, resulting in high *F*st between the domestic vs. Rupert and Laval strains [mean *F*st = 0.187 ± 0.009). The Laval and Rupert strains were even more genetically differentiated than the domestic vs. Laval or domestic vs. Rupert strains [mean *F*st = 0.427 ± 0.020 (Martin et al. 1997). Finally, Martin et al. (1997) found no evidence for pronounced inbreeding in any of these three strains with inbreeding coefficient (*F*) values varying between 0.18 and 0.35.

Breeding design

Hybrid and purebred crosses were made from mid-November 2005 until the end of December 2005 at LARSA using eggs and milt obtained from the different fish rearing locations. Three purebred strains

were produced: ♀ domestic × ♂ domestic ($D_{♀}D_{♂}$), ♀ Laval × ♂ Laval ($L_{♀}L_{♂}$), and ♀ Rupert × ♂ Rupert ($R_{♀}R_{♂}$). Five reciprocal hybrids were produced: $D_{♀}R_{♂}$, $D_{♀}L_{♂}$, $L_{♀}D_{♂}$, $L_{♀}R_{♂}$, and $R_{♀}L_{♂}$. It was not possible to obtain the $R_{♀}D_{♂}$ cross because of the temporal differences in sexual maturation between these two strains (October for domestic males and December for Rupert females). All breeders were used only once. For each cross, 10 full-sib families were obtained through single-pair matings, but 8 of these 80 families were eliminated (because of low hatching success for some due to poor egg or milt quality and random elimination of two families with high hatching success rate to get similar numbers of families in each rearing tank). The final numbers of families were 10 $D_{♀}D_{♂}$, 10 $L_{♀}L_{♂}$, 9 $R_{♀}R_{♂}$, 9 $D_{♀}R_{♂}$, 7 $D_{♀}L_{♂}$, 9 $L_{♀}D_{♂}$, 10 $L_{♀}R_{♂}$ and 8 $R_{♀}L_{♂}$.

Family rearing

During the first six months, i.e., from egg incubation (January) to exogenous feeding (June), families were kept separate in recirculating fresh water and reared in seven troughs, each of which was divided into twelve units. Water temperature was maintained at 6°C during egg incubation and at 8°C after hatching. In June, families were marked and, later identified, by different combinations of adipose and pelvic fin clippings and transferred to nine 3 m^3 tanks, with eight families per tank. All families were brought to the same fry stage by the end of the summer and maintained at 10°C in recirculating fresh water. The photoperiod followed the natural seasonal cycle and fish were fed according to commercial charts.

In September, fish from each family were randomly divided among three rearing environments. At ISMER, 230 fish per family were reared in ten 0.5 m^3 indoor tanks, with six to eight families per tank according to the initial pool conditions set up at LARSA, under natural temperature and photoperiod conditions in running dechlorinated fresh water. To maintain sustainable rearing densities, the number

of fish per family was gradually reduced to 60 by the end of the experiment (Table 1), with all reductions in number being done randomly. Fish were fed daily (1% w/w ration) with commercial dry pellets. At LARSA, 150 fish per family were reared in nine 3 m^3 tanks under natural photoperiod conditions at 10°C in recirculating indoor freshwater tanks. Fish numbers were gradually decreased to 50 fish per family by the end of the experiment (Table 1). Fish were fed daily (1% w/w ration) with commercial dry pellets. At the fish farm (Pisciculture de la Jacques Cartier facility), it was not possible to follow individual families and only cross-type comparisons were done. Two hundred fish per cross-type were reared in one outdoor pond under natural temperature and photoperiod conditions. The experiment lasted from September 2006 (7-month-old fish) to November 2007 (21-month-old fish).

Performance traits

Every eight weeks at ISMER and LARSA, 25 fish per family (n = 1800 for each location: 250 fish [25 fish × 10 families] for $D_♀D_♂$, $L_♀L_♂$, and $L_♀R_♂$ cross-types; 225 fish [25 fish × 9 families] for the $R_♀R_♂$, $D_♀R_♂$, and $L_♀D_♂$ cross-types; 200 fish [25 fish × 8 families] for the $R_♀L_♂$ cross-type; and 175 fish [25 fish × 7 families] for the $D_♀L_♂$ cross-type) were anaesthetized in MS 222 (0.16 g/L [3-aminobenzoic acid ethyl ester]) and their body mass (0.1 g) and fork length (0.1 cm) were measured. At the fish farm, mass and length were measured only twice: on 25 fish per cross-type in July (n = 200), and on every remaining fish in November (n = 710). In the two others environments, mass and length were also recorded for every remaining fish at the final sampling in November (LARSA, n = 3500: $D_♀D_♂$, and $L_♀R_♂$: 500 fish [50 fish × 10 families]; $L_♀L_♂$: 477 fish [≈ 48 fish × 10 families]; $R_♀R_♂$ and $D_♀R_♂$: 450 fish [50 × 9 families]; $R_♀L_♂$: 400 fish [50 × 8 families]; $L_♀D_♂$: 373 fish [≈ 42 fish × 9 families]; and $D_♀L_♂$: 350 fish [50 × 7 families]; (2) ISMER, n = 4115: $D_♀D_♂$, $L_♀L_♂$, and $L_♀R_♂$: 600 fish [60 × 10 families];, $D_♀R_♂$ and $L_♀D_♂$: 540 fish [60 × 9 families]; $R_♀R_♂$: 39 fish [≈ 49 fish × 9 families]; $D_♀L_♂$:

420 fish [60 × 7 families]; and $R_{♀}L_{♂}$: 376 fish [≈ 47 fish × 8 families]. Condition factor was estimated according to the equation:

$$(\text{mass} / \text{length}^3) \times 100 \qquad (1)$$

In November 2007, the presence or absence of sexual maturation was determined at the three rearing environments. For 25 fish per family at ISMER and LARSA and 25 fish per cross-type at Pisciculture de la Jacques Cartier, gonads were excised and weighed and the gonadosomatic index was calculated as:

$$(\text{gonad mass} / \text{total mass}) \times 100 \qquad (2)$$

A daily record of mortalities was made at ISMER and LARSA. The relative mortality was determined for each family in these two environments. At Pisciculture de la Jacques Cartier, all fish were captured and counted at the end of the experiment and the relative mortality determined for each cross-type.

Statistical analysis

Data normality and homogeneity of variance were tested with the Kolmogorov-Smirnov and the Brown-Forsythe tests respectively. Mass data (log), condition factor (rank), and all percentage indexes (arcsin) were transformed to obtain normality and account for heteroscedasticity. Since body mass and length were highly correlated ($r = 0.98$, $P < 0.05$), we only tested models using body mass.

To test for the presence of heterosis (objective 1), hybrid performance was compared to the performance of parental strains using ANOVAs and post-hoc tests. We used a conservative approach and considered that heterosis was present only when hybrids significantly outperformed both parental strains. Mass and condition factor were analyzed using two linear mixed models:

$$y_{ijkl} = \mu + AS_i + E_j + C_k + (AS\times E)_{ij} + (AS\times C)_{ik} + (E\times C)_{jk} + (AS\times E\times C)_{ijk} + F_{kl} + e_{ijkl} \quad \text{Model A}$$

$$y_{ijkl} = \mu + AS_i + E_j + C_k + (AS\times E)_{ij} + (AS\times C)_{ik} + (E\times C)_{jk} + (AS\times E\times C)_{ijk} + e_{ijkl} \quad \text{Model B}$$

where y_{ijkl} is the phenotypic observation; μ is the sample mean; AS_i is the effect of the ith age stage; E_j is the effect of the jth environment; C_k is the effect of the kth cross-type, all of which were fitted as fixed effects as well as their interactions; F_{kl} is the effect of the lth full-sib families nested in kth cross-types fitted as a random effect; and e_{ijkl} is the random residual effect. Model A includes the two environments, ISMER and LARSA, at each age stage while model B includes the three environments at two age stages (17 and 21 months). The a posteriori Tukey's HSD tests applied on least square means were used to detail significant factor or interaction effects. Sexual maturity and survival were analyzed using two-way ANOVAs with environment and cross-type as factors. The a posteriori Tukey test was used for mean comparisons when possible or replaced by the Games and Howell test when variances were not homogenous (Sokal and Rohlf 1981).

When the presence of significant heterosis or outbreeding depression was found, the intensity was expressed in percentage according to Shikano and Taniguchi (2002):

$$[(f_1/m) - 1] \times 100 \qquad (3)$$

where f_1 is the mean performance of the F_1 hybrids and m the mean performance of parental strains. To test for the effects of cross direction (objective 2) and environment (objective 3) on the intensity of heterosis, we either took into account the presence or absence of significant heterosis, or when heterosis was present in both reciprocal hybrids or for a same hybrid in different environments, the intensity was compared with ANOVAs.

The relative importance of additive, dominant, and epistatic genetic interactions in determining the performance of hybrids were calculated according to Wu and Li (2002) and based on the partitioning of the phenotypic variance of the full-sibs F_1 into each component of the variance.

$$V_{A(f1)} = (1/2)\,[V_{f1} + V_m - V_H] \qquad (4)$$

$$V_{NA(f1)} = (1/2)\,[V_{f1} + V_H - V_m] \qquad (5)$$

$$d/a = 2\,(f_1 - m) / (P_i - P_j) \qquad (6)$$

$$V_{D(f1)} = [(d/a)^2 \times V_{A(f1)}] / 2 \qquad (7)$$

$$V_{I(f1)} = V_{NA(f1)} - V_{D(f1)} \qquad (8)$$

where $V_{A(f1)}$ is the additive variance and $V_{NA(f1)}$ the non-additive variance of the F_1 hybrids; V_{f1}, V_m, and V_H are the variance of the performance of the F_1 hybrids, the variance of the mean performance of the parental strains, and of the variance of heterosis respectively; d/a is the dominance ratio; f_1 is the mean performance of the F_1 hybrids; m is the mean performance of parental strains; P_i and P_j are the mean performance of each i and j parental strains; $V_{D(f1)}$ is the dominance variance and $V_{I(f1)}$ the epistasis variance of the F_1 hybrids.

Mixed model analyses were performed using JMP 7 (SAS Institute, NC, USA); other statistical analyses were conducted using Statistica version 6.0 for Windows (StatSoft, USA). The statistical analyses were not corrected for multiple tests. A significance level of $\alpha = 0.05$ was used in all statistical tests.

Results

Body mass differed among environments, age stages and cross-types (significant interaction, $P < 0.001$; Table 2). The mixed models explained a large proportion of the total variance with an adjusted R^2 of

0.82 (Model A) and 0.64 (Model B) for body mass (Table 2). All cross-types were significantly heavier when raised in the constant temperature environment (LARSA), except for domestic fish, which showed similar weights at the three different environments at the end of the experiment (Table 3). When the three pure cross-types were compared, domestic fish were always significantly bigger than the two other strains in all three environments ($P < 0.05$; Table 3). In the constant temperature environment at LARSA, the Rupert strain was significantly heavier than the Laval strain ($P < 0.05$; Table 3). At ISMER, such a difference could only be observed at 17 months of age (Table 3).

When hybrid body mass was compared to those of their respective parental lines, heterosis was present but varied according to the type of hybrid cross; no outbreeding depression was observed (Tables 3 and 4). The $D_{♀}R_{♂}$ hybrid was intermediate to the values measured for the two parental strains in all three environments (Table 3) and never expressed heterosis. $L_{♀}R_{♂}$ hybrids were significantly heavier than their two parental lines ($P < 0.01$; Table 3). They also expressed heterosis at each age stage and in all three environments (Table 4). Globally, the intensity of heterosis expressed by $L_{♀}R_{♂}$ hybrids was higher at ISMER than at LARSA (14.6 ± 1.5 vs. 10.2 ± 1.0; df =1, $F = 6.6294$, $P = 0.011$) and decreased over time, i.e., the intensities in 18- and 21-month-old fish were significantly lower than in 9-, 11-, 13- and 15-month-olds (df = 6, $F = 4.0388$, $P < 0.001$; Interaction site × age stage: $P > 0.05$). In contrast, $R_{♀}L_{♂}$ hybrids were usually intermediate to their parental lines, except for 17- and 21-month-old animals, which were significantly heavier than their two parental lines in the two environments with less controlled rearing conditions, i.e., ISMER (17 month-old only) and the fish farm (Table 3). The intensity of heterosis expressed by the $R_{♀}L_{♂}$ hybrids was similar in both LARSA and ISMER environments for 17-month-old animals, similar between 17-month-old and 21-month-old animals at the fish farm, and similar to the heterosis intensity expressed by the $L_{♀}R_{♂}$ hybrids when occurring simultaneously at the farm and at ISMER ($P < 0.05$ for all statistical comparisons). The $D_{♀}L_{♂}$ and

$L_{♀}D_{♂}$ hybrids both had intermediate mass compared to the parental lines in the varying temperature environments (ISMER and the fish farm) and presented no heterosis (Table 3). However, under constant temperature at LARSA, $L_{♀}D_{♂}$ hybrids were significantly heavier than the two parental lines ($P < 0.05$; Table 3) and expressed heterosis, but only starting at 15 months of age. The intensity of heterosis did not vary over time (df = 3, $F = 0.2544$, $P > 0.05$; Table 4). In contrast, the reciprocal hybrid $D_{♀}L_{♂}$, remained intermediate to its parental lines and never expressed heterosis (Table 3).

The calculated dominance ratio (d/a) revealed that hybrids expressing heterosis also had a high dominance ratio and seemed therefore to be more susceptible to non-additive than to additive effects (Table 5). The dominance variance (V_D) was also greater in hybrids that expressed heterosis than in hybrids that did not while no clear pattern emerged from the additive variance (V_A) values. On the other hand, the epistasis variance component was null in all hybrid crosses with the exception of the $D_{♀}R_{♂}$ cross-type at LARSA.

Condition factor, sexual maturity and survival

Even though some hybrid crosses differed from parental lines at certain ages or locations, the effects of hybridization on condition factor were less consistent and marked than those for mass; we thus only present results for mass. The occurrence of sexual maturity varied among cross-types ($P < 0.05$; Fig. 1) and was also greater in males than in females ($P < 0.001$). However, there was no significant effect of rearing environment, and no significant interaction between environment, sex and cross-type on the expression of early sexual maturation (df = 14; $F = 0.65$; $P = 0.82$). The percentage of early sexual maturation was significantly higher in the domestic strain (more than 25%) than in the other two pure crosses (less than 10% in both Laval and Rupert) ($P < 0.001$; Fig. 1). In hybrids, the percentage of

308 animals reaching early sexual maturation was intermediate ($L_{♀}D_{♂}$) or similar (all other hybrid cross-
309 types) to the percentage observed in the parental line expressing the lowest percentage of sexual
310 maturation. Thus, no heterosis or outbreeding depression was observed for the occurrence of early
311 sexual maturity. Finally, survival differed among environments, and mortalities were more numerous in
312 the variable temperature environments ($P < 0.05$; fish farm 58 ± 32%; ISMER 7.25 ± 8.7%; LARSA 1
313 ± 1.3%), but there was no cross-type effect. It is noteworthy that, at the fish farm, predation played an
314 important role in mortalities occurring in the outdoor pond. Overall then, no heterosis or outbreeding
315 depression was observed in the three environments.
316
317 **Discussion**
318
319 This experiment highlights the presence of heterosis for variables related to growth—i.e. mass) —in
320 brook trout using inter-strain crosses and provides no evidence for outbreeding depression. Strong
321 heterosis expression was observed in a few cases that were as high as 24% for mass in some crosses. In
322 general, however, heterosis expression levels were slightly higher or similar to those reported for the
323 same traits in chinook salmon (*Oncorhynchus tshawytscha*, up to 10%; Bryden et al. 2004), Nile and
324 Mphende tilapia (*O. niloticus*; Bentsen et al. 1998; *O. shiranus*; Maluwa and Gjerde 2006; 12% to
325 17%), guppy (*P. reticulata*, 4.5%; Nakadate et al. 2003), and carp (*Labeo rohita*, 10%; Gjerde et al.
326 2002). Also, the expression of heterosis for growth variables varied according to rearing environments
327 and to the strains involved in the cross. No evidence for heterosis was observed for sexual maturity or
328 survival.
329
330 *Genetic distance*

331 The genetic distance between strains involved in hybridization may partly explain the variable patterns
332 of heterosis being expressed (Shikano et al. 2000; Linhart et al. 2002; Wang and Xia 2002; Stelkens et
333 al. 2009). Heterosis is known to be linked to the extant of genetic differentiation between the parental
334 strains owing to local adaptations that can fix different alleles in populations (Falconer and Mackay
335 1996). Yet, some authors found no correlation between genetic distance and heterosis (Bentsen et al.
336 1998), and it was argued that the genetic diversity and dissimilarity among individuals in strains
337 (Shikano and Taniguchi 2002b) or the degree of inbreeding (Nakadate et al. 2003) would be more
338 important factors for the expression of heterosis. Here, it is noteworthy that we observed the highest
339 occurrence of heterosis in intra-specific crosses involving parental populations with the highest level of
340 genetic differentiation, that is between the Rupert and Laval strains with Fst = 0.427 (Martin et al.
341 1997). As mentioned in the Introduction, the three strains used here previously showed no sign of
342 inbreeding, suggesting that genetic divergence more than inbreeding may have been responsible in
343 explaining variable patterns of heterosis observed between the different crosses.
344
345 *Cross direction*
346 The cross direction also played a role in the intensity of heterosis expression for growth. This was
347 particularly evident in hybrid crosses between the Rupert and Laval strains. More generally, the extent
348 of heterosis was more pronounced when the Laval strain was used as dam than when it was used as sire
349 in hybrid crosses involving either the Rupert or the domestic strains. The importance of cross direction
350 in heterosis expression has been reported in other species for different performance traits (resistance to
351 infections in poeciliid fish, Clayton and Price 1994; growth in tilapias, Bentsen et al. 1998; swimming
352 performance in largemouth bass, Cooke et al. 2001). Different factors may explain such reciprocal
353 effects: maternal effects, paternal effects, and genetic linkage between sex genes and performance
354 genes. Maternal effects are generally involved in cross direction, but are more often observed during

355 the early fry development (Klupp 1979; Wangila and Dick 1996; Bentsen et al. 1998; Heath et al. 1999;
356 Perry et al. 2004; Wang et al. 2006b). Paternal effects have also been reported, but their underlying
357 genetic mechanisms are still unclear (Cheng et al. 1987; Bentsen et al. 1998; Gjerde et al. 2002; Wang
358 et al. 2006b). The genetic linkage between sex genes and genes associated with specific traits of
359 performance can result in sex-biased gene expression that may influence the predominance of a specific
360 strain as dam or sire (Nilsson 1993; Bentsen et al. 1998; Ellegren and Parsch 2007; Derome et al.
361 2008). Further investigations are needed to discriminate the influence of each of these factors on
362 heterosis expression.
363
364 *Family effects*
365 Within cross-types, significant family effects were present; some families expressed strong and
366 significant heterosis, while others did not (data not shown). Such differences among families have also
367 previously been observed in carp (Moav and Wohlfarth 1976), rainbow trout (*Salmo gairdneri*; Klupp
368 1979), and guppy (Shikano et al. 2000). However, familial variability was lowest in the L♀R♂ hybrid,
369 which constantly expressed significant heterosis, while in most other crosses, even though some
370 families expressed heterosis, there was no significant outperformance when the cross-type was
371 considered as a whole. Shikano et al. (2000) explained that such family differences could result from
372 differences in the degree of genetic differentiation among parental strains. As already demonstrated by
373 Martin et al. (1997), the Rupert and Laval strains were the most genetically distant.
374
375 *Environment interaction*
376 Genomic influence on performance and heterosis expression is also dependent on environmental
377 conditions. The environment may modify gene expression as previously shown for the physiological
378 pathway of growth in brook trout (Côté et al. 2007). Here, such a modification by the environment was

more important in the L♀D♂ hybrid, which expressed heterosis only in the constant temperature environment. Therefore, heterosis expression in this hybrid seemed to be phenotypically plastic. Other studies have reported the occurrence of heterosis modified by environment in rainbow trout (*Oncorhynchus mykiss*; Ayles and Baker 1983), Nile tilapia (Bentsen et al. 1998) and common carp (Wohlfarth 1993). It should be emphasized that the three environmentals used in this study differed in many other ways, including temperature regime, indoor/outdoor environment, flow-through/recirculation, and tank size and type. Moreover, the limited number of samplings at the fish farm may have limited our capacity to obtain detailed information about hybrid performances at this site, although highly significant heterosis was also detected at this site. Also, it is difficult to identify the specific rearing factors that most influence fish performances. Nevertheless, our primary objective was to assess of different rearing conditions (more than deciphering the precise role of specific environmental parameters) to test if some hybrids would always outperform parental strains independently of the conditions.

In our study, environmental interactions were not observed for all hybrid crosses, suggesting that different genomes are not influenced the same way by environmental variability and therefore revealed the occurrence of genotype (strain combination) by environment interaction. Because of such interactions, the phenotypes of laboratory-reared animals may not reflect the phenotypes that would develop heterosis in other rearing or natural environments (Wohlfarth 1993; Fishback et al. 2002; Sundstrom et al. 2007; Tymchuk et al. 2007). In the absence of an interaction between additive genetic effect and environment, a given breeding program can combine the best strains into a synthetic population (Eknath et al. 1993; Maluwa and Gjerde 2006; Maluwa et al. 2006). An analogous approach could potentially be used in breeding programs related to heterosis expression using hybrids that express heterosis in all environments tested. For example, the L♀R♂ hybrid could be a good candidate

403 for the application of such an approach in brook trout as it expressed heterosis in the three tested
404 rearing environments. On the other hand, in the presence of genotype–environment interactions, the
405 response to selection will be less predictable; it may then be desirable to develop strains for
406 crossbreeding that are specific to each particular environment (Gjedrem 1992). Such an approach could
407 also be adjusted in the presence of heterosis by environment interactions to take full advantage of
408 heterosis expression in aquaculture production. In our study, heterosis expression observed for the
409 $L_{♀}D_{♂}$ hybrid was sensitive to environmental conditions, and the use of such hybrids in production may
410 require that the test and the farm environments be very similar (Bentsen et al. 1998).
411
412 *Variation with ontogeny*
413 We observed that heterosis expression in some hybrid crosses varied over time and was influenced by
414 age or developmental stage in addition to genomic and environmental components. During ontogeny,
415 genes associated with different biological processes can be expressed differentially, and gene
416 expression can also be modified by interactions with other genes (Perry et al. 2005; Wang et al. 2006a;
417 Darias et al. 2008; Nolte et al. 2009) that would affect heterosis expression. Heterosis expression later
418 in development may also result from a larger differentiation among strains with increasing age (Klupp
419 1979; Wang et al. 2006a; Nolte et al. 2009).
420
421 *The genetic basis of heterosis*
422 Even though estimates of the different components of genetic variance were used in a qualitative
423 manner, they provide potential explanatory genetic mechanisms underlying the expression of heterosis.
424 For instance, these estimates point to the importance of dominance effects in the expression of heterosis
425 rather than additive or epistasis effects. This is in accordance with the dominance hypothesis of
426 heterosis expression (Hochholdinger and Hoecker 2007). A previous study of gene expression during

early growth, which used the same hybrid crosses as in this study, revealed that gene expression in hybrid crosses was highly dependent on the specific genetic architecture of parental lines with a prevalence of dominance in heterosis expression. Thus, Bougas et al. (2010) compared transcription profiles among the same three populations of brook charr and their hybrids using microarrays to assess the influence of hybrid origin on modes of transcription regulation inheritance and on the mechanisms underlying growth. They found that hybrids exhibited strikingly different patterns of mode of transcription regulation, being mostly additive (94%) for domestic, and nonadditive for the Laval (45.7%) and Rupert-Laval hybrids (37.5%). Their results also indicated that prevalence of dominance in transcription regulation was related to growth heterosis. In fact, the study of Bougas et al. (2010) clearly showed, for the first time in vertebrates, that the consequences of hybridization on both the transcriptome level and the phenotype are highly dependent on the specific genetic architectures of crossed populations and therefore hardly predictable. As such the parallelism in patterns of heterosis observed here for growth and in Bougas et al. (2010) at the transcriptome level is quite striking.

Conclusion

Intra-specific heterosis is present in brook trout. However, its expression seems complex and difficult to predict, being influenced by a variety of biotic and abiotic factors, including genetic distance between parental lines, strain combination, cross direction, and developmental stage as well as rearing environment. However, one hybrid cross, $L_{♀}R_{♂}$, stood out as the best candidate for using heterosis to enhance brook trout production in various types of environments. Further studies combining the analysis of gene expression and quantitative genetics performed in both F1 hybrids and backcrosses should provide a better understanding of the mechanisms underlying heterosis in fish.

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References

Ayles, G. B. and R. F. Baker. 1983. Genetic differences in growth and survival between strains and hybrids of rainbow trout (*Salmo gairdneri*) stocked in aquaculture lakes in the Canadian prairies. Aquaculture 33:269-280.

Bentsen, H. B., A. E. Eknath, M. S. Palada-de Vera, J. C. Danting, H. L. Bolivar, R. A. Reyes, E. E. Dionisio, F. M. Longalong, A. V. Circa, M. M. Tayamen and B. Gjerde. 1998. Genetic improvement of farmed tilapias: growth performance in a complete diallel cross experiment with eight strains of *Oreochromis niloticus*. Aquaculture 160:145-173.

Birchler, J. A., D. L. Auger and N. C. Riddle. 2003. In search of the molecular basis of heterosis. The Plant Cell 15:2236-2239.

Bougas, B., S. Granier, C. Audet and L. Bernatchez. 2010. The transcriptional landscape of cross-specific hybrids and its possible link with growth in brook charr (*Salvelinus fontinalis* Mitchill). Genetics 186:97-107.

474 Bryden, C. A., J. W. Heath and D. D. Heath. 2004. Performance and heterosis in farmed and wild
475 Chinook salmon (*Oncorhynchus tshawyacha*) hybrid and purebred crosses. Aquaculture
476 235:249-261.
477 Cheng, K. M., I. M. McCallum, R. I. McKay and B. E. March. 1987. A comparison of survival and
478 growth of two strains of chinook salmon (*Oncorhynchus tshawytscha*) and their crosses reared
479 in confinement. Aquaculture 67:301-311.
480 Clayton, G. M. and D. J. Price. 1994. Heterosis in resistance to *Ichthyophthirius multifiliis* infections in
481 poeciliid fish. Journal of Fish Biology 44:59-66.
482 Comings, D. E. and J. P. MacMurray. 2000. Molecular heterosis: A review. Molecular Genetics and
483 Metabolism 71:19-31.
484 Cooke, S. J., T. W. Kassler and D. P. Phillipp. 2001. Physiological performance of largemouth bass
485 related to local adaptation and interstock hybridization: implications for conservation and
486 management. Journal of Fish Biology 59:248-268.
487 Côté, G., G. Perry, P. Blier and L. Bernatchez. 2007. The influence of gene-environment interactions
488 on GHR and IGF-I expression and their association with growth in brook charr, *Salvelinus*
489 *fontinalis* (Mitchill). BMC Genetics 8:87.
490 Darias, M. J., J. L. Zambonino-Infante, K. Hugot, C. L. Cahu and D. Mazurais. 2008. Gene expression
491 patterns during the larval development of European sea bass (*dicentrarchus labrax*) by
492 microarray analysis. Marine Biotechnology (NY) 10:416-28.
493 Derome, N., B. Bougas, S. M. Rogers, A. R. Whiteley, A. Labbe, J. Laroche and L. Bernatchez. 2008.
494 Pervasive sex-linked effects on transcription regulation as revealed by expression quantitative
495 trait loci mapping in lake whitefish species pairs (*Coregonus* sp., Salmonidae). Genetics
496 179:1903-1917.

497 Edmands, S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and
498 outbreeding for conservation and management. Molecular Ecology 16:463-475.
499 Einum, S. and I. A. Fleming. 1997. Genetic divergence and interactions in the wild among native,
500 farmed and hybrid Atlantic salmon. Journal of Fish Biology 50:634-651.
501 Eknath, A. E., M. M. Tayamen, M. S. Paladadevera, J. C. Danting, R. A. Reyes, E. E. Dionisio, J. B.
502 Capili, H. L. Bolivar, T. A. Abella, A. V. Circa, H. B. Bentsen, B. Gjerde, T. Gjedrem and R. S.
503 V. Pullin. 1993. Genetic-improvement of farmed tilapias - the growth-performance of 8 strains
504 of *Oreochromis niloticus* tested in different farm environments. Aquaculture 111:171-188.
505 Ellegren, H. and J. Parsch. 2007. The evolution of sex-biased genes and sex-biased gene expression.
506 Nature Reviews Genetics 8:689-698.
507 Falconer, D. S. and T. F. C. Mackay. 1996. Introduction to quantitative genetics, 4th edition. Longman
508 Group, Essex, UK.
509 Fishback, A. G., R. G. Danzmann, M. M. Ferguson and J. P. Gibson. 2002. Estimates of genetic
510 parameters and genotype by environment interactions for growth traits of rainbow trout
511 (*Oncorhynchus mykiss*) as inferred using molecular pedigrees. Aquaculture 206:137-150.
512 Fraser, J. M. 1981. Comparative survival and growth of planted wild, hybrid, and domestic strains of
513 brook trout (*Salvelinus fontinalis*) in Ontario lakes. Canadian Journal of Fisheries and Aquatic
514 Sciences 38:1672-1684.
515 Garcia de Leaniz, C., I. A. Fleming, S. Einum, E. Verspoor, W. C. Jordan, S. Consuegra, N. Aubin-
516 Horth, D. Lajus, B. H. Letcher, A. F. Youngson, J. H. Webb, L. A. Vollestad, B. Villanueva, A.
517 Ferguson and T. P. Quinn. 2007. A critical review of adaptive genetic variation in Atlantic
518 salmon: implications for conservation. Biological Reviews 82:173-211.
519 Gharrett, A. J., W. W. Smoker, R. R. Reisenbichler and S. G. Taylor. 1999. Outbreeding depression in
520 hybrids between odd- and even-broodyear pink salmon. Aquaculture 173:117-129.

521 Gjedrem, T. 1992. Breeding plans for rainbow trout. Aquaculture 100:73-83.
522 Gjerde, B., P. V. G. K. Reddy, K. D. Mahapatra, J. N. Saha, R. K. Jana, P. K. Meher, M. Sahoo, S.
523 Lenka, P. Govindassamy and M. Rye. 2002. Growth and survival in two complete diallele
524 crosses with five stocks of Rohu carp (*Labeo rohita*). Aquaculture 209:103-115.
525 Gjerde, B. and T. Refstie. 1984. Complete diallel cross between five strains of Atlantic salmon.
526 Livestock Production Science 11:207-226.
527 Glover, K. A., O. Bergh, H. Rudra and O. Skaala. 2006. Juvenile growth and susceptibility to
528 *Aeromonas salmonicida* subsp *salmonicida* in Atlantic salmon (*Salmo salar* L.) of farmed,
529 hybrid and wild parentage. Aquaculture 254:72-81.
530 Heath, D. D., C. W. Fox and J. W. Heath. 1999. Maternal effects on offspring size: variation through
531 early development of chinook salmon. Evolution 53:1605-1611.
532 Hochholdinger, F. and N. Hoecker. 2007. Towards the molecular basis of heterosis. Trends in Plant
533 Science 12:427-432.
534 Hoffmann, A. A. and J. Merilä. 1999. Heritable variation and evolution under favourable and
535 unfavourable conditions. Trends in Ecology & Evolution 14:96-101.
536 Hulata, G. 1995. A review of genetic improvement of the common carp (*Cyprinus carpio* L) and other
537 cyprinids by crossbreeding, hybridization and selection. Aquaculture 129:143-155.
538 Klupp, R. 1979. Genetic variance for growth in rainbow trout (*Salmo gairdneri*) Aquaculture 18:123-
539 134.
540 Linhart, O., D. Gela, M. Rodina, V. Slechtova and V. Slechta. 2002. Top-crossing with paternal
541 inheritance testing of common carp (*Cyprinus carpio* L.) progeny under two altitude conditions.
542 Aquaculture 204:481-491.
543 Lippman, Z. B. and D. Zamir. 2007. Heterosis: revisiting the magic. Trends in Genetics 23:60-66.

544 Maluwa, A. O. and B. Gjerde. 2006. Genetic evaluation of four strains of *Oreochromis shiranus* for
545 harvest body weight in a diallel cross. Aquaculture 259:28-37.
546 Maluwa, A. O., B. Gjerde and R. W. Ponzoni. 2006. Genetic parameters and genotype by environment
547 interaction for body weight of *Oreochromis shiranus*. Aquaculture 259:47-55.
548 Marengoni, N. G., Y. Onoue and T. Oyama. 1998. Offspring growth in a diallel crossbreeding with
549 three strains of Nile tilapia *Oreochromis niloticus*. Journal of the World Aquaculture Society
550 29:114-119.
551 Martin, S., J.-Y. Savaria, C. Audet and L. Bernatchez. 1997. Microsatellites reveal no evidence for
552 inbreeding effects but low inter-stock genetic diversity among brook charr stocks used for
553 production in Quebec. Bulletin of the Aquaculture Association of Canada 97:21-23.
554 McClelland, E. K. and K. A. Naish. 2007. What is the fitness outcome of crossing unrelated fish
555 populations? A meta-analysis and an evaluation of future research directions. Conservation
556 Genetics 8:397-416.
557 Moav, R. and G. W. Wohlfarth. 1976. Two-way selection for growth rate in the common carp
558 (*Cyprinus carpio* L.). Genetics 82:83-101.
559 Nakadate, M., T. Shikano and N. Taniguchi. 2003. Inbreeding depression and heterosis in various
560 quantitative traits of the guppy, *Poecilia reticulata*. Aquaculture 220:219-226.
561 Nielsen, H. M., J. Odegard, I. Olesen, B. Gjerde, L. Ardo, G. Jeney and Z. Jeney. 2010. Genetic
562 analysis of common carp (*Cyprinus carpio*) strains. I: Genetis parameters and heterosis for
563 growth traits and survival. Aquaculture 304:7-13.
564 Nilsson, J. 1993. Arctic charr strain crosses - effects on growth and sexual maturity. Journal of Fish
565 Biology 43:163-171.

566 Nolte, A. W., S. Renaut and L. Bernatchez. 2009. Divergence in gene regulation at young life history
567 stages of whitefish (*Coregonus* sp.) and the emergence of genomic isolation. BMC
568 Evolutionary Biology 9:59.
569 Perry, G. M. L., C. Audet and L. Bernatchez. 2005. Maternal genetic effects on adaptive divergence
570 between anadromous and resident brook charr during early life history. Journal of Evolutionary
571 Biology 18:1348-1361.
572 Perry, G. M. L., C. Audet, B. Laplatte and L. Bernatchez. 2004. Shifting patterns in genetic control at
573 the embryo-alevin boundary in brook charr. Evolution 58:2002-2012.
574 Shikano, T., M. Nakadate and Y. Fujio. 2000. An experimental study on strain combinations in
575 heterosis in salinity tolerance of the guppy *Poecilia reticulata*. Fisheries Science 66:625-632.
576 Shikano, T. and N. Taniguchi. 2002a. Heterosis for neonatal survival in the guppy. Journal of Fish
577 Biology 60:715-725.
578 Shikano, T. and N. Taniguchi. 2002b. Using microsatellite and RAPD markers to estimate the amount
579 of heterosis in various strain combinations in the guppy (*Poecilia reticulata*) as a fish model.
580 Aquaculture 204:271-281.
581 Sogard, S. M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin of
582 Marine Science 60:1129-1157.
583 Sokal, R. R. and F. J. Rohlf. 1981. Biometry: the principles and practice of statistics in biological
584 research, 2nd edition. W H Freeman, San francisco, USA.
585 Stelkens, R. B., C. Schmid, O. Selz and O. Seehausen. 2009. Phenotypic novelty in experimental
586 hybrids is predicted by the genetic distance between species of cichlid fish. BMC Evolutionary
587 Biology 9:283.

588 Sundstrom, L. F., M. Lohmuis, W. E. Tymchuk and R. H. Devlin. 2007. Gene-environment interactions
589 influence ecological consequences of transgenic animals. Proceedings of the National Academy
590 of Sciences of the United States of America 104:3889-3894.
591 Thériault, V., D. Garant, L. Bernatchez and J. J. Dodson. 2007. Heritability of life-history tactics and
592 genetic correlation with body size in a natural population of brook charr (*Salvelinus fontinalis*).
593 Journal of Evolutionary Biology 20:2266-2277.
594 Tymchuk, W. E., L. F. Sundstrom and R. H. Devlin. 2007. Growth and survival trade-offs and
595 outbreeding depression in rainbow trout (*Oncorhynchus mykiss*). Evolution 61:1225-1237.
596 Wang, C. H., S. F. Li, Z. G. Liu, S. P. Xiang, J. Wang, Z. Y. Pang and J. P. Duan. 2006a.
597 Developmental quantitative genetic analysis of body weight and morphological traits in red
598 common carp, *Cyprinus carpio* L. Aquaculture 251:219-230.
599 Wang, I. A., S. E. Gilk, W. W. Smoker and A. J. Gharrett. 2007. Outbreeding effect on embryo
600 development in hybrids of allopatric pink salmon (*Oncorhynchus gorbuscha*) populations, a
601 potential consequence of stock translocation. Aquaculture 272:S152-S160.
602 Wang, J. and D. Xia. 2002. Studies on fish heterosis with DNA fingerprinting. Aquaculture Research
603 33:941-947.
604 Wang, X. X., K. E. Ross, E. Saillant, D. M. Gatlin and J. R. Gold. 2006b. Quantitative genetics and
605 heritability of growth-related traits in hybrid striped bass (*Morone chrysops* female x *Morone*
606 *saxatilis* male). Aquaculture 261:535-545.
607 Wangila, B. C. C. and T. A. Dick. 1996. Genetic effects and growth performance in pure and hybrid
608 strains of rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Order: Salmoniformes, Family:
609 Salmonidae). Aquaculture Research 27:35-41.

610 Webster, D. A. and W. A. Flick. 1981. Performance of indigenous, exotic, and hybrid strains of brook
611 trout (*Salvelinus fontinalis*) in waters of the Adirondack Mountains, New York. Canadian
612 Journal of Fisheries and Aquatic Sciences 38:1701-1707.
613 Wilson, A. J., J. A. Hutchings and M. M. Ferguson. 2003. Selective and genetic constraints on the
614 evolution of body size in a stream-dwelling salmonid fish. Journal of Evolutionary Biology
615 16:584-594.
616 Wohlfarth, G. W. 1993. Heterosis for growth-rate in common carp. Aquaculture 113:31-46.
617 Wu, Z. X. and M. D. Li. 2002. Analysis of heterosis by a direct method using the concept of
618 heritability. Genetica 114:163-170.
619
620

621 **Figure Caption**

622

623 Fig. 1: Early maturation in the three purebred strains and their hybrids. No environment effect was
624 observed, so data from the three study sites were pooled. The first letter of the cross-type indicates the
625 dam and the second letter the sire. Solid bars are for females and open bars for males. Statistical
626 analyses were done on arcsin-transformed data but results are presented as arithmetical means ± SE.
627 Number of families (n) is indicated in parenthesis. Cross-types with different letters are significantly
628 different ($P < 0.05$).

629

Table 1: Number of fish per family in the different rearing environments (indoor, running freshwater, seasonal temperature variations [ISMER]; indoor, recirculating water, constant 10°C temperature conditions [LARSA]) for each age stage. Percentages refer to the reduction in fish number compared to the initial number**.**

Environment	7 months	9 months	11 months	13 months	15 months	17 months	18 months	21 months
ISMER	230	230	190 (-17%)	120(-48%)	120 (-48%)	110(-52%)	60 (-74%)	60 (-74%)
LARSA	150	150	150	150	100 (-33%)	100 (-33%)	50 (-67%)	50 (-67%)

Table 2: Summary of statistical analyses for body mass. Model A includes two environments (indoor, running freshwater, seasonal temperature variations [ISMER]; indoor, recirculating water, constant 10°C temperature conditions [LARSA]) at each age stage; Model B includes the three environments (ISMER; LARSA; outdoor, seasonal temperature variations, fish farm pond [Farm]) at the two age stages (17 and 21 months) measured at the farm.

	Model A				Model B			
	df	mean squares	*F*	*P*-value	df	mean squares	*F*	*P*-value
Age stage	6	444.18	12,635.9	< 0.001	1	135.91	3,320.5	< 0.001
Environment	1	591.98	16,840.5	< 0.001	2	102.24	2,497.9	< 0.001
Cross-type	7	92.20	34.4	< 0.001	7	14.29	349.2	< 0.001
Age stage × Environment	6	21.28	605.4	< 0.001	2	16.74	409.0	< 0.001
Age stage × Cross-type	42	0.49	13.9	< 0.001	7	0.05	1.2	0.28
Environment × Cross-type	7	6.48	184.4	< 0.001	14	2.21	54.0	< 0.001
Age stage × Environment × Cross-type	42	0.33	9.5	< 0.001	14	0.18	4.3	< 0.001
Family (nested in Cross-type), random	64	2.93	83.3	< 0.001				
Error	28,022	0.04			11,587	0.04		
Model R^2	0.82				0.64			
R^2 adjusted	0.82				0.64			

Table 3: Growth performance measured as body mass (g) in the purebred strains (bold) and their hybrids in the three different environments (indoor, running freshwater, seasonal temperature variations [ISMER]; indoor, recirculating water, constant 10°C temperature conditions [LARSA]; outdoor, seasonal temperature variations, fish farm pond [Farm]) for each age stage. Statistical analyses were done on log-transformed data, and post-hoc analyses on least square means, but results are presented as arithmetical means ± SE (n [number of families] = 10 for $D_{♀}D_{♂}$, $L_{♀}L_{♂}$, and $L_{♀}R_{♂}$; 9 for $R_{♀}R_{♂}$, $D_{♀}R_{♂}$, and $L_{♀}D_{♂}$; 8 for $R_{♀}L_{♂}$; and 7 for $D_{♀}L_{♂}$). Different letters indicate significant differences among cross-types for one environment and one age stage ($P < 0.05$). Grey highlights indicate hybrids that are significantly higher than both of their parental lines (heterosis).

Cross	9 months	11 months	13 months	15 months	17 months	18 months	21 months
ISMER							
$D_{♀}R_{♂}$	18.4 ± 1.2 w	25.1 ± 1.7 w	25.8 ± 2.2 w	34.2 ± 3.0 x	42.5 ± 4.2 v	58.7 ± 4.3 x	121.7 ± 6.7 x
$D_{♀}D_{♂}$	**23.6 ± 2.2 v**	**39.7 ± 3.7 v**	**34.6 ± 3.6 v**	**45.2 ± 4.6 w**	**65.1 ± 6.7 u**	**100.5 ± 8.2 w**	**197.6 ± 11.9 w**
$D_{♀}L_{♂}$	16.7 ± 1.0 w	24.5 ± 1.4 w	25.3 ± 1.7 w	29.6 ± 2.2 x	41.0 ± 1.9 v	60.6 ± 3.5 x	124.3 ± 6.4 x
$L_{♀}D_{♂}$	16.4 ± 1.1 w	25.6 ± 1.9 w	25.2 ± 1.8 w	32.3± 2.6 x	46.2 ± 3.5 v	66.9 ± 4.5 x	128.8 ± 5.2 x
$L_{♀}L_{♂}$	**6.8 ± 0.2 z**	**9.1 ± 0.4 z**	**7.9 ± 0.3 z**	**8.4 ± 0.3 z**	**16.2 ± 0.5 z**	**35.3 ± 1.4 z**	**68.8 ± 2.1 z**
$L_{♀}R_{♂}$	11.9 ± 0.9 x	16.7 ± 1.7 x	16.2 ± 1.8 x	19.2 ± 2.2 x	29.2 ± 2.3 w	41.6 ± 2.7 y	83.2 ± 4.2 y
$R_{♀}L_{♂}$	9.3 ± 0.6 y	15.0 ± 0.9 yx	14.2 ± 1.2 y	16.1 ± 1.4 y	23.9 ± 2.1 x	36.8 ± 3.7 zy	71.8 ± 6.0 z
$R_{♀}R_{♂}$	**9.5 ± 0.6 y**	**12.6 ± 0.8 y**	**12.6 ± 0.8 y**	**14.8 ± 0.8 y**	**20.1 ± 1.3 y**	**31.5 ± 2.0 z**	**66.9 ± 4.5 z**
LARSA							

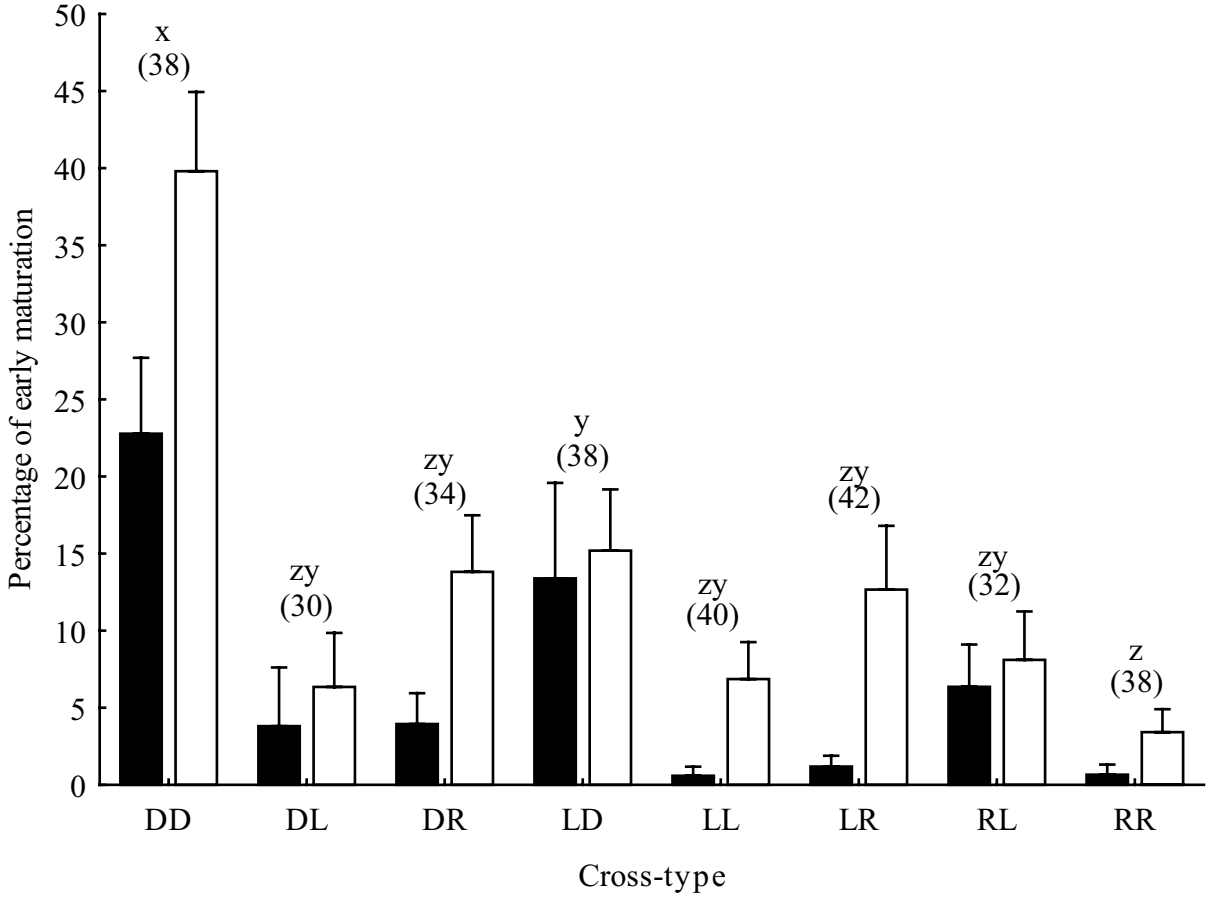
D♀R♂	23.5 ± 1.8 wv	43.0 ± 4.0 wv	69.0 ± 7.2 v	88.9 ± 11.0 w	103.7 ± 11.9 x	123.4 ± 13.5 x	183.8 ± 20.1 w
D♀D♂	**29.0 ± 3.0 v**	**50.1 ± 4.7 v**	**82.4 ± 6.4 vu**	**109.6 ± 10.8 v**	**121.5 ± 10.2 w**	**148.0 ± 12.5 w**	**217.6 ± 15.5 v**
D♀L♂	20.7 ± 1.4 w	33.4 ± 2.2 x	47.5 ± 3.4 xw	62.6 ± 4.1 yx	68.7 ± 3.3 y	83.3 ± 4.0 y	134.1 ± 7.4 y
L♀D♂	24.3 ± 1.9 wv	50.3 ± 4.9 v	86.0 ± 9.9 u	114.9 ± 14.3 u	133.6 ± 16.1 v	165.1 ± 21.4 v	241.1 ± 27.3 u
L♀L♂	**9.4 ± 0.5 z**	**18.8 ± 1.4 z**	**30.4 ± 2.7 z**	**43.1 ± 3.0 z**	**54.8 ± 4.1 z**	**67.1 ± 4.6 z**	**106.3 ± 6.4 z**
L♀R♂	15.3 ± 0.9 x	30.5 ± 2.6 x	56.2 ± 5.4 w	70.5 ± 4.6 x	85.5 ± 7.8 x	107.1 ± 9.2 x	155.7 ± 9.7 x
R♀L♂	13.2 ± 0.9 yx	23.0 ± 2.1 y	39.1 ± 4.3 y	56.6 ± 5.8 y	73.5 ± 7.6 yx	79.9 ± 7.5 zy	129.7 ± 12.9 y
R♀R♂	**11.8 ± 0.8 y**	**23.6 ± 1.3 y**	**41.9 ± 2.2 yx**	**54.7 ± 2.0 y**	**72.1 ± 3.2 y**	**82.3 ± 4.5 y**	**126.9 ± 7.7 y**
Farm							
D♀R♂					46.0 ± 3.0 w		125.6 ± 4.8 v
D♀D♂					**87.4 ± 7.4 v**		**199.8 ± 13.1 wv**
D♀L♂					43.7 ± 1.8 xw		117.9 ± 3.9 xw
L♀D♂					35.8 ± 2.3 xw		97.8 ± 2.6 w
L♀L♂					**16.6 ± 0.8 z**		**39.4 ± 2.2 z**
L♀R♂					29.8 ± 3.4 y		67.6 ± 4.7 y
R♀L♂					36.6 ± 5.3 yx		97.8 ± 4.4 yx
R♀R♂					**16.0 ± 1.4 z**		**35.1 ± 8.6 z**

Table 4: Heterosis intensity for each cross presenting a trait performance significantly higher than the performance of its two parental lines in the three environments (indoor, running freshwater, seasonal temperature variations [ISMER]; indoor, recirculating water, constant 10°C temperature conditions [LARSA]; outdoor, seasonal temperature variations, fish farm pond [Farm]), and for each age stage. Heterosis intensity was calculated as $[(f_1/m) - 1] \times 100$, where f_1 is the mean performance of the F_1 hybrids and m the mean performance of parental strains. Mean ± SE.

	Cross	9 months	11 months	13 months	15 months	17 months	18 months	21 months
ISMER	$L_{♀}R_{♂}$	18.5 ± 3.9	17.0 ± 4.5	19.0 ± 5.3	20.3 ± 5.0	16.1 ± 2.7	6.1 ± 1.8	4.9 ± 1.1
	$R_{♀}L_{♂}$					9.2 ± 3.0		
LARSA	$L_{♀}D_{♂}$				11.7± 2.7	10.7 ± 2.6	10.3 ± 2.7	8.7 ± 2.1
	$L_{♀}R_{♂}$	16.4 ± 2.5	11.8 ± 3.0	12.3 ± 3.1	9.6 ± 1.8	7.0 ± 2.4	8.2 ± 2.1	6.2 ± 1.4
Farm	$L_{♀}R_{♂}$					18.1 ± 3.7		16.5± 1.8
	$R_{♀}L_{♂}$					23.8 ± 4.2		22.8 ± 1.2

Table 5: Dominance ratio (d/a) at each age stage and contribution of the different genetic components (V_A: additive variance; V_D: dominance variance; V_I: epistasis variance) to the phenotypic variance (Wu et al. 2002) expressed in each cross-type and in two different environments (indoor, running freshwater, seasonal temperature variations [ISMER]; indoor, recirculating water, constant 10°C temperature conditions [LARSA]). Negative values were defined to be equal to zero.

	9 months	11 months	13 months	15 months	17 months	18 months	21 months	Pooled sampling times			
Cross	d/a	d/a	d/a	d/a	d/a	d/a	d/a	d/a	V_A	V_D	V_I
ISMER											
D♀R♂	0.26	0.08	0.21	0.27	0.01	0.22	0.18	0.07	1248.8	3.2	0
D♀L♂	0.18	0.00	0.31	0.15	0.01	0.22	0.13	0.04	1338.3	1.0	0
L♀D♂	0.15	0.08	0.30	0.30	0.23	0.03	0.07	0.07	1388.3	3.1	0
L♀R♂	2.81	3.36	2.58	2.36	5.61	4.56	28.92	6.04	472.0	8611.6	0
R♀L♂	0.97	2.40	1.70	1.40	2.90	1.79	4.86	2.70	409.6	1494.7	0
LARSA											
D♀R♂	0.36	0.46	0.34	0.25	0.28	0.25	0.51	0.36	2466.1	155.5	129.0
D♀L♂	0.15	0.07	0.34	0.42	0.59	0.60	0.47	0.43	1520.0	140.2	0
L♀D♂	0.52	1.01	1.14	1.16	1.36	1.45	1.56	1.31	3055.4	2606.7	0
L♀R♂	3.83	3.81	3.51	3.91	2.56	4.28	3.62	3.56	1448.2	9188.0	0
R♀L♂	2.08	0.74	0.52	1.41	1.16	0.69	1.62	1.15	1243.4	817.1	0


50
45
40
35
30
25
20
15
10
5
0
Percentage of early maturation
x
(38)
zy
(30)
zy
(34)
y
(38)
zy
(40)
zy
(42)
zy
(32)
z
(38)
DD
DL
DR
LD
LL
LR
RL
RR
Cross-type