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Abstract: Selection for stress resistance to transport has been identified as a target for genetic improvement in fish production. However, few studies have investigated the potential use of heterosis (i.e., hybrid vigour) to improve this trait, as well as specifically testing if hybrids are less sensitive to stress exposure than their parental lines. Three strains (domestic [D], Laval [L], and Rupert [R]) of brook charr (Salvelinus fontinalis) and their reciprocal hybrids were submitted to transport stress to measure stress resistance. Primary (cortisol) and secondary (glucose, osmolality, and haematocrit) stress responses were measured for each cross. Significant heritabilities were observed for both levels of stress response, with h2 = 0.60 (± 0.20) for plasma cortisol and 0.61 (± 0.20) for plasma glucose. We observed strain differences whereby the Rupert strain was the least sensitive to stress at the primary and secondary levels. No heterosis was detected, and only one case of outbreeding depression was present. The outbreeding depression was observed in the D♀R♂ hybrid, which had a 27% increase of plasma glucose compared to parental strains. The D♀R♂ and R♀L♂ hybrids had more pronounced variations (increase or decrease) in plasma osmolality than their respective parental strains, but these variations were difficult to relate definitively with the potential secondary stress response. These results indicate a strong potential for genetic improvement in the stress response to transport with the use of purebred crosses while hybridization has little value in this regard.

Quantitative genetic analysis of the physiological stress response in three strains of brook charr, *Salvelinus fontinalis* (Mitchill), and their hybrids

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ABSTRACT

Selection for stress resistance to transport has been identified as a target for genetic improvement in fish production. However, few studies have investigated the potential use of heterosis (i.e., hybrid vigour) to improve this trait, as well as specifically testing if hybrids are less sensitive to stress exposure than their parental lines. Three strains (domestic [D], Laval [L], and Rupert [R]) of brook charr (*Salvelinus fontinalis*) and their reciprocal hybrids were submitted to transport stress to measure stress resistance. Primary (cortisol) and secondary (glucose, osmolality, and haematocrit) stress responses were measured for each cross. Significant heritabilities were observed for both levels of stress response, with h^2 = 0.60 (± 0.20) for plasma cortisol and 0.61 (± 0.20) for plasma glucose. We observed strain differences whereby the Rupert strain was the least sensitive to stress at the primary and secondary levels. No heterosis was detected, and only one case of outbreeding depression was present. The outbreeding depression was observed in the D♀R♂ hybrid, which had a 27% increase of plasma glucose compared to parental strains. The D♀R♂ and R♀L♂ hybrids had more pronounced variations (increase or decrease) in plasma osmolality than their respective parental strains, but these variations were difficult to relate definitively with the potential secondary stress response. These results indicate a strong potential for genetic improvement in the stress response to transport with the use of purebred crosses while hybridization has little value in this regard.

Key words: stress resistance; heterosis; heritability; brook charr

INTRODUCTION

During aquaculture and stocking activities, fish are faced with several potential stressors. In particular, transportation, but also capture and handling procedures, a highly crowded and confined farming environment, possible air exposure, variation in water quality are all factors that may increase the stress level of organisms (Barton & Iwama, 1991; Iwama *et al.*, 1999; Barton, 2002; Hur *et al.*, 2007). Such stressors may disturb the organism's homeostatic equilibrium, and fish need to compensate by physiological and biochemical changes (Barton & Iwama, 1991; Iwama *et al.*, 1999; Barton, 2002). Three main levels of stress response have been identified (Barton & Iwama, 1991; Iwama *et al.*, 1999; Barton, 2002). The primary neuroendocrine response involves the release of stress hormones—catecholamines and cortisol—into the blood. Biochemical and physiological secondary responses associated with the release of stress hormones activate metabolic pathways that result in the modification of blood chemistry and haematology, including a rapid release of glucose to provide sufficient energy, changes in osmolarity, and lysozyme activity. Finally, tertiary whole-organism and population responses are characterized by changes in the energy supply to the different biological pathways and in population productivity, resulting in negative impacts on growth rate, reproductive success, disease and parasite resistance, saltwater tolerance, and survival (Barton & Iwama, 1991; Fevolden *et al.*, 1991; Pickering, 1993; Barton, 2002; Davis, 2006; Liebert & Schreck, 2006). Therefore, fish with reduced stress response may have an advantage in farming conditions compared to more stress-prone individuals (Fevolden *et al.*, 1991; Fevolden *et al.*, 1993; Pickering, 1993).

Differences in the intensity of the stress response have been reported among families and strains of rainbow trout (*Oncorhynchus mykiss* Walbaum) and Atlantic salmon (*Salmo salar* Linnaeus), among strains of fighting fish (*Betta splendens* Regan), and among species of tilapia (*Oreochromis* spp.), guppy (*Poeciliopsis* spp.), and charr (*Salvelinus* spp.) (Bulger & Schultz, 1982; Fevolden *et al.*, 1991; McDonald *et al.*, 1993; Pottinger & Moran, 1993; Cnaani *et al.*, 2004; Verbeek *et al.*, 2008). For example, brook charr (*Salvelinus fontinalis* Mitchill) are less sensitive to transport and net confinement stress (reduced ion loss) compared to lake trout (*Salvelinus namaycush* Walbaum) (McDonald *et al.*, 1993). Furthermore, quantitative genetic studies have revealed a moderate to high degree of heritability of the cortisol response for different fishes including carp (*Cyprinus carpio* Linnaeus, 0.60, Tanck *et al.*, 2001) and rainbow trout (*O. mykiss*, 0.56 for North American lines, Weber *et al.*, 2008; 0.50 for European lines, Fevolden *et al.*, 2002). Given such additive genetic components, stress resistance—and more specifically variation in stress-induced cortisol concentration—has been identified as a trait of interest for genetic improvement (Fevolden *et al.*, 1991; Lankford & Weber, 2006). However, studies using selective breeding programs for disease resistance or growth that aim to improve fish performance via a lower cortisol response have met with limited success thus far (Lankford & Weber, 2006; Weber & Silverstein, 2007).

Another approach that can be considered for the genetic improvement of physiological traits is the production of hybrid crosses that may result in heterosis (i.e., hybrid vigour), which is the improved performance of first generation progeny compared to parental lines (Falconer & Mackay, 1996). Heterosis is the most important non-additive effect on cross performance and is usually stronger when parental lines are genetically distant from each other (Shikano *et al.*, 2000; Wang & Xia, 2002). This phenomenon is now being used in improvement schemes concerning traits of interest in aquaculture, including growth rate, survival, and salinity tolerance (Bentsen *et al.*, 1998; Shikano & Taniguchi,

2002; Bryden *et al.*, 2004; Hena *et al.*, 2005). Until now, very few studies have investigated the importance of heterosis on stress response in fish (Campbell *et al.*, 1998; Bryden *et al.*, 2004).

The main objective of this study was to test for the occurrence and to quantify the importance of heterosis in the physiological stress response by comparing three pure strains of brook charr (*S. fontinalis*) and their F1 hybrids. More specifically, the effects of stress induced by transportation, a common activity in aquaculture that often results in mortality, were investigated. A second objective was to estimate heritability values for primary (plasma cortisol) and secondary (plasma glucose, plasma osmolality, and haematocrit) stress indicators for the first time in brook charr and to compare the observed values with other fishes. In this way, the present study planned to evaluate the relative merits of hybrid crosses and selective breeding for improving the response of brook charr to stress in an aquaculture context.

MATERIALS AND METHODS

BROOK CHARR STRAINS

Three genetically distinct strains of brook charr (Martin *et al.*, 1997) were used as parental lines. The Laval strain originates from a wild population of anadromous brook charr from the Laval River (48°44′N; 69°05′W) on the north shore of the St. Lawrence Estuary (QC, Canada). The fish used were third generation breeders reared in captivity at the Station aquicole ISMER/UQAR (Rimouski, QC,

Canada). The Rupert strain originates from a freshwater-resident wild population inhabiting the Rupert River system (51°05′N; 73°41′W) (QC, Canada). The fish used as breeders were also from the third generation produced in captivity at the Laboratoire régional en sciences aquatiques (LARSA, Université Laval, Québec, QC, Canada). Finally, the so-called "Domestic" strain is the main one being used by the Québec fish farming industry and it originates from two strains (Nashua and Baldwin). Breeders used in this study were obtained from the Pisciculture de la Jacques Cartier (Cap-Santé, QC, Canada). The two wild strains were selected for breed improvement because adults from these populations exhibit late sexual maturation and large adult size.

BREEDING DESIGN

Hybrid and purebred crosses were made from mid-November to the end of December 2005 at LARSA using eggs and milt obtained from the different fish rearing locations. Three purebred crosses were produced: ♀ domestic × ♂ domestic ($D_{♀}D_{♂}$), ♀ Laval × ♂ Laval ($L_{♀}L_{♂}$), and ♀ Rupert × ♂ Rupert ($R_{♀}R_{♂}$). Five hybrid and reciprocal hybrid crosses were also 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 long time lag in sexual maturation between these two strains (October for the domestic males and December for the Rupert females). All breeders were used only once; their mass and length measurements are presented in Table I. For each cross, 10 full-sib families were obtained through single-pair mating. Milt was used fresh (immediately after collection) without any additive. The numbers of eggs fertilized for each female were not counted and all were incubated. The number of fry per family was equalized after exogenous feeding had begun. Eight of the resulting 80 families were eliminated due to the limited number of individuals that could be pooled in each tank.

FAMILY REARING

From egg incubation (January) to exogenous feeding (June), each family was incubated separately in individual clays, and each incubation tank contained 12 clays. Water temperature was maintained at 6°C during egg incubation and at 8°C after hatching. The photoperiod was set at 12L:12D. In June, families were identified using different combinations of adipose and pelvic fin clippings and transferred to nine 3 m^3 tanks, with eight families pooled per tank. All families were brought to 2136 degrees-days by the end of the summer and maintained at 10°C in recirculating fresh water. Photoperiod followed the natural seasonal cycle, and fish were fed according to commercial charts with commercial pellets. In September, fish were transferred in transport bags (one family per bag) immediately to the Station aquicole ISMER/UQAR. Here they were reared in ten 0.5 m^3 indoor tanks, with six to eight families per tank, under natural temperature and photoperiod conditions in running dechlorinated fresh water. Fish were fed daily (1% w/w ration) with commercial dry pellets. No mortality difference was observed among cross types during the whole rearing period. There was no disease occurrence, and prophylactic treatments (chloramines T) were applied following marking and weight and length measurements.

STRESS EXPOSURE

A simulation of fish transfer procedures in transport bags was conducted in June 2007 to induce stress in 16-month-old fish. Twenty fish per cross were used for this experiment. The fish were captured in tanks, taking care that a similar number of fish from the different families within each cross-type were chosen, i.e., 2 to 3 fish per family, and randomly distributed among bags. Each transport bag (30 cm in diameter, 100 cm in length) contained 10 fish that were kept in 1/3 dechlorinated fresh water (same water source as the holding tanks) and 2/3 compressed oxygen (16 bags with a total of 160 fish). Transportation bags were kept in the dark and shaken every 30 min for 10 s. Fish were kept in the bags for 4 h, which is long enough to induce an intense stress response in brook charr (McDonald *et al.*, 1993). After 4 h, the bags were put into fresh water to let the temperature gradually decrease to the tank temperature (about 20 min), and fish were then sampled. Twenty fish per cross were also sampled directly from fish tanks and used as controls. No mortality was observed in transport bags or rearing tanks during the experiment.

SAMPLING PROCEDURES

All samplings were made between 16:00 and 19:00 to avoid bias due to endocrine circadian rhythms. Stressed and control fish were anaesthetized in MS 222 (0.16 g l^{-1} [3-aminobenzoic acid ethyl ester]) and their body mass (to the nearest 0.1 g) and fork length (0.1 cm) were measured (Tables II and III). Blood was collected by caudal puncture using ammonium-heparinized syringes. A small quantity of blood was transferred to capillary tubes for haematocrit determination and the remainder was centrifuged at 7200*g* for 3 min. The plasma was drawn off, quickly frozen in liquid nitrogen, and then stored at -80°C until analysis. Plasma osmolality was measured with an Advanced Micro-osmometer

3MO, plasma glucose was measured by enzymatic determination (Alexander & Griffiths, 1993), and cortisol levels were measured using a cortisol ^{125}I RIA kit (MP Biomedicals, Orangeburg, NY, USA).

STATISTICAL ANALYSES

Data normality and homogeneity of variance were tested with Kolmogorov-Smirnov and Brown-Forsythe tests, respectively. Plasma cortisol concentrations were log transformed to obtain normality. The variability between replicate transport bags was tested using ANOVA and was not significant (results not shown). The different variables were analyzed using two-way ANOVAs with cross-type, stress treatment, and stress treatment × cross-type interaction as fixed effects. The effect of dam and sire origin (domestic, Laval, or Rupert) on each physiological variable after stress exposure was analyzed using two-way ANOVAs with dam and sire origin as factors. The presence of heterosis or outbreeding depression was determined by the presence of a significant difference between the mean performance of hybrids compared to the mean performance of both parental strains (Bryden *et al.*, 2004). Heterosis was expressed when there was a lower stress response in hybrids compared to parental lines. *A posteriori* Tukey tests were used for mean comparisons when possible or replaced by Games and Howell tests when variances were not homogenous. The influence of fish mass on variables was examined using mass as a covariate in ANCOVAs. Analyses were made using Statistica version 6.0 for Windows (StatSoft, Tulsa, OK, USA). A significance level of $\alpha = 0.05$ was used in all statistical tests.

HERITABILITY ANALYSES

Our breeding design was used to fit animal models (Lynch & Walsh, 1998) with the ASReml software (V2.0; Gilmour *et al.*, 2006). Univariate analyses were used to decompose the phenotypic variance (V_P) of each trait for the whole fish population (including pure and hybrid crosses) into their additive genetic (V_A) and residual (V_R) variances. The model was the following:

$$y = \mu + C + A + e$$

where y is the phenotypic observation, μ is the overall mean, C is the fixed effect of the cross-type, A is the random additive genetic effect, and e is the random residual effect. The narrow-sense heritability (h^2) for each trait was estimated as the ratio of the additive genetic variance (V_A) to the total phenotypic variance (V_P): $h^2 = V_A/V_P$. The statistical significance of the additive genetic component for each trait was tested by re-running a restricted model where the additive variance was set to zero and then comparing the difference the in log-likelihood ratio between the original and the restricted model against the chi-square distribution (df = 1), where χ^2 = -2*difference in log likelihood.

RESULTS

PLASMA CORTISOL RESPONSE

A stress response was noted in every cross-type, as shown by a significant increase in cortisol between control and stressed fish (Table IV; Fig. 1). However, the intensity of the cortisol response was variable depending on the cross, with significant interactions observed between stress treatment and cross-types (Table IV; Fig. 1). All control fish had the same level of initial plasma cortisol (Fig. 1).

246 The stress treatment in purebred crosses induced a significantly lower cortisol response in the Rupert
247 fish than in Laval and domestic fish, with the last two being similar (Fig. 1A). In hybrids, when the
248 Rupert strain was used as either dam or sire, the post-stress cortisol level did not differ significantly
249 from either parental line (Fig. 1B; 1D). In crosses involving the domestic and the Laval strains, all
250 hybrids and parental lines showed similar cortisol responses (Fig. 1C). These results are indicative of
251 an additive response rather than a non-additive effect. Mass had no significant effect on this trait (Table
252 IV).
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254
255 SECONDARY STRESS RESPONSE INDICATORS
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257
258 A significant interaction was observed between stress treatment and cross-type for glucose
259 concentration (Table IV). Plasma glucose concentrations were similar for all controls (Fig. 2) while
260 they were significantly higher after stress exposure in all cross-types (Table IV; Fig. 2). The glucose
261 response was similar among the three purebred lines (Fig. 2A), and hybrids showed concentrations
262 similar to their parental lines (Fig. 2C; 2D). The only exception was the D♀R♂ hybrid, which had a
263 significantly higher glucose concentration after stress exposure (Fig. 2B), hence expressing outbreeding
264 depression. Glucose concentration was 27% higher in this hybrid after stress exposure compared to the
265 average glucose concentration in parental lines. There was no significant co-factor effect for mass
266 (Table IV).
267
268
269 A significant interaction was observed between stress treatment and cross-type in the plasma
270 osmolality response to transport stress (Table IV; Fig. 3). In purebred lines, controls were not different

(Fig. 3A). Following stress exposure, Laval fish had significantly higher plasma osmolality levels than controls while osmolality did not vary in the other two purebred lines (Fig. 3A). Pre-stress levels of plasma osmolality were similar to both parental lines in the D♀R♂ and D♀L♂ hybrids (Fig. 3B and 3C), similar to the Laval line in the L♀D♂ hybrid (Fig. 3C), and similar to the Rupert line in hybrids between the Rupert and the Laval lines (Fig. 3D). After stress exposure, there was a significant increase in plasma osmolality in the D♀R♂ hybrid while no change was observed in the parental lines (Fig. 3B). The reverse was observed in the R♀L♂ hybrid, with a significant decrease in plasma osmolality (Fig. 3D). As with the Rupert line, no osmolality change was observed in the L♀R♂ hybrids (Fig. 3D), and hybrids between the domestic and the Laval strains behaved in a way similar to their maternal strain (Fig. 3C). The interaction between stress treatment and cross-type was significant for the blood haematocrit response (Table IV). Blood haematocrit was similar among controls and increased only in the domestic line after stress exposure (Fig. 4). For both plasma osmolality and blood haematocrit, the mass co-factor was significant (Table IV) but correlations were weak ($r = 0.15$ for both).

HERITABILITY

Significant additive genetic variance and heritability were obtained at both stress response levels for the whole population. Heritability estimates for cortisol ($h^2 = 0.60 \pm 0.20$) and glucose ($h^2 = 0.61 \pm 0.20$) following stress exposure were high and significant (Table V), while estimates were not significant for osmolality ($h^2 = 0 \pm 0$) or haematocrit ($h^2 = 0.46 \pm 0.25$) (Table V).

PARENTAL ORIGIN EFFECTS

Dam and sire origin significantly affected the stress response depending on the trait as was the case for heritability, the parental origin effect was strong for cortisol (Table VI). However, the results for the secondary response show different tendencies ~~(Table VI)~~: (i) there were significant effects of both dam and sire origin in the cortisol response, with fish issued from the Rupert strain having lower plasma cortisol than other fish ~~(Table VI)~~; (ii) no significant dam or sire effect was observed for the glucose response ~~(Table VI)~~; and (iii) there was a significant dam origin effect on the osmolality and haematocrit stress responses (Table VI). Progeny of Rupert dams had lower plasma osmolality following stress exposure than progeny of the other two strains when used as dams, and progeny of Laval dams had lower haematocrit after stress exposure than when domestic dams were used.

DISCUSSION

Our main objectives were to determine whether heterosis occurred and to estimate the heritability of primary and secondary stress indicators in brook charr (*S. fontinalis*). While our results revealed no clear evidence of heterosis, relatively high heritability was found for endocrine and physiological responses. A third objective was to compare the stress response between strains of brook charr. Inter-strain differences have been previously reported between unselected lines of fighting fish (*B. splendens*) and also between lines selected for different response to stress in rainbow trout (*O. mykiss*) and Atlantic salmon (*S. salar*) (Fevolden *et al.*, 1991; Pottinger, 2006; Verbeek *et al.*, 2008). In these studies, the stress cortisol response varied by 1.25 to 2 times when the most sensitive population is

compared to the least sensitive one. Our results indicate a similar range, with the Rupert strain response being about half those of the other purebred strains.

PUREBRED LINES

As previously indicated based on the primary and secondary stress responses, the Rupert strain displayed a less pronounced response to transport stress while the Laval strain seemed to be the most sensitive. The osmoregulatory disturbance in the Laval strain is not easy to interpret since a secondary stress response would have resulted in decreased osmolality in a freshwater fish. The domestic strain was the only one to show an increase in haematocrit, which may reflect a need for oxygen to compensate stress (Casillas & Smith, 1977). Some studies have revealed an impact of growth selection on stress performance, with a greater response to stress challenge and a longer stress recovery in heavier fish (Casillas & Smith, 1977; Lankford & Weber, 2006; Weber & Silverstein, 2007), while others observed no such correlation (Fevolden *et al.*, 1991; Millot *et al.*, 2009). Here, only weak correlations were present between mass and either the primary or secondary stress responses, indicating a weak link and therefore limited effect of body mass on stress resistance in brook charr.

NON-ADDITIVE GENETIC EFFECTS

No non-additive components seemed to influence the cortisol response; this is similar to findings on other species (channel catfish, *Ictalurus punctatus* [Rafinesque], Bosworth *et al.*, 2004; Chinook

345 salmon, *Oncorhyncus tshawytsha* [Walbaum], Bryden *et al.*, 2004). Studies on hybrids have rarely
346 provided evidence of non-additive effects, but they generally focussed on survival or cortisol response
347 (Bulger & Schultz, 1982; Bosworth *et al.*, 2004; Bryden *et al.*, 2004). However, heterosis related to
348 survival time (tertiary response) was reported in F1 hybrids after salinity stress in *Poecilia reticulata*
349 Peters (Chiyokubo *et al.*, 1998) and heat stress in *Poeciliopsis monacha-occidentalis* Angus (Bulger &
350 Schultz, 1982).
351
352
353 A weak but significant non-additive component was present at the physiological level (secondary
354 response), especially for plasma glucose concentration in the D♀R♂ hybrid. A non-additive component
355 was also observed for plasma osmolality in D♀R♂ and R♀L♂ hybrids, but this is more difficult to
356 interpret for the D♀R♂ hybrid, as previously mentioned. Our observations of non-additive components
357 only at the secondary response level reveal the presence of genetic divergence in purebred strains at the
358 physiological level rather than a neuroendocrine response to stress stimuli. The extents of non-additive
359 genetic phenomena are thought to be principally linked to the genetic distance between parental lines
360 (Falconer & Mackay, 1996; Tymchuk *et al.*, 2007). If the lines are too genetically distant or adapted to
361 their own environment, hybrids can show outbreeding depression with a breakdown of genetic complex
362 associations; on the other hand, when the genetic distance between parental strains is closer, hybrids
363 can express heterosis (Falconer & Mackay, 1996; Shikano *et al.*, 2000; Cooke *et al.*, 2001). Our results
364 do not support any of these expectations according to genetic distance: (1) the Laval and Rupert strains
365 were the most genetically distant strains (Martin *et al.*, 1997), and one of their hybrids (R♀L♂)
366 expressed a response significantly different from the parental responses for osmolality; and (2) the
367 D♀R♂ hybrid expressed outbreeding depression (glucose) while the two parental lines were more
368 genetically similar. In addition, the results obtained for the other hybrids do not support the hypothesis
369 that the genetic distance would be the main effect involved in non-additive expression in our crosses.

Other effects related to genetic architecture (e.g., epistasis, pleitropy, or genetic linkage) should be explored to explain our results. Overall, the presence of non-additive genetic effects only in secondary stress responses suggests that the use of hybrids to improve transport stress resistance in aquaculture has limited potential.

ADDITIVE GENETIC EFFECTS

The primary response to stress, i.e., cortisol response, seems to be principally under additive genetic control. The plasma cortisol concentration in hybrids was always similar to both parental lines. Both dam and sire origin significantly affected this trait, indicating the importance of an additive genetic basis underlying this stress response. Other studies on hybrids also revealed additive effects on plasma cortisol level after exposure to stress: Bryden *et al.* (2004) exposed wild and farm Chinook salmon (*O. tshawytscha*) hybrids and purebred crosses to an "aerial emersion" stressor, and the cortisol response in hybrids was equal to both parental lines. The high additive component for cortisol regulation translated into high and significant heritability estimate for this trait ($h^2 = 0.60 \pm 0.20$). The cortisol response to stress is already used for genetic improvement in other fish species, especially in rainbow trout (*O. mykiss*), in which heritability values similar to those obtained in our study have been documented in the F1 generation (h^2 ranging from 0.41 to 0.56 depending on strain origin) (Pottinger & Carrick, 1999; Fevolden *et al.*, 2002; Overli *et al.*, 2005; Weber & Silverstein, 2007; Weber *et al.*, 2008). The selection procedure for stress response in rainbow trout was based on the mean post-stress plasma cortisol response across five episodes of confinement stress testing on parental lines, with the highest responding (HR) or lowest responding (LR) individuals used to produce the next generation. This breeding program was repeated several times to obtain F2 and F3 generations with improved stress

resistance and other possibly related traits, such as increased growth or disease resistance (Pottinger & Carrick, 1999; Overli *et al.*, 2005; Ruiz-Gomez *et al.*, 2008). Our results suggest that such a program could also be applied in brook charr.

For the secondary stress response, plasma glucose concentration also displayed significant heritability estimates. This trait had higher heritability (h^2 = 0.61 ± 0.20) than values reported in previous studies on androgenetic carp (*C. carpio*, 0.19; Tanck *et al.*, 2001), Atlantic salmon (*S. salar*, 0.03; Fevolden *et al.*, 1993), and rainbow trout (*O. mykiss*, 0.07; Fevolden *et al.*, 1993). The low heritability observed in carp could be related to the androgenetic design, i.e., the UV irradiation and heat shock treatment might induce additional environmental variation due to embryonic damage caused by the androgenetic shock treatment and therefore reduce heritability (Tanck *et al.*, 2001). On the other hand, our own estimates could have been inflated due to our full-sib design, which may include other sources of variance including maternal effects (Falconer & Mackay, 1996; Pante *et al.*, 2002). However, previous studies in brook charr revealed that while maternal effects are apparent during the very first stages of development, they vanish within several months following hatching (Perry *et al.*, 2004; Perry *et al.*, 2005). This suggests that maternal effect should have a limited impact on our results. No significant heritability was found for osmolality or haematocrit response. Until now, no study has documented the heritability of osmolality variations related to stress resistance, but a very low heritability for haematocrit was reported in clonal lines of ayu (*Plecoglossus altivelis* [Temminck & Schlegel], 0.072; DelValle *et al.*, 1996).

In summary, the significant heritability of stress response at both the primary (cortisol) and secondary (glucose) levels indicates a good potential for selective breeding and genetic improved

resistance to transport stress in brook charr, and particularly so for the Rupert strain. Future work should aim at determining whether the difference expressed among strains is the result of global stress sensitivity variations or if some stains are more sensitive than others to different types of stress. On the opposite, hybridization does not seem to be a promising avenue to improve stress resistance in brook charr. Nevertheless, it would be worth further investigating this issue by comparing strains specifically selected for different sensitiviy to stress response which was not the case here. Thus, fixation of alleles related to the stress response in different strains could produce different, non-additive physiological effects in mixed progenies.

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Table

Table I: Total mass (Kg) and length (cm) of the breeders used to produce the different purebred (bold) and hybrid cross-types. Mean ± SE; n is the number of individuals; different letters indicate significant differences among cross-types ($\alpha = 0.05$).

	Female			Male		
Cross	n	mass	length	n	mass	length
D♀R♂	10	0.59 ± 0.02^{ab}	35.72 ± 0.40^{a}	10	0.63 ± 0.04^{a}	37.72 ± 1.02^{a}
D♀D♂	**10**	$\mathbf{0.70 \pm 0.02^{c}}$	$\mathbf{36.75 \pm 0.36^{a}}$	**10**	$\mathbf{0.81 \pm 0.03^{a}}$	$\mathbf{38.42 \pm 0.70^{a}}$
D♀L♂	10	0.78 ± 0.07^{bcd}	38.05 ± 1.30^{ab}	10	1.03 ± 0.12^{ab}	43.95 ± 0.66^{bc}
L♀D♂	10	0.97 ± 0.10^{cd}	41.25 ± 0.73^{b}	10	0.71 ± 0.03^{a}	37.68 ± 0.42^{a}
L♀L♂	**10**	$\mathbf{1.07 \pm 0.08^{d}}$	$\mathbf{42.60 \pm 0.87^{b}}$	**10**	$\mathbf{1.25 \pm 0.06^{bc}}$	$\mathbf{44.83 \pm 0.63^{bc}}$
L♀R♂	10	1.16 ± 0.14^{c}	42.21 ± 0.74^{b}	10	0.85 ± 0.09^{ab}	40.26 ± 1.27^{ab}
R♀L♂	10	1.39 ± 0.21^{bcd}	45.46 ± 2.01^{b}	10	1.46 ± 0.17^{c}	46.34 ± 0.62^{a}
R♀R♂	**10**	$\mathbf{0.47 \pm 0.04^{a}}$	$\mathbf{35.71 \pm 1.01^{a}}$	**10**	$\mathbf{0.77 \pm 0.11^{a}}$	$\mathbf{40.33 \pm 1.75^{abc}}$

Table II: Total mass (g) and length (cm) of the three purebred strains (bold) and their hybrids used as controls or for the stress challenge. Mean ± SE; n is the number of individuals; different letters indicate significant differences among cross-types ($\alpha = 0.05$).

	Control			Stressed		
Cross	n	mass	length	n	mass	length
$D_{♀}R_{♂}$	20	41.87 ± 2.07^{de}	15.69 ± 0.25^{c}	20	49.26 ± 4.16^{de}	16.53 ± 0.43^{c}
$D_{♀}D_{♂}$	**20**	**58.24 ± 5.48^{e}**	**16.63 ± 0.49^{c}**	**20**	**61.53 ± 5.25^{e}**	**17.25 ± 0.49^{c}**
$D_{♀}L_{♂}$	20	37.82 ± 3.47^{cd}	15.02 ± 0.45^{bc}	20	39.12 ± 4.02^{cd}	15.38 ± 0.45^{bc}
$L_{♀}D_{♂}$	20	33.36 ± 2.39^{cd}	14.73 ± 0.34^{bc}	20	45.39 ± 4.05^{cd}	16.41 ± 0.41^{c}
$L_{♀}L_{♂}$	**26**	**15.59 ± 1.01^{a}**	**11.94 ± 0.29^{a}**	**30**	**14.03 ± 0.70^{a}**	**11.49 ± 0.18^{a}**
$L_{♀}R_{♂}$	20	24.48 ± 2.09^{bc}	13.56 ± 0.39^{ab}	20	31.85 ± 3.23^{bc}	14.93 ± 0.53^{bc}
$R_{♀}L_{♂}$	20	23.91 ± 2.25^{b}	13.53 ± 0.36^{ab}	20	21.27 ± 1.72^{b}	13.13 ± 0.33^{ab}
$R_{♀}R_{♂}$	**21**	**22.75 ± 1.50^{b}**	**13.20 ± 0.28^{ab}**	**20**	**22.42 ± 1.48^{b}**	**13.23 ± 0.28^{b}**

Table III: Summary of two-way ANOVAs for body mass and length. df is degrees of freedom; MS is mean square; F is the F-ratio.

	Mass (g)				Length (cm)			
	df	MS	F	*P*-value	df	MS	F	*P*-value
Stress treatment	1	633.0	1.6	> 0.1	1	21.4	7.3	< 0.01
Cross-type	7	9455.7	49.9	< 0.001	7	137.2	46.6	< 0.001
Stress treatment × Cross-type	7	278.4	1.5	> 0.1	7	6.5	2.2	< 0.05
Error	321	189.4			321	2.9		
Model R^2	0.53				0.52			
Adjusted R^2	0.51				0.50			

Table IV: Summary of two-way ANOVAs for cortisol, glucose, osmolality, and haematocrit. df is degrees of freedom; MS is mean square; F is the F-ratio.

	Cortisol (ng ml^{-1})				Glucose (mg ml^{-1})				Osmolality (mosm kg^{-1})				Haematocrit (%)			
	df	MS	F	*P*-value	df	MS	F	*P*-value	df	MS	F	*P*-value	df	MS	F	*P*-value
Mass (co-variable)	1	0.2	1.7	> 0.1	1	0.2	2.2	> 0.1	1	468.0	8.7	< 0.01	1	0.03	7.9	< 0.01
Stress treatment	1	108.6	1132.0	< 0.001	1	28.4	410.8	< 0.001	1	127.0	2.4	> 0.1	1	0.02	6.4	< 0.01
Cross-type	7	0.2	2.2	< 0.05	7	0.3	4.2	< 0.001	7	303.0	5.6	< 0.001	7	0.01	1.9	> 0.05
Stress treatment × Cross-type	7	0.2	2.2	< 0.05	7	0.2	2.3	< 0.05	7	431.0	8.0	< 0.001	7	0.01	2.3	< 0.05
Error	300	0.1			289	0.1			274	54.0			278	0.01		
Model R^2	0.80				0.62				0.29				0.14			
Adjusted R^2	0.79				0.60				0.25				0.09			

Table V: Genetic components of the different traits in the stress responses. Estimates of total phenotypic (V_P), additive (V_A), and residual (V_R) variance components and heritability (h^2) with their standard errors (± SE); n is the number of individuals. *P*-values were obtained from a likelihood ratio test.

	n	V_P	V_R	V_A	h^2	*P*-value
Cortisol	159	0.14 ± 0.03	0.06 ± 0.02	0.08 ± 0.04	0.60 ± 0.20	< 0.05
Glucose	158	0.17 ± 0.04	0.07 ± 0.02	0.11 ± 0.06	0.61 ± 0.20	< 0.05
Osmolality	148	58.92 ± 7.04	58.92 ± 7.04	0	0	> 0.1
Haematocrit	146	0.004 ± 0.001	0.002 ± 0.001	0.002 ± 0.002	0.46 ± 0.25	> 0.1

Table VI: Dam and sire origin effects on the different traits after stress exposure. Physiological traits are expressed as mean ± SE. Different letters indicate significant differences among cross-types ($\alpha = 0.05$); *P*-value indicates the significance level.

	Dam				Sire			
	Domestic	Laval	Rupert	*P*-value	Domestic	Laval	Rupert	*P*-value
Cortisol (ng ml^{-1})	46.39 ± 4.85^{b}	47.06 ± 4.60^{b}	28.96 ± 3.07^{a}	< 0.05	53.19 ± 5.85^{b}	44.78 ± 3.78^{b}	32.78 ± 4.40^{a}	< 0.01
Glucose (mg ml^{-1})	1.33 ± 0.04	1.24 ± 0.05	1.19 ± 0.05	> 0.05	1.23 ± 0.05	1.21 ± 0.04	1.34 ± 0.05	> 0.05
Osmolality (mosm kg^{-1})	310.42 ± 0.94^{b}	309.05 ± 1.14^{b}	303.52 ± 1.47^{a}	< 0.01	307.87 ± 1.25	307.57 ± 0.96	309.85 ± 1.33	> 0.05
Haematocrit (%)	0.40 ± 0.01^{b}	0.37 ± 0.01^{a}	0.37 ± 0.01^{ab}	< 0.01	0.39 ± 0.01	0.38 ± 0.01	0.37 ± 0.01	> 0.1

Figure Captions

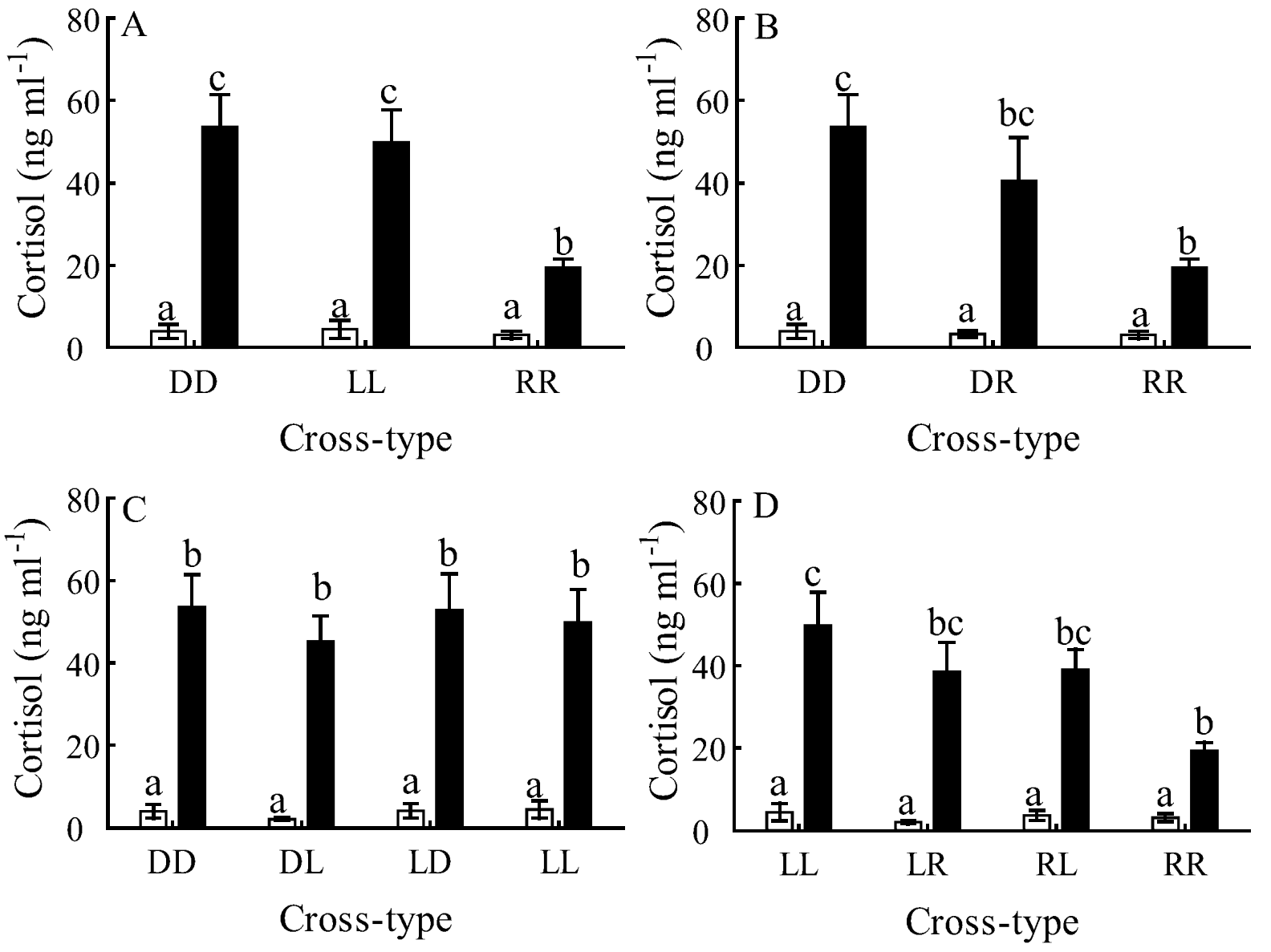
Fig. 1: Cortisol (ng ml^{-1}) stress response in the three purebred strains (A) and hybrids between (B) domestic and Rupert strains, (C) domestic and Laval strains, and (D) Laval and Rupert strains. The first letter of the cross-type indicates the dam and the second letter the sire. Open bars are for controls and solid bars for stressed. Statistical analyses were made on log-transformed data but results are presented as mean ± SE. Different letters indicate significantly different means ($\alpha = 0.05$).

Fig. 2: Plasma glucose (mg ml^{-1}) stress response in the three purebred strains (A) and hybrids between (B) domestic and Rupert strains, (C) domestic and Laval strains, and (D) Laval and Rupert strains. The first letter of the cross-type indicates the dam and the second letter the sire. Open bars are for controls and solid bars for stressed. Mean ± SE. Different letters indicate significantly different means ($\alpha = 0.05$).

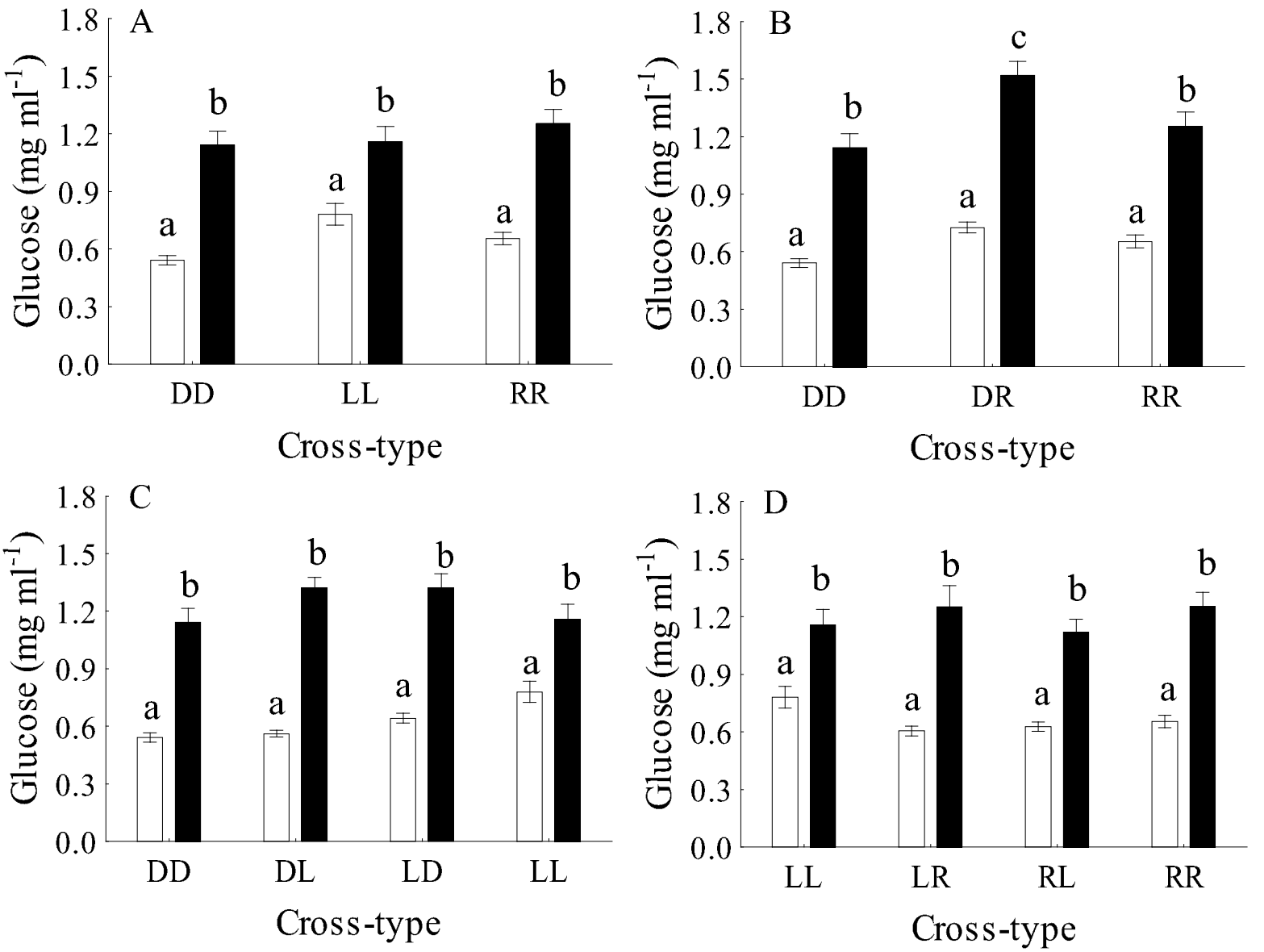
Fig. 3: Osmolality (mosm kg^{-1}) stress response in the three purebred strains (A) and hybrids between (B) domestic and Rupert strains, (C) domestic and Laval strains, and (D) Laval and Rupert strains. The first letter of the cross-type indicates the dam and the second letter the sire. Open bars are for controls and solid bars for stressed. Mean ± SE. Different letters indicate significantly different means among controls and asterisks indicate significantly different means between control and stressed ($\alpha = 0.05$).

Fig. 4: Haematocrit (%) stress response in the three purebred strains and their hybrids. The first letter of the cross-type indicates the dam and the second letter the sire. Open bars are for controls and solid bars for stressed. Mean ± SE. Asterisks indicate significantly different means between control and stressed ($\alpha = 0.05$).

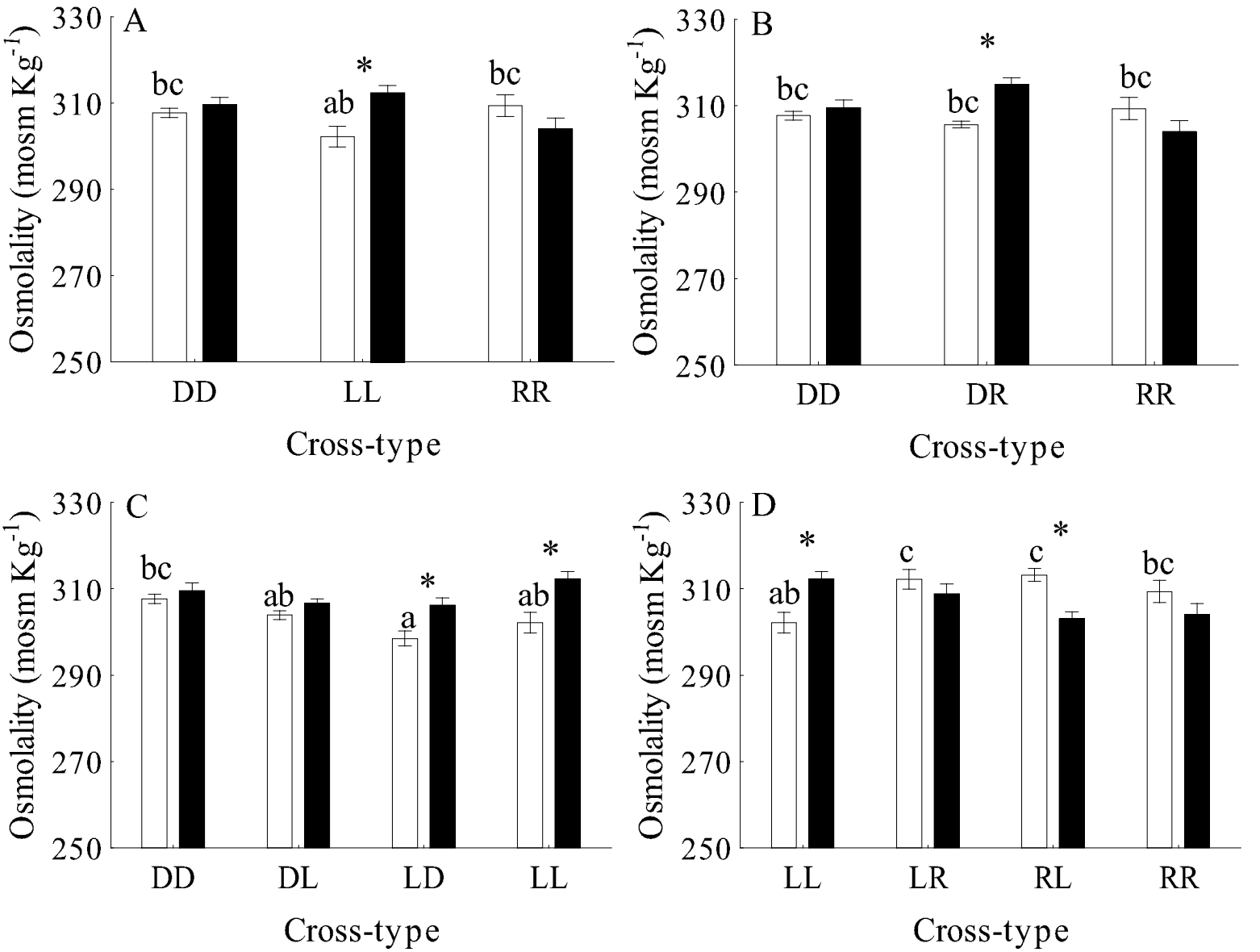
Figure


A
B
C
D
Cortisol (ng ml-1)
0
20
40
60
80
a
b
c
bc
DD
DR
DL
LD
LL
LR
RL
RR
Cross-type

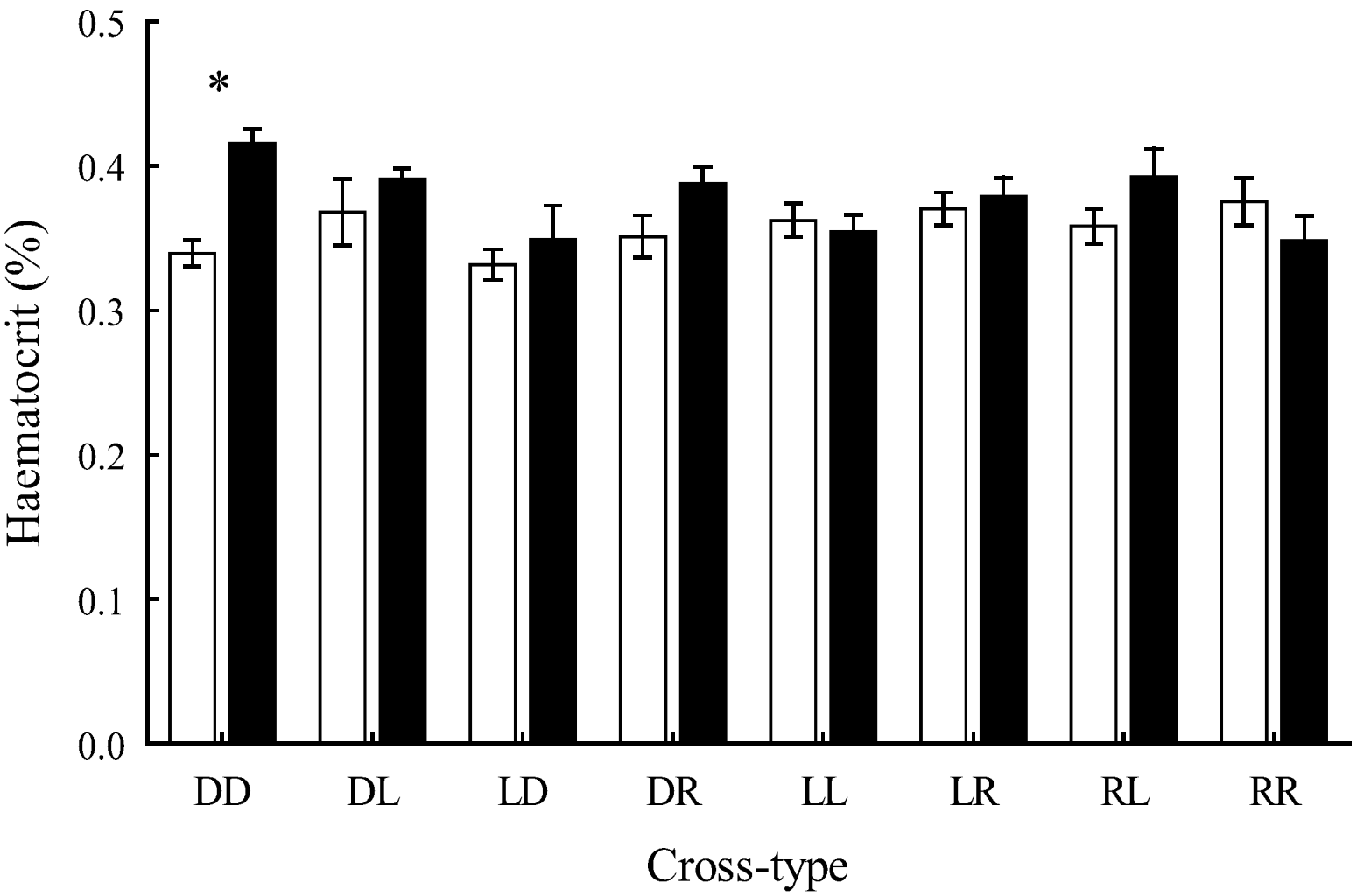
Figure


A
B
C
D
Glucose (mg ml-1)
1.8
1.5
1.2
0.9
0.6
0.3
0.0
a
b
c
DD
DR
DL
LD
LL
LR
RL
RR
Cross-type

Figure


A
B
C
D
Osmolality (mosm Kg-1)
330
310
290
270
250
bc
ab
*
a
c
DD
DL
LD
LL
DR
RR
LR
RL
Cross-type

Figure



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