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The Transcriptional Landscape of Cross-Specific Hybrids and Its Possible Link With Growth in Brook Charr (*Salvelinus fontinalis* Mitchill)

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ABSTRACT

The genetic mechanisms underlying hybridization are poorly understood despite their potentially important roles in speciation processes, adaptative evolution, and agronomical innovation. In this study, transcription profiles were compared among 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. We found that twice as many transcripts were differently expressed between the domestic population and the two wild populations (Rupert and Laval) than between wild ones, despite their deeper genetic distance. This could reflect the consequence of artificial selection during domestication. We detected that hybrids exhibited strikingly different patterns of mode of transcription regulation, being mostly additive (94%) for domestic × Rupert, and nonadditive for Laval × domestic (45.7%) and Rupert × Laval hybrids (37.5%). Both heterosis and outbreeding depression for growth were observed among the crosses. Our results indicated that prevalence of dominance in transcription regulation seems related to growth heterosis, while prevalence of transgressive transcription regulation may be more related to outbreeding depression. Our study clearly shows, 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.

LONG viewed as a dead end in animals, hybridization is increasingly considered as potentially positive from an evolutionary perspective (Barton 2001; Burke and Arnold 2001; Rieseberg *et al.* 2003; Tallmon *et al.* 2004; Nolte *et al.* 2006; Baak and Rieseberg 2007). Hybridization is also of interest for plant and animal production, because it may generate desirable phenotypic novelty through heterosis, in which the offspring have a more advantageous phenotype than their parents. However, hybridization can also lead to outbreeding depression, by which the offspring express a disadvantageous phenotype relative to their parents. Such hybrid phenotypic traits, expressed outside the range normally observed in the parental lines, are also referred to as transgressive phenotypes.

Numerous genetically based mechanisms have been invoked to explain transgressive phenotypes, including epistasis, overdominance, and additive allelic interactions (reviewed by Rieseberg *et al.* 1999). It has also been hypothesized that the crossing of inbred lines characterized by distinct gene expression levels could lead to advantageous transgressive phenotypic (Birchler *et al.* 2003; Cui *et al.* 2006; Stupar and Springer 2006; Swanson-Wagner *et al.* 2006; Stupar *et al.* 2008). One approach used to study the genetics of phenotypic traits is to measure transcript expression in the hybrids relative to their parents, but such studies have been in the past limited to mouse and Drosophila sp. (Reiland and Norr 2002; Michalak and Noor 2003; Gibson *et al.* 2004; Ranz *et al.* 2004; Cui *et al.* 2006; Hughes *et al.* 2006; Rottscheidt and Harr 2007). Other studies have also focused on elucidating the genomic basis of heterosis of mollusc production (Hedgecock *et al.* 2007) and plant production (Auger *et al.* 2005; Swanson-Wagner *et al.* 2006; Lippman and Zamir 2007; Ge *et al.* 2008; Stupar *et al.* 2008). Comparative studies of genome-wide transcriptomes of parental and hybrid crosses also revealed that hybrids tended to express numerous genes at a level outside the range observed in their parental lines (Gibson *et al.* 2004; Ranz *et al.* 2004; Auger *et al.* 2005; Cui *et al.* 2006; Hughes *et al.* 2006; Stupar and Springer 2006; Swanson-Wagner *et al.* 2006; Hedgecock *et al.* 2007; Rottscheidt and Harr 2007; Ge *et al.* 2008; Roberge *et al.* 2008; Stupar *et al.* 2008; Normandeau *et al.* 2009; Renaut *et al.* 2009). Thus, divergent lineages can accumulate divergences in gene regulation networks

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through time and these divergences often result in misexpressed genes (reviewed by Landry *et al.* 2007), which could affect the viability or fitness of individuals. In hybrids from subspecies of Mus, an overabundance of misexpressed transcripts was found in the testis relative to other tissues, including brain and liver (Rottscheidt and Harr 2007). In addition to this variation across tissues, the proportions of additive *vs.* nonadditive expression inheritance may vary between hybrids from different populations within species (Rottscheidt and Harr 2007; Stupar *et al.* 2008), sometimes in a contradictory manner. For example, in Drosophila, Gibson *et al.* (2004) found a prevalence of nonadditivity, whereas Hughes *et al.* (2006) found the opposite. These varying results seemed to depend on both methodological and genetic factors. Thus, the prevalence of nonadditivity could be linked with the X chromosome, as a bias was observed between males and females and very few additive genes were X linked (Gibson *et al.* 2004). Furthermore, these differences could also be linked to the highly homozygous lines used by Hughes *et al.* (2006), a situation which tends to increase the additive portion of expression inheritance observed. These two studies also differed in the methods used for the analysis; they used different criteria to calculate the dominance effects and different α level in their statistical analysis. However, Hughes *et al.* (2006) suggested that the differences between these two studies likely lie in genetic architecture divergence among the lines being crossed.

Genetic architecture is defined as the sum of the interacting genetic dimensions that lead to a given phenotype (Hansen 2006; Lynch 2007) and has been shown to vary in a population-specific manner (Lavagnino *et al.* 2008). As a result, it is especially difficult to predict the phenotype of hybrids. To date, very few studies have investigated the influence of population-specific genetic architecture on gene transcription regulation among populations of a same species. To our knowledge, only one study in maize (Stupar *et al.* 2008) examined hybrids between more than two crosses. These authors aimed to evaluate whether there was a link between heterosis at phenotypic traits and the genetic distance of the parental strains crossed, as well as the observed patterns of transcription regulation inheritance. However, they did not find any solid link between these parameters.

Brook charr (*Salvelinus fontinalis*) is an economically important salmonid native to eastern North America; in Québec, it represents 50% of all freshwater aquaculture production. Using microarrays, the general objective of this study was to document the differences between pure brook charr populations in terms of gene expression and their modes of transcription regulation inheritance in the F1 hybrids. Thus, F1 hybrid crosses descending from three genetically distinct populations were performed in order to investigate the phenotypic and genomic responses of these F1 hybrids relative to their parental populations. Our results show that the three parental populations and their hybrids differ strikingly in their overall patterns of gene expression. Namely, the number of significant transcripts and impacted biological functions vary in the three possible comparisons among pure and hybrid crosses. Moreover, the mode of transcription regulation (additivity or nonadditivity) in hybrids was dependent on which parental population it was compared to. We interpreted these patterns of genome-wide transcription in terms of their possible link to the contrasting size phenotypes observed in the same hybrid crosses.

MATERIALS AND METHODS

Fish crosses: Juvenile brook charr (yolk sac resorption stage) were produced using breeders from three genetically distinct populations: a domestic (D) population, in use for aquaculture in Québec, Canada, for more than a hundred years, as well as the Laval (L) and Rupert (R) populations. The L population is derived from an anadromous population originating from the Laval River near Forestville (north of the St. Lawrence River, Québec) (Castric and Bernatchez 2003), whereas the R population originates from a freshwater wild population of the Rupert River, which drains into James Bay in northwestern Quebec (Fraser *et al.* 2005). On the basis of estimates of Shriver *et al.* (1995) using microsatellite data, these three populations were highly differentiated; L and R populations were separated by 13.3 Dsw (genetic distance), with the D population being about equally genetically distant (about 6.7 Dsw) from the two others (Martin *et al.* 1997).

Breeders from the L population were kept in captivity for three generations at the Institut des Sciences de la Mer à Rimouski (Québec) and at the Laboratoire de Recherche des Sciences Aquatiques (Laboratoire de Recherche des Sciences Aquatiques, LARSA, Laval University, Québec) for the R population. Breeders from the D population were obtained from the Pisciculture de la Jacques Cartier (Cap-Santé, Québec). In 2005, 10 sires of each population (L, R, or D) were crossed with 10 dams (L, R, or D) to generate 10 full-sib outbreed families per pure and hybrid crosses. Three pure and three hybrid crosses were generated (RL, LD, DR, L, D, and R). For the hybrid crosses, the first letter corresponds to the mother's origin. All families were kept separately at the LARSA under identical controlled conditions. Fertilized eggs were incubated at 6°. After hatching, the progeny were kept at 8°, with a photoperiod of 12 hours of light and 12 hours of darkness.

Microarray experiment: *Sampling:* One sexually undifferentiated juvenile was randomly sampled for eight families in each of the crosses and immediately frozen in a mix of dry ice and alcohol. Experiments were conducted on whole, unpooled individuals.

RNA extraction, labeling, and cDNA hybridization: Total RNA was extracted with the PureLink Micro-to-Midi total RNA purification system kit and then treated using DNase I, amplification grade (1 unit/μl) (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. Total RNA was further purified by ultracentrifugation using microcon (Millipore) spin columns. RNA quality and integrity was controlled with an Experion automated electrophoresis station and RNA HighSens chips (Bio-Rad, Hercules, CA). For each sample, 12 μg total RNA was retrotranscribed and the cDNA samples labeled using Genisphere 3DNA Array 50 kit, Invitrogen's Superscript II retrotranscriptase, and cyanine 3 and Alexa 647 fluorescent dyes (Genisphere),

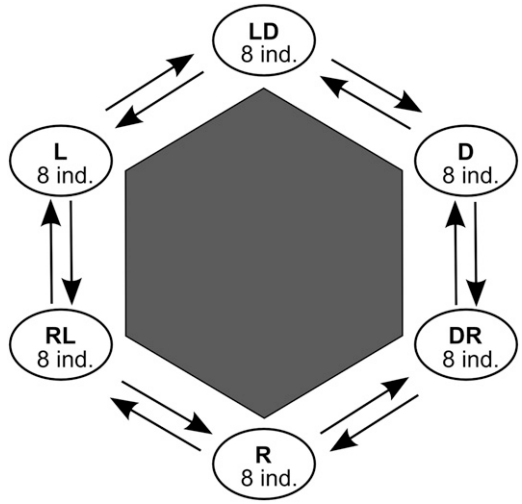

Figure 1.—Loop experimental design including direct comparisons between hybrids and their parental populations and indirect comparisons between parental populations. Each sample was hybridized twice using dye swap. L, Laval; R, Rupert; D, domestic; ind., individuals.

following the procedures at http://web.uvic.ca/cbr/grasp/ (Genisphere Array 50 Protocol).

The transcription profiles were measured using cDNA microarrays produced by the consortium for Genomic Research on All Salmon Project (cGRASP, http://web.uvic.ca/grasp/, Rise *et al.* 2004). The chip contains 16,006 salmonid cDNAs of Atlantic salmon and rainbow trout (von Schalburg *et al.* 2005) and was successfully tested and applied to other salmonids species, including brook charr (Rise *et al.* 2004; von Schalburg *et al.* 2005; Koop *et al.* 2008; Mavarez *et al.* 2009; Sauvage *et al.* 2010). Sequence differences between samples are not likely to result in spurious differential expression since the divergence between the studied populations are similar among themselves relative to Atlantic salmon and much less important than is found among salmonid species, which showed similar hybridization performances for this chip (von Schalburg *et al.* 2005; Koop *et al.* 2008).

A total of 48 microarrays were analyzed. Each individual was technically replicated on two bicolored distinct microarrays and dye swapped. A loop design (Figure 1) was used to include pairwise direct comparisons between a hybrid and its parental population and indirect comparisons between pure crosses. This design allowed the comparison of all the groups with a similar statistical power while minimizing the use of individuals and microarrays.

Data acquisition, preparation, statistical analysis, and functional classification of genes: Microarrays were scanned using a ScanArray scanner (Packard BioScience). Spots were localized and quantified with the QuantArray 3.0 software, using the histogram quantification method. Local background and the data from bad spots were removed. Spots with signal intensities smaller than the mean intensity of empty spots plus twice their standard deviations were removed from the analysis, leaving a total of 3263 analyzed spots. After a $\log_2$ transformation, the data were normalized with the lowess method (regional lowess procedure) implemented in the R/MAANOVA package (Kerr *et al.* 2000) to remove signal intensity-dependant dye effects on each slide (regional lowess procedure). Normalized data for each slide/dye combination were median centered to allow comparable expression values within and between slides without global patterns of expression showing any individual or population biases (Swanson-Wagner *et al.* 2006).

To detect differences between crosses, data were analyzed using a mixed-model ANOVA (Wolfinger *et al.* 2001) and the R/MAANOVA package (Kerr *et al.* 2000; Kerr *et al.* 2002). We tested the presence of cross-type effects with the following ANOVA model,

$$Y_{ijlk} = \mu + G + A_i + D_j + C_{l(ij)} + S_{k(ij)} + (AD_{ij}) + (GA_i) + (GD_j) + (GC_{l(ij)}) + (GS_{k(ij)}) + \varepsilon_{ijlk},$$

with *A*, array; *D*, dye; *C*, cross type; G, gene; *S*, sample (two replicates per samples); and terms in parentheses are interaction terms. This model included "array" and "sample" as random terms and "dye" and "cross type" as fixed terms.

We tested the null hypothesis that the residuals for each gene were normally distributed using the Kolmogorov test from the R Nortest package. We also evaluated departure from homoscedasticity of gene-specific variance using the Fligner–Killeen test, as implemented in R. A permutation-based *F*-test (*Fs*, with 1000 sample ID permutations) was then performed, and restricted maximum likelihood was used to solve the mixed-model equations (Cui *et al.* 2005). A false discovery rate correction (FDR = 0.05) was applied using the R/MAANOVA package, to generate a first list of transcripts with significant differential expression between crosses. Further contrast tests were conducted on this list with 1000 permutations for the 12 possible comparisons among the six crosses. A FDR of 0.05 was also applied for each contrast test.

All differentially expressed transcripts between parental crosses were examined to assess their mode of transcription regulation in the hybrid crosses. Each transcript was classified as additive if the contrast *P* value did not allow rejecting the null hypothesis of no statistical difference in expression between hybrid and the average value of its parental populations. Transcripts were classified as nonadditive if the contrast *P* value indicated that the hybrid was expressing the transcript at a significantly different level than the average of the parental populations (FDR = 0.05). Among nonadditive transcripts, dominant transcripts were defined as transcripts for which the contrast *P* value with one of the parental populations was nonsignificant. Transcripts were defined as maternal dominant if the mother/hybrid crosses contrast was nonsignificant and paternal dominant if the father/hybrid crosses contrast was nonsignificant (FDR $<$ 0.05). Nonadditive transcripts were defined as underdominant (UD) if their expression was significantly lower than both parental populations and as overdominant (OD) if their expression was significantly higher than both parental populations (contrast *P* value $<$ 0.05). For transcripts that were not differentially expressed between parental populations, all nonadditive transcripts were classified as UD or OD in the same way. The OD and UD transcripts were also defined as transgressive. A chi-square test (exact binomial with 1 d.f.) was applied to determine whether the modes of transcription regulation differed among the crosses, using the R software package.

Functional classification and assessment of significant differential representation of functional classes were performed in the DAVID and PANTHER environments. DAVID 2.1 gene accession conversion tool was first used to convert gene ontology-linked identifications from the salmonid microarrays to UNIGEN clusters. Assessment of significant differential representation of functional categories was performed using PANTHER (significance threshold: $P = 0.05$). The overrepresentation of a given function corresponds to a significant differentially overrepresented function among the ones represented by all significantly expressed transcripts (3263). Gene ontology annotation was possible for 45% of

the significant cDNA clones and 49% of all clones on the cGRASP microarray.

Body size measurement: Twenty juveniles per family (10 families for RR, RL, LL, LD, and DD crosses, and 9 families for DR cross) were sampled and their fork lengths measured. Normality and homoscedasticity of data were tested respectively with the Kolmogorov–Smirnov and the Brown and Forsythe tests (Quinn and Keough 2005). To detect cross effects, data were analyzed with the following ANOVA mixed model implemented in ASReml version 2 (VSN International, UK),

$$Y_{ijk} = \mu + C_i + F_{j(i)} + \varepsilon_{ijk},$$

with C (cross type) as the fixed effect and F (family) nested within C as the random effect followed by *a posteriori* analysis when significant. If a cross effect was confirmed, the differences between parental populations and their hybrid crosses were tested to detect the presence of heterosis or outbreeding depression with the one-way ANOVA model ($Y_{ijk} = \mu + C_i + \varepsilon_{ijk}$ with C as the fixed effect) using Statistica version 6.0 (StatSoft, Tulsa, OK, USA). The mean differences were tested with Tukey tests or Games and Howell tests for nonhomoscedastic data, using SPSS version 13.0. The intensity of heterosis or outbreeding depression is expressed in percentage as $[(f1\,m^{-1}) - 1] \times 100$ for heterosis and as $[1 - (f1\,m^{-1})] \times 100$ for outbreeding depression (Shikano and Taniguchi 2002), where $f1$ and m represent the mean hybrid value and the mean pure-population value, respectively. Size data per family are presented in supporting information, File S1.

RESULTS

Overall sample differences: Results from the Kolmogorov test revealed that 4.68% (153 of 3263) transcripts showed departure of normality at $\alpha = 0.05$. However, the permutation-based ANOVA makes no assumption about normality, and although normality is assumed in the procedure used for assessing genetic parameters, restricted maximum-likelihood estimators, such as the one we used for the *Fs* statistic, are robust to deviations from the assumption of normality (Kruuk 2004; Cui *et al.* 2005). Results from the Fligner–Killeen test revealed that only 4.1% (135 out of 3263) transcripts showed departure from homogeneity of gene-specific variance at α=0.05, which should not significantly affect our main conclusions.

A total of 863 of 3263 (26.4%) analyzed transcripts showed significant differential expression in at least one of the six crosses ($P < 0.015$, FDR < 0.05). Among the 863 significant transcripts, 129 (15%) were labeled "unknown" because they did not generate any significant BLAST hits (von Schalburg *et al.* 2005, 2008). A total of 151 functional categories were represented by the 3263 significantly expressed transcripts. However, only those that were identified as significantly overrepresented in parental populations and their hybrid comparisons by the Panther analysis are presented (Figure 2) and interpreted, whereas details about the genes representing other functional groups are presented in supporting tables.

Comparing the parental populations: The three parental populations differed substantially in terms of the number of differentially expressed genes as well as transcribed functional categories. Between the R and L populations, 104 transcripts representing 72 different genes (23 unknown) were differentially expressed (Table S1). In contrast, 265 transcripts representing 178 different genes (Table S2) including 43 unknown were differentially expressed between R and D populations, and 276 transcripts representing 191 different genes (Table S3) with 45 unknown between L and D populations. These differences were all significant (chi-square test, exact binomial with 1 d.f: R/L *vs.* L/D $P <$ 2.2 E-16, R/L *vs.* R/D $P <$ 2.2 E-16).

Gene ontology analysis identified nine functional categories that were overrepresented among parental populations (Figure 2A). Between the R and D populations, three functional categories (cell adhesion, pyrimidin metabolism, and rRNA metabolism), were overrepresented. Pyrimidin metabolism and rRNA metabolism genes were overtranscribed in the D relative to the R population. Genes falling in the cell adhesion category were either undertranscribed or overtranscribed in the D relative to the R population (Table S2). Between L and D populations, five functional categories (calcium metabolism, cell motility, chromosome segregation, lipid and fatty acid metabolism, and signal transduction) were overrepresented. The genes playing a role in the calcium metabolism and the chromosome segregation categories were undertranscribed in the L relative to the D population. Genes related to the cell motility, lipid and fatty acid metabolism, and signal transduction categories were either undertranscribed or overtranscribed in the L relative to the D population (Table S3). Finally, two functional categories (carbohydrate metabolism and pyrimidin metabolism) were overrepresented among the genes differently expressed between the L and R populations. Pyrimidin metabolism genes were undertranscribed in the R relative to the L populations. Inversely, genes related to the carbohydrate metabolism category were overtranscribed in the R relative to the L population (Table S1). Only one category, pyrimidin metabolism, was overrepresented in two parental population comparisons. Gene ontology annotation was possible for only 28 of 72 significant genes between R and L, 62 of the 178 significant genes between D and R, and 83 out of 191 genes between L and D populations, respectively.

Comparing hybrid and parental crosses: The differences observed between the three hybrid crosses and their parental populations varied considerably depending on which of the three hybrids was compared to its parental populations. For the DR hybrids, the number of transcripts differentially expressed between the hybrids and their parental populations was smaller than between the latter ones; 130 (89 genes) and 155 (113 genes) transcripts were differentially expressed between

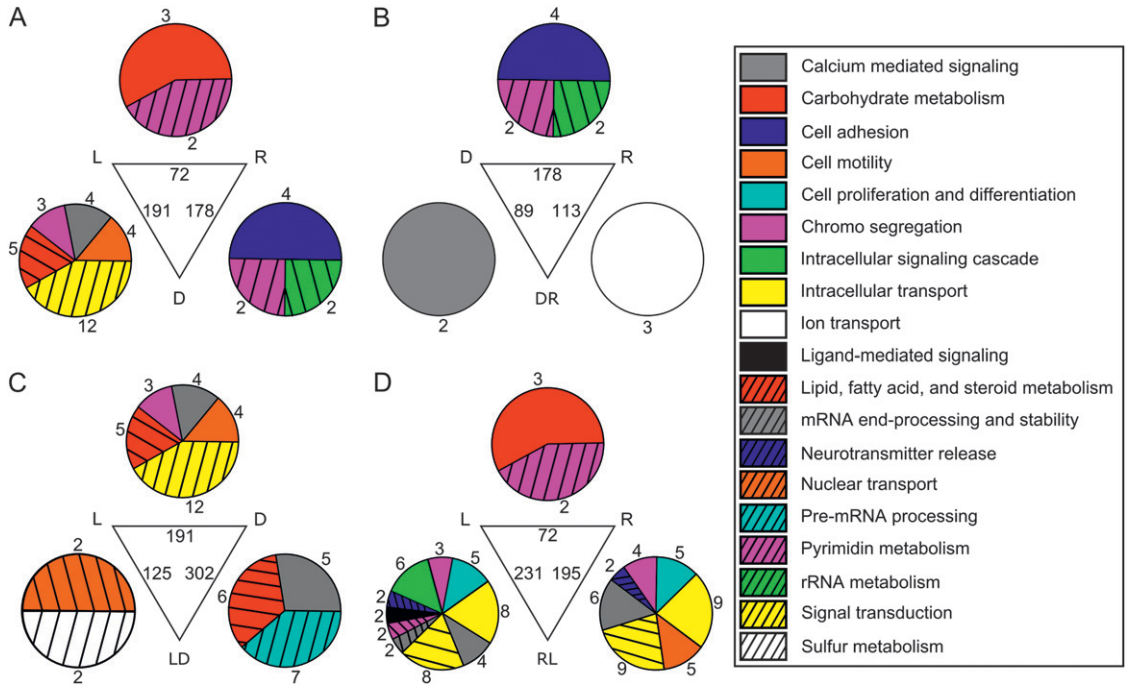

FIGURE 2.—Pie chart representation of overrepresented biological functions as revealed by Panther analysis in the comparisons between the parental populations and hybrid crosses and parental populations. The numbers inside the triangles correspond to the number of significant genes with differential expression in these comparisons, and those near the pie splices correspond to the number of genes in each functional category labeled in the legend. (A) Comparison between the three parental populations. (B) Comparison between DR hybrids and parental populations. (C) Comparison between LD hybrids and parental populations. (D) Comparison between RL hybrids and parental populations.

DR *vs.* D and DR *vs.* R, respectively, compared to 265 differentially expressed transcripts (178 genes) between the parental populations. In contrast, the LD hybrids showed twice as many differently expressed transcripts when compared to their D parental population than when compared to L; 177 transcripts (125 genes) were differentially expressed between LD *vs.* L, compared to 455 (302 genes) between LD and D. The RL hybrids presented a third distinct pattern of transcription profile, whereby the number of significant differences in transcript expression between hybrids and either parents was higher than in the parental populations comparison. Thus, 350 and 294 transcripts were differentially expressed between RL *vs.* L and RL *vs.* R, representing 231 and 195 different genes, respectively, compared to 104 differentially expressed transcripts (72 genes) between parental populations.

Gene ontology analysis identified 16 different functional categories that were overrepresented in comparisons between hybrid and parental populations (Figure 2, B, C, and D). Between DR and D, one functional category, calcium-mediated signaling, was overrepresented whereas between DR and R, only the ion transport category was overrepresented (Figure 2B). Moreover, no category was overrepresented in both of the hybrids *vs.* parental and the parental population comparisons. Gene ontology annotation was possible for 44 out of 89 genes for DR *vs.* D and 46 out of 113 genes for DR *vs.* R. Two functional categories, nuclear transport and sulfur metabolism, were overrepresented when LD was compared to L and three (calcium-mediated metabolism, lipid and fatty acid metabolism, and pre-mRNA processing) when LD was compared to D (Figure 2C). Two functional categories were overrepresented in both hybrids *vs.* parental and parental population comparisons: calcium mediated metabolism and lipid and fatty acid metabolism. Gene ontology annotation was possible for 64 out of 125 genes for LD *vs.* L and 141 out of 302 genes for LD *vs.* D. Ten functional categories (calcium-mediated signaling, cell proliferation and differentiation, chromosome segregation, intracellular signaling cascade, ligand-mediated signaling, mRNA end-processing and stability, neurotransmitter release, pyrimidin metabolism, signal transduction, and intracellular transport) were overrepresented between RL and L crosses (Figure 2D). Seven functional categories (calcium-mediated signaling, cell motility, cell proliferation and differentiation, chromosome segregation, neurotransmitter release, signal transduction, and intracellular transport) were overrepresented when RL hybrid was compared to the R population (Figure 2D). Only one category, pyrimidin metabolism, was overrepresented in both hybrid *vs.* parental and parental population comparisons. Gene ontology annotation was possible for 108 of 231 genes for RL *vs.* L and 91 of 195 genes for RL *vs.* R.

TABLE 1

Additivity, dominant (maternal and paternal), and over-/under dominantly (OD/UD) transcript expression among differentially expressed transcripts between parental populations and OD/UD transcript expression among nondifferentially expressed transcripts between parental populations in hybrid crosses (FDR = 0.05)

	Differentially expressed transcripts between parental populations					Nondifferentially expressed transcripts between parental populations	
		Dominant					
Hybrid strains	Additive	Maternal	Paternal	OD	UD	OD	UD
DR	249	4	12	0	0	10	7
LD	150	110	14	2	0	59	55
RL	65	21	17	0	1	90	56

DR, domestic × Rupert hybrids; LD, Laval × domestic hybrids; RL, Rupert × Laval hybrids.

Additivity *vs.* nonadditivity: *Differentially expressed transcripts between parental populations:* The majority of transcripts differently expressed between the parental populations exhibited an additive mode of transcription regulation but the proportion of additivity differed among crosses: 94% of transcripts in DR, compared to 54.3% in LD and 62.5% in RL hybrids (Table 1). The proportion of additivity in the DR cross was statistically different from RL and LD crosses (chi-square test, exact binomial with 1 d.f.: DR *vs.* LD $P < 2.2$ E-16, DR *vs.* RL $P < 2.2$ E-16). Maternal influence was also important in LD hybrids since 88% of the transcripts with a dominant mode of transcription regulation presented maternal dominance, compared with 55 and 25% for RL and DR hybrids, respectively. Very few transcripts were over- or underdominant (Table 1).

Nondifferentially expressed transcripts between parental populations: All transcripts that were differentially expressed in hybrids, but did not show significant differential expression between parental populations, were included in this analysis. The number of transcripts showing either OD or UD modes of transcription was variable among crosses with the highest number for RL hybrids (Table 1). The numbers of OD *vs.* UD transcripts were only significantly different in the RL hybrid ($P < 0.05$, chi-square test, exact binomial with 1 d.f.). The fold change of expression for the transcripts showing OD in LD hybrids was significantly different from DR and RL hybrids crosses (LD: 1.68, DR: 1.19, RL: 1.31, *t*-test, $P < 0.05$), whereas the fold change of the transcripts showing UD was not significantly different between the three hybrids crosses (LD: 0.81, DR: 0.81, RL: 0.79). The list of misexpressed transcripts and their functional categories are available in supporting Table S4.

Cross-specific modes of transcription regulation: A total of 116 transcripts (62 genes) involved in various biological functions showed varying modes of transcription regulation (additivity *vs.* nonadditivity) among the different hybrid crosses. For example, the *Heat shock protein HSP 90-beta* gene had an additive mode of transcription regulation in DR whereas it presented a nonadditive mode of transcription regulation in the LD hybrid. The *Apolipoprotein B-100 precursor* gene had a nonadditive mode of transcription regulation in DR and RL hybrids but an additive one in the LD hybrid. The *CD63 antigen* gene also showed different modes of transcription regulation: additive in DR and nonadditive in RL hybrids. Only the genes for which gene ontology annotation could be found are presented in Table 2, while the rest of the list is presented in Table S5. When genes were represented by several clones, all the repeated clones within a given hybrid cross displayed the same inheritance pattern, except for the three following genes: *H-2 class II histocompatibility antigen gamma chain*, *ADP/ATP translocase 2*, and *Tubulin alpha chain* (Table S5).

Body size differences among crosses: Fork lengths at the yolk-sac resorption stage were statistically different between the three parental populations (Tukey tests, all $P < 0.05$). The D population was the smallest with 22.3 ± 0.9 mm of length. The L population was the longest (25.7 ± 1.0 mm) whereas the length of the R population was of 23.9 ± 1.4 mm. The DR hybrids, with a fork length of 23.1 ± 1.2 mm, displayed intermediate size (additivity) compared to the average of the juvenile from their parental populations (Figure 3A). Heterosis was observed in the LD hybrids, where hybrids were 19.6% longer (28.7 ± 1.7 mm) than the average of the juveniles from their parental populations (Tukey tests, $P < 0.05$) (Figure 3B). In contrast, RL hybrids showed outbreeding depression, whereby the hybrids were 9.2% shorter (22.5 ± 1.4 mm) than the average of the juveniles from their parental populations (Tukey tests, $P < 0.05$) (Figure 3C).

DISCUSSION

Differentiation among the parental populations: On the basis of microsatellite markers, the three parental populations were previously found to be highly genetically distinct from one another with the domestic population being genetically intermediate to the L and R populations (Martin *et al.* 1997). However, genetic information obtained from microsatellites is more likely

TABLE 2

Genes exhibiting an additive mode of transcription regulation in one hybrid cross and a nonadditive mode in one or two of the other hybrid crosses

Gene names	No. clones	DR	LD	RL	Functional categories
Peroxiredoxin-5, mitochondrial precursor	1	A	N-A	*	Antioxydation
Tubulin α-1A chain	1	*	A	N-A*	C.M./I.P.T./chromosome segregation
Tubulin α-1C chain	1	A	A	N-A*	C.M./I.P.T./chromosome segregation
UV excision repair protein RAD23 homolog B	1	*	N-A	A	DNA repair
Heat shock protein HSP 90-β	1	A	N-A	*	Immunity and defense
Peroxisomal multifunctional enzyme type 2	1	A	N-A	*	L.F.A metabolism
H-2 class II histocompatibility antigen γ chain	2	N-A	A	A	MHCII-mediated immunity
H-2 class II histocompatibility antigen γ chain	1	A	N-A	*	MHCII-mediated immunity
NADH dehydrogenase [ubiquinone] 1 α subcomplex subunit 8	1	A	N-A	*	Oxydative phosphorilation
Heterogeneous nuclear ribonucleoprotein A/B	1	*	A	N-A	Pre-mRNA processing
Heterogeneous nuclear ribonucleoprotein A0	1	A	N-A	*	Pre-mRNA processing
26S proteasome non-ATPase regulatory subunit 14	1	*	A	N-A	Protein metabolism and modification
40S ribosomal protein S2	1	A	N-A	*	Protein metabolism and modification
Elongation factor 1-δ	1	A	N-A	N-A*	Protein metabolism and modification
Nucleoside diphosphate kinase A	5	A	*	N-A	Pyrimidin metabolism
Nucleoside diphosphate kinase B	4	A	*	N-A	Pyrimidin metabolism
Cold-inducible RNA-binding protein	2	A	N-A	*	RNA binding
CD63 antigen	1	A	*	N-A	Signal transduction
Acyl-CoA-binding protein	1	A	*	N-A	Intracellular transport
ADP/ATP translocase 2	2	A	N-A	*	Intracellular transport
ADP/ATP translocase 2	1	A	N-A	N-A*	Intracellular transport
Myelin expression factor 2	1	A	*	N-A	Intracellular transport
Oncorhynchus mykiss invariant chain S25-7 mRNA	1	N-A	A	*	Intracellular transport
Vacuolar ATP synthase 16-kDa proteolipid subunit	2	A	N-A	*	Intracellular transport
Apolipoprotein B-100 precursor	2	N-A	A	N-A*	Intracellular transport/ L.F.A. metabolism

Only genes for which gene ontology annotation could be found are presented here. Genes without gene ontology annotation can be found in Table S5.

No. clones, number of clones; DR, Domestic × Rupert hybrids; LD, Laval × Domestic hybrids; RL, Rupert × Laval hybrids; N-A, nonadditive mode; A, additive mode; *, transcripts did not show significant difference between their parental strains. Abbreviation for the functional categories: C.M., cell motility, I.P.T., intracellular protein traffic, L.F.A., Lipid and fatty acid. The functional category is defined for unique gene in Panther online classification system (http://www.pantherdb.org).

to reflect the outcome of differentiation resulting from neutral processes. Contrary to Martin *et al.* (1997), our results reveal that, from a transcriptome standpoint, the D population is the most differentiated, with the R and L populations being more similar to each other. Previous studies have suggested that much of the variation in transcription profiles among populations or species resulted from neutral divergence, while others considered that this is more likely the result of selection (reviewed by Rodríguez-Trelles *et al.* 2005). Whitehead and Crawford (2006) also showed that only variation exceeding the phylogenetic variance may be considered as resulting from selection. Our results, combined with those of Martin *et al.* (1997), show that transcription profiles and neutral patterns of divergence among the three parental populations of brook charr are distinct. This has also been reported in other studies on fish, including salmonids (Oleksiak *et al.* 2002; Giger *et al.* 2006). Although further investigation will be necessary to test this hypothesis, the more pronounced transcriptional distinctness observed in the D population could be a direct consequence of artificial selection or of inbreeding depression caused by the domestication process. Although we cannot strictly rule it out, the hypothesis of inbreeding depression may be less compelling than the artificial selection hypothesis, since the inbreeding coefficient has been found to be smaller for the D ($F = 0.18$) relative to the Rupert and Laval populations (mean $F = 0.35$) (Martin *et al.* 1997).

Previous studies in Atlantic salmon and brook charr demonstrated that only four to seven generations of domestication could lead to significant changes in transcription profiles (Roberge *et al.* 2006; Sauvage *et al.* 2010). Since the domestic (D) population used in this study has undergone at least 15 generations of domestication, it is plausible that some of the differentiation has been generated by directional selection for traits of commercial interest (*i.e.*, growth, disease resistance, or swimming resistance). For instance, the growth factor gene *(CTGF)* and genes involved in lipid

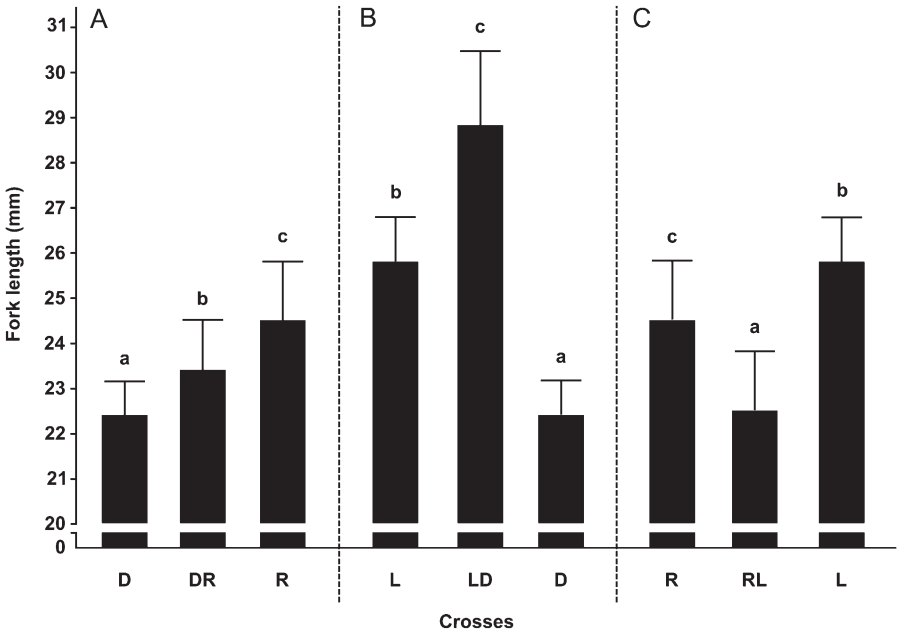

FIGURE 3.—Comparison of fork length at yolk-sac resorption stage between hybrid crosses and parental populations. Means ±SD. Different letters indicate significantly different means ($P < 0.05$). (A) DR hybrid compared to D and R parental populations. (B) LD hybrid compared to L and D parental populations. (C) RL hybrid compared to R and L parental populations.

metabolism were significantly overtranscribed in the D population. Namely, the D population showed 34 and 30% overtranscription of the *CTGF* gene, 27 and 19% overtranscription of the *apolipoprotein B-100 precursor* gene, and 55 and 67% overtranscription of the *apolipoprotein A-I precursor* gene against L and R populations, respectively (Table S2 and Table S3). Similar results were obtained by ROBERGE *et al.* (2006), who observed 23% of overtranscription for GH gene and 89% of overtranscription for *apolipoprotein A-I-1 precursor* gene in farmed salmon. Interestingly, when comparing their results on domestication effects in brook charr to those on Atlantic salmon, SAUVAGE *et al.* (2010) also observed that genes with similar biological functions were found to be under selection in both studies. Finally, the D population is known for its disease susceptibility, for example to furuncunlosis (CIPRIANO *et al.* 2002). This susceptibility could be partially linked to the fact that the MHC antigen coding genes were undertranscribed in the domestic relative to the other two populations (Table S2 and Table S3).

The transcriptional differences observed between the R and L populations may partially reflect adaptive responses to their distinct environment (local adaptation). For example, R and L populations exhibited significant bioenergetic-related physiological differences at young ages, which could be the result of environmental constraints. Thus, some carbohydrate metabolism genes are undertranscribed, whereas genes related to lipid metabolism (*apolipoprotein A, fatty acid-binding protein*) are overtranscribed in the L population. This could reflect a reduction in basal metabolism and higher growth-related resource allocation, which, in turn, would result in higher growth performance, as observed in the L population, which is bigger at this life stage than the R population. Modifications of lipid metabolism have also been suggested to be linked to migration-related osmoregulatory changes associated with transition from fresh water to salt water, which anadromous salmonids undergo (SHERIDAN *et al.* 1985; LI and YAMADA 1992). Thus, the differential expression of lipid metabolism-related genes between R and L populations could also be a consequence of differential adaptation to a purely freshwater lifecycle in the former *vs.* anadromous life cycle in the latter. This, however, remains to be rigorously investigated. In summary, the MARTIN *et al.* (1997) study on neutral markers and our gene expression data in controlled conditions showed that the three pure populations used in this study are genetically very distinct. They also suggest that both artificial selection and adaptation by natural selection to their respective environments may be responsible for this differentiation.

Hybrid response: Despite the important role potentially played by hybridization in speciation processes, adaptative evolution, and agronomical innovation, little is known about transcription regulation inheritance in hybrids, particularly in fishes. Our results showed that the differences in numbers of significant genes, as well as affected biological functions, varied considerably depending on which of the three hybrids was compared to its parental populations. In addition, although individuals from the three hybrid crosses expressed a majority of transcripts in an additive manner, the proportions of additivity in transcription regulation inheritance varied among the hybrid crosses. Previous studies using single species reported contrasting results about transcription regulation inheritance. These reported a predominance of either additivity (fruit flies, HUGHES *et al.* 2006; mice, CUI *et al.* 2006 and ROTTSCHEIDT and HARR 2007; maize,

Stupar and Springer 2006; Swanson-Wagner *et al.* 2006; Stupar *et al.* 2008) or nonadditivity (fruit flies, Gibson *et al.* 2004; oyster, Hedgecock *et al.* 2007; maize, Auger *et al.* 2005; rice, Ge *et al.* 2008; Salmonid species, Roberge *et al.* 2008; Normandeau *et al.* 2009; Renaut *et al.* 2009) as the major mode of transcription regulation inheritance. As also noted by Rottscheidt and Harr (2007), the variability in the observed major mode of transcription regulation inheritance seemed dependent on different factors such as the methods, tissues, and types of hybrids (backcross or F1) used. Hybrids from divergent species exhibited numerous misexpressed transcripts resulting from the accumulation of regulatory incompatibilities (Landry *et al.* 2007). However, Rottscheidt and Harr (2007) showed that neither the level of inbreeding nor time since divergence could be associated in previous studies with the frequency of nonadditivity. The only exception to this was found in patterns of gene expression in testis, which were interpreted as a consequence of the role of sexual organ differentiation in reproductive isolation. In contrast, within inbred lines of maize, the hybrids from less distant parental lines exhibited the greatest proportion of nonadditivity of transcripts (Stupar *et al.* 2008). Moreover, frequency of additivity varied highly depending on the tissue studied. Thus, genetic distance has not yet firmly been linked with the frequency of additivity at the intraspecific level of comparison. Here, our results regarding the extent of nonadditive transcription regulation inheritance observed in hybrids are suggestive of a positive correlation with genetic distance between the parental populations. Thus, hybrids produced by crosses between the two most distant populations (R and L) showed more nonadditive transcripts than any other hybrid crosses. Predominance of nonadditivity was also observed in hybrids resulting from crosses between two divergent populations of lake whitefish (*Coregonus clupeaformis*), another salmonid species (Renaut *et al.* 2009). In addition, we found that transcription regulation inheritance (additive or nonadditive modes of transmission regulation) was often not correlated for a given gene among the three hybrid crosses (Table 2). Transcription regulation inheritance is affected by many factors, such as DNA binding sites, transcription factor abundance, and the affinity of the latter for the binding sites. However, epigenetic changes could be responsible for transcriptional changes among hybrids (reviewed by Michalak 2009). These parameters and their complex interactions result in the diversity of transcript expression patterns that in turn contribute to the biological diversity observed at the population level (Brown 2006; Maston *et al.* 2006; Gerke *et al.* 2009). Moreover, because of interactions in the regulatory network, hybrids with different phenotypes than those of their parents can be obtained from crossing parental populations with equivalent phenotypes (Brem and Kruglyak 2005; Landry *et al.* 2007). Here, the hybridization process resulted in complex patterns of transcript regulation and affected biological functions among crosses. Given the differences detected between parental populations, such variability of the transcriptome among the three hybrid crosses could depend on the unique genetic architectures of each of the parental populations.

Hypothetical links between patterns of gene expression and hybrid phenotypes: In this section, we discuss how size phenotypes in the hybrid crosses could be indirectly linked to the differences in inheritance patterns of transcripts that we observed. Thus, as observed at the transcriptome level, we observed three different inheritance patterns of size at age in hybrids relative to their parental populations (Figure 3). Namely, the DR hybrids, which showed an additive size phenotype response, also exhibited over 94% of additively expressed transcripts. This was in sharp contrast with LD and RL hybrids, which respectively showed heterosis and outbreeding depression for size, as well as a higher proportion of nonadditivity (dominant and over-/underdominance) in transcription regulation. What could the molecular causes underlying the expression of heterosis *vs.* outbreeding depression at the phenotypic level be? Among the most common hypotheses regarding the mechanisms underlying heterosis are: (i) dominance, which explains heterosis by masking the effect of deleterious alleles by superior alleles from the parents; (ii) overdominance, for which allelic interactions at heterozygous locus result in positive effect superior to homozygote; (iii) pseudo-overdominance, which is a positive dominance complementation of linked alleles (Lippman and Zamir 2007). Moreover, dominant patterns of expression regulation have been proposed to result from allelic dosage effects (Auger *et al.* 2005), monoallelic expression (Birchler *et al.* 2003), or epistatic gene interactions (Hedgecock *et al.* 2007). The main differences observed between LD and RL hybrids lie in the proportions of maternal/paternal dominant transcripts and over-/underdominance (Table 1). Given that LD hybrids present a higher number of dominant transcripts relative to OD and UD compared to RL hybrids, the dominance hypothesis could explain the predominance of size heterosis observed in LD hybrids. In particular, there was a clear prevalence of maternal population dominance in the LD hybrids. Thus, gene dominance from the biggest parental population may explain the performance of these hybrids. At early life stages, the maternal genome may influence size through gene products contained in the egg (Hebert *et al.* 1998; Nakajima and Taniguchi 2002). Indeed, previous studies on the L population have revealed that maternal effects are acting until yolk sac resorption and are correlated with the size of the mother (Perry *et al.* 2005). Heterosis may be further enhanced by the presence of numerous overdominant transcripts in hybrids (Table S4). Therefore, at this

stage of development, heterosis could result from advantageous dominance and overdominance of transcripts inheritance, and maternal effects could further contribute to the heterosis observed in the LD individuals.

On the other hand, outbreeding depression can also result in the loss of favorable genetic interactions or/and the disruption of epistatic interaction between coevolved genes (Mcclelland and Naish 2007). Transgressive phenotypes in hybrids may thus be the consequence of a deeper genetic distance between parental populations (Stelkens and Seehausen 2009). For instance, in rainbow trout (*Oncorhynchus mykiss*), outbreeding depression did not appear in introgressed genomes, possibly because coadapted genotypes were too similar for recombination to have a negative impact (Tymchuk *et al.* 2007). Moreover, divergent genomes may accumulate regulatory incompatibilities resulting in occurrence of misexpressed transcripts (OD or UD) in hybrids (Landry *et al.* 2007). Here, the deeper genetic distance between the R and L populations could explain the higher prevalence of transgressive transcripts involved in many biological functions in their hybrids, as compared to those involving the D population. It is also noteworthy that outbreeding depression for length was observed in the RL hybrids, which also showed the highest number of transgressive transcripts (either UD or OD) (Table 1).

Our results thus support the hypothesis that transgressivity of phenotypes may depend both on genetic distance between the parental populations and on interactions between dominant *vs.* transgressive transcription regulation mechanisms arising from divergent genetic architectures. Therefore, prevalence of dominance combined with overdominance in transcription regulation seems related with heterosis, while prevalence of transgressive transcription regulation seems to be more related with outbreeding depression. To conclude, while our results suggest that size and transcriptional regulation phenotypes are at least partially associated, they also illustrate the complexity of predicting hybrid phenotypes on the sole basis of previous knowledge of parental phenotypes or their genetic divergence.

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LITERATURE CITED

Auger, D. L., A. D. Gray, T. S. Ream, A. Kato, E. H. Coe *et al.*, 2005 Nonadditive gene expression in diploid and triploid hybrids of maize. Genetics **169:** 389–397.

Baack, E. J., and L. H. Rieseberg, 2007 A genomic view of introgression and hybrid speciation. Curr. Opin. Genet. Dev. **17**(6): 513–518.

Barton, N. H., 2001 The role of hybridization in evolution. Mol. Ecol. **10:** 551–568.

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

Brem, R. B., and L. Kruglyak, 2005 The landscape of genetic complexity across 5,700 gene expression traits in yeast. Proc. Natl. Acad. Sci. USA **102:** 1572–1577.

Brown, T. A., 2006 *Genomes 3.* Garland Science, New York.

Burke, J. M., and M. L. Arnold, 2001 Genetics and the fitness of hybrids. Annu. Rev.Genet. **35:** 31–52.

Castric, V. and L. Bernatchez, 2003 The rise and fall of isolation by distance in the anadromous brook charr (*Salvelinus fontinalis* Mitchill). Genetics **163:** 983–996.

Cipriano, R. C., D. Marchant, T. E. Jones and J. H. Schachte, 2002 Practical application of disease resistance: a brook trout fishery selected for resistance to furunculosis. Aquaculture **206:** 1–17.

Cui, X. G., J. T. G. Hwang, J. Qiu, N. J. Blades and G. A. Churchill, 2005 Improved statistical tests for differential gene expression by shrinking variance components estimates. Biostatistics **6:** 59–75.

Cui, X. Q., J. Affourtit, K. R. Shockley, Y. Woo and G. A. Churchill, 2006 Inheritance patterns of transcript levels in F-1 hybrid mice. Genetics **174:** 627–637.

Fraser, D. J., P. Duchesne and L. Bernatchez, 2005 Migratory charr schools exhibit population and kin associations beyond juvenile stages. Mol. Ecol. **14:** 3133–3146.

Ge, X., W. Chen, S. Song, W. Wang, S. Hu *et al.*, 2008 Transcriptomic profiling of mature embryo from an elite super-hybrid rice LYP9 and its parental lines. BMC Plant Biol. **8:** 114.

Gerke, J., K. Lorenz and B. Cohen, 2009 Genetic interactions between transcription factors cause natural variation in yeast. Science **323:** 498–501.

Gibson, G., R. Riley-Berger, L. Harshman, A. Kopp, S. Vacha *et al.*, 2004 Extensive sex-specific nonadditivity of gene expression in *Drosophila melanogaster.* Genetics **167:** 1791–1799.

Giger, T., L. Excoffier, P. J. R. Day, A. Champigneulle, M. M. Hansen *et al.*, 2006 Life history shapes gene expression in salmonids. Curr. Biol. **16:** R281–R282.

Hansen, T. F., 2006 The evolution of genetic architecture. Annu. Rev. Ecol. Evol. System. **37:** 123–157.

Hebert, K. P., P. L. Goddard, W. W. Smoker and A. J. Gharrett, 1998 Quantitative genetic variation and genotype by environment interaction of embryo development rate in pink salmon (*Oncorhynchus gorbuscha*). Can. J. Fisheries Aquatic Sci. **55:** 2048–2057.

Hedgecock, D., J.-Z. Lin, S. DeCola, C. D. Haudenschild, E. Meyer *et al.*, 2007 Transcriptomic analysis of growth heterosis in larval Pacific oysters (*Crassostrea gigas*). Proc. Natl. Acad. Sci. USA **104:** 2313–2318.

Hughes, K. A., J. F. Ayroles, M. M. Reedy, J. M. Drnevich, K. C. Rowe *et al.*, 2006 Segregating variation in the transcriptome: *cis* regulation and additivity of effects. Genetics **173:** 1347–1355.

Kerr, M. K., M. Martin and G. A. Churchill, 2000 Analysis of variance for gene expression microarray data. J. Comput. Biol. **7:** 819–837.

Kerr, M. K., C. A. Afshari, L. Bennett, P. Bushel, J. Martinez *et al.*, 2002 Statistical analysis of a gene expression microarray experiment with replication. Statist. Sinica **12:** 203–217.

Kruuk, L. E. B., 2004 Estimating genetic parameters in natural populations using the 'animal model'. Philos. Trans. R. Soc. Lond. B Biol. Sci. **359:** 873–890.

Koop, B. F, K. R. von Schalburg, J. Leong, N. Walker, R. Lieph *et al.*, 2008 A salmonid EST genomic study: genes, duplications, phylogeny and microarrays. BMC Genomics **9:** 545.

Landry, C. R., D. L. Hartl and J. M. Ranz, 2007 Genome clashes in hybrids: insights from gene expression. Heredity **99:** 483–493.

Lavagnino, N. J., R. R. H. Anholt and J. J. Fanara, 2008 Variation in genetic architecture of olfactory behaviour among wild-derived populations of *Drosophila melanogaster.* J. Evol. Biol. **21:** 988–996.

Li, H. O., and J. Yamada, 1992 Changes of the fatty acid composition in smolts of masu salmon (*Oncorhynchus masou*) associated with desmoltification and sea-water transfer. Comp. Biochem. Physiol. **103:** 221–226.

Lippman, Z. B., and D. Zamir, 2007 Heterosis: revisiting the magic. Trends Genet. **23:** 60–66.

Lynch, M., 2007 *The Origins of Genome Architecture.* Sinauer Associates, Sunderland, MA.

Martin, S., J.-Y. Savaria, C. Audet and L. Bernatchez, 1997 Microsatellites reveal no evidence for inbreeding effects but low inter-stock genetic diversity among brook charr stocks used for production in Québec. Bull. Aquaculture Assoc. Can., 21–23.

Maston, G. A., S. K. Evans and M. R. Green, 2006 Transcriptional regulatory elements in the human genome. Annu. Rev. Genomics Hum. Genet. **7:** 29–59.

Mavarez, J., C. Audet and L. Bernatchez, 2009 Major disruption of gene expression in hybrids between young sympatric anadromous and resident populations of brook charr (*Salvelinus fontinalis* Mitchill). J. Evol. Biol. **8:** 1708–1720.

McClelland, E. K. and K. A. Naish, 2007 What is the fitness outcome of crossing unrelated fish populations? A meta-analysis and an evaluation of future research directions. Conserv. Genet. **8:** 397–416.

Michalak, P., 2009 Epigenetic, transposon and small RNA determinants of hybrid dysfunctions. Heredity **102:** 45–50.

Michalak, P., and M. A. F. Noor, 2003 Genome-wide patterns of expression in drosophila pure species and hybrid males. Mol. Biol. Evol. **20:** 1070–1076.

Nakajima, M., and N. Taniguchi, 2002 Genetic control of growth in the guppy (*Poecilia reticulata*). Aquaculture **202:** 393–405.

Nolte, A., J. Freyhof and D. Tautz, 2006 When invaders meet locally adapted types: rapid moulding of hybrid zones between sculpins (*Cottus, Pisces*) in the Rhine system. Mol. Ecol. **15:** 1983–1993.

Normandeau, E., J. A. Hutchings, D. J. Fraser and L. Bernatchez, 2009 Population-specific gene expression responses to hybridization between farm and wild Atlantic salmon. Evol. Appl. **2:** 489–503.

Oleksiak, M. F., G. A. Churchill and D. L. Crawford, 2002 Variation in gene expression within and among natural populations. Nat. Genet. **32:** 261–266.

Perry, G. M. L., C. Audet and L. Bernatchez, 2005 Maternal genetic effects on adaptive divergence between anadromous and resident brook charr during early life history. J. Evol. Biol. **18:** 1348–1361.

Quinn, G. P., and M. J. Keough 2005 Experimental design and data analysis for biologists. Fourth edition. Cambridge University Press, Cambridge, UK.

Ranz, J. M., K. Namgyal, G. Gibson and D. L. Hartl, 2004 Anomalies in the expression profile of interspecific hybrids of *Drosophila melanogaster* and *Drosophila simulans*. Genome Res. **14:** 373–379.

Reiland, J., and M. A. Noor, 2002 Little qualitative RNA misexpression in sterile male F1 hybrids of *Drosophila pseudoobscura* and *D. persimilis*. BMC Evol. Biol. **2:** 16.

Renaut, S., A. W. Nolte and L. Bernatchez, 2009 Transcriptomic investigation of post-zygotic isolation in lake whitefish (*Coregonus clupeaformis*). Mol. Biol. Evol. **26:** 925–936.

Rieseberg, L. H., M. A. Archer and R. K. Wayne, 1999 Transgressive segregation, adaptation and speciation. Heredity **83:** 363–372.

Rieseberg, L. H., A. Widmer, A. Michele Arntz and B. Burke, 2003 The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations. Philos. Trans. R. Soc. Lond. B Biol. Sci. **358:** 1141–1147.

Rise, M. L., K. R. von Schalburg, G. D. Brown, M. A. Mawer, R. H. Devlin *et al.*, 2004 Development and application of a salmonid EST database and cDNA microarray: data mining and interspecific hybridization characteristics. Genome Res. **14:** 478–490.

Roberge, C., S. Einum, H. Guderley and L. Bernatchez, 2006 Rapid parallel evolutionary changes of gene transcription profiles in farmed Atlantic salmon. Mol. Ecol. **15:** 9–20.

Roberge, C., E. Normandeau, S. Einum, H. Guderley and L. Bernatchez, 2008 Genetic consequences of interbreeding between farmed and wild Atlantic salmon: insights from the transcriptome. Mol. Ecol. **17:** 314–324.

Rodríguez-Trelles, F., R. Tarrío and F. J. Ayala, 2005 Is ectopic expression caused by deregulatory mutations or due to gene-regulation leaks with evolutionary potential? BioEssays **27:** 592–601.

Rottscheidt, R. and B. Harr, 2007 Extensive additivity of gene expression differentiates subspecies of the house mouse. Genetics **177:** 1553–1567.

Sauvage, C., N. Derôme, E. Normandeau, J. St-Cyr, C. Audet *et al.*, 2010 Fast transcriptional responses to domestication in the brook charr *Salvelinus fontinalis*. Genetics **185:** 105–112.

Sheridan, M. A., N. Y. S. Woo and H. A. Bern, 1985 Changes in the rates of glycogenesis, glycogenolysis, lipogenesis, and lipolysis in selected tissues of the coho salmon (*Oncorhynchus kisutch*) associated with parr-smolt transformation. J. Exp. Zool. **236:** 35–44.

Shikano, T. and N. Taniguchi, 2002 Heterosis for neonatal survival in the guppy. J. Fish Biol. **60:** 715–725.

Shriver, M. D., L. Jin, E. Boerwinkle, R. Deka, R. E. Ferrell *et al.*, 1995 A novel measure of genetic distance for highly polymorphic tandem repeat loci. Mol. Biol. Evol. **12:** 914–920.

Stelkens, R., and O. Seehausen, 2009 Genetic distance between species predicts novel trait expression in their hybrids. Evolution **63:** 884–897.

Stupar, R. M., and N. M. Springer, 2006 *Cis*-transcriptional variation in maize inbred lines B73 and Mo17 leads to additive expression patterns in the F1 hybrid. Genetics **173:** 2199–2210.

Stupar, R. M., J. M. Gardiner, A. G. Oldre, W. J. Haun, V. L. Chandler *et al.*, 2008 Gene expression analyses in maize inbreds and hybrids with varying levels of heterosis. BMC Plant Biol. **8:** 33.

Swanson-Wagner, R. A., Y. Jia, R. DeCook, L. A. Borsuk, D. Nettleton *et al.*, 2006 All possible modes of gene action are observed in a global comparison of gene expression in a maize F-1 hybrid and its inbred parents. Proc. Natl. Acad. Sci. USA **103:** 6805–6810.

Tallmon, D. A., G. Luikart and R. S. Waples, 2004 The alluring simplicity and complex reality of genetic rescue. Trends Ecol. Evol. **19:** 489–496.

Tymchuk, W. E., L. F. Sundström and R. H. Devlin, 2007 Growth and survival trade-offs and outbreeding depression in rainbow trout (*Oncorhynchus mykiss*). Evolution **61–5:** 1225–1237.

von Schalburg, K. R., M. L. Rise, G. A. Cooper, G. D. Brown, A. R. Gibbs *et al.*, 2005 Fish and chips: various methodologies demonstrate utility of a 16,006-gene salmonid microarray. BMC Genomics **6;** 126.

von Schalburg, K. R., G. A. Cooper, J. Leong, A. Robb, R. Lieph *et al.*, 2008 Expansion of the genomics research on Atlantic salmon (*Salmo salar*) L. project (GRASP) microarray tools. J. Fish Biol. **72:** 2051–2070.

Whitehead, A., and D. L. Crawford, 2006 Neutral and adaptive variation in gene expression. Proc. Natl. Acad. Sci. USA **103:** 5425–5430.

Wolfinger, R. D., G. Gibson, E. D. Wolfinger, L. Bennett, H. Hamadeh *et al.*, 2001 Assessing gene significance from cDNA microarray expression data via mixed models. J. Comput. Biol. **8:** 625–637.

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The Transcriptional Landscape of Cross-Specific Hybrids and Its Possible Link With Growth in Brook Charr (*Salvelinus fontinalis* Mitchill)

Bérénice Bougas, Sarah Granier, Céline Audet and Louis Bernatchez

DOI: 10.1534/genetics.110.118158

File S1

Size data at yolk sac resorption stage for DD, LL, RR, DR, LD, RL individual

File S1 is available for download as an Excel file http://www.genetics.org/cgi/content/full/genetics.110.118158/DC1.

TABLE S1

List of differentially expressed genes between Rupert and Laval populations

Gene names	#clones	Pvalue(FDR)	Fold-change	Functional categories
Over-transcribed in R				
Acidic mammalian chitinase precursor	1	0.0371	1.79	Carbohydrate metabolism
NADP-dependent malic enzyme, mitochondrial precursor	1	0.0465	1.20	Carbohydrate metabolism
Transaldolase	1	0.0369	1.15	Carbohydrate metabolism
Glutathione peroxidase 2	2	0.0060	1.26	**Cell cycle regulation / response to stress*
UV excision repair protein RAD23 homolog B	1	0.0494	1.24	DNA repair
Endoplasmin precursor	1	0.0363	1.25	**Intracellular transport / response to stress*
Tubulin beta-6 chain	1	0.0210	1.20	**Intracellular transport / Energy metabolism*
Sodium/potassium-transporting ATPase subunit beta-233	1	0.0221	1.25	**Ion transport*
H-2 class II histocompatibility antigen gamma chain	2	0.0423	1.38	MHCII-mediated immunity
Heterogeneous nuclear ribonucleoprotein A/B	1	0.0287	1.19	Pre-mRNA processing
Eukaryotic translation initiation factor 3 subunit 4	1	0.0147	1.27	Protein metabolism and modification
Glucose-regulated protein 94 [Paralichthys olivaceus]	1	0.0369	1.20	**Protein process*
Protein transport protein Sec61 subunit beta	1	0.0230	1.20	**Protein transport*
Membrane-bound transcription factor site-1 protease precursor	1	0.0207	1.21	Proteolysis
Keratin, type II cytoskeletal 8	1	0.0474	1.17	**Strutural molecule activity*
60S ribosomal protein L10a	1	0.0291	1.18	None
60S ribosomal protein L19	1	0.0286	1.28	None

60S ribosomal protein L4-A	1	0.0417	1.19	None
Adenylyl cyclase-associated protein 1	1	0.0363	1.16	None
Intermediate filament protein ON3	1	0.0217	1.33	None
Lipocalin precursor	3	0.0316	1.60	None
Myotrophin	1	0.0369	1.16	None
Myristoylated alanine-rich C-kinase substrate	1	0.0291	1.18	None
NADH-ubiquinone oxidoreductase chain 3	1	0.0073	1.44	None
Oncorhynchus mykiss toxin-1 mRNA, complete cds	1	0.0134	1.43	None
PREDICTED: Danio rerio hypothetical LOC556254 (LOC556254), mRNA	1	0.0494	1.17	None
Protein involved in cell morphogenesis and proliferation, associated with protein kinase Cbk1p	1	0.0435	1.19	None
Serum albumin 1 precursor	1	0.0122	1.50	None
Serum albumin 2 precursor	2	0.0099	1.56	None
Tubulin alpha chain	2	0.0463	1.20	None
UDP-glucuronosyltransferase	1	0.0455	1.19	None
Uncharacterized protein C6orf58 homolog precursor	1	0.0435	1.25	None
Under- transcribed in R				
ATP synthase lipid-binding protein, mitochondrial precursor	1	0.8099	0.81	B.P.U
SPARC precursor	1	0.7173	0.72	B.P.U
Diamine acetyltransferase 1	1	0.8173	0.82	**Cell cycle regulation*
ADP/ATP translocase 2	1	0.7905	0.79	Intracellular transport
Myelin expression factor 2	1	0.7330	0.73	Intracellular transport
Immune-related Hdd11	1	0.7121	0.71	**Immunity*
Lysosomal acid lipase/cholesteryl ester hydrolase precursor	1	0.6654	0.67	**L.F.A metabolism*

Creatine kinase, sarcomeric mitochondrial precursor	1	0.8314	0.83	Muscle contraction
Myosin light chain 1, cardiac muscle	3	0.7422	0.74	**Muscle contraction*
Troponin I, fast skeletal muscle	2	0.7617	0.76	**Muscle contraction*
Cathepsin L precursor	2	0.6889	0.69	**Peptidase activity*
26S proteasome non-ATPase regulatory subunit 14	1	0.7512	0.75	Protein metabolism and modification
40S ribosomal protein S27	1	0.8171	0.82	Protein metabolism and modification
Nucleoside diphosphate kinase A	7	0.7611	0.76	Pyrimidin metabolism
Nucleoside diphosphate kinase B	5	0.7035	0.70	Pyrimidin metabolism
CD63 antigen	1	0.8629	0.86	Signal transduction
Collagen alpha-1(X) chain precursor	1	0.7857	0.79	Signal transduction/cell adhesion
Actin, cytoplasmic 1	1	0.8309	0.83	**Strutural molecule activity*
Guanidinoacetate N-methyltransferase	2	0.7803	0.78	**Transferase activity*
Apolipoprotein A-I precursor	1	0.6521	0.65	None
Apolipoprotein A-IV precursor	1	0.6576	0.66	None
Canalicular multispecific organic anion transporter 2	1	0.7972	0.80	None
Complement C3-1	2	0.8177	0.82	None
Fatty acid-binding protein, heart	1	0.8059	0.81	None
Pachymedusa dacnicolor partial mRNA for ribosomal protein S16 (rps16 gene)	1	0.6866	0.69	None
PREDICTED: Danio rerio similar to type V collagen (LOC799369), mRNA	1	0.8129	0.81	None
Proteasome subunit beta type 1-A	1	0.8466	0.85	None

Fold-change represents the fold changes of average ratio; p-value (FDR) corresponds to the FDR-corrected (1000 permutations) p-value of the ANOVA test. #clones: number of clones. Abbreviation for the functional categories: A.A: Amino acid; B.P.U.: Biological process unclassified; L.F.A.: Lipid, fatty acid; None: no functional category defined for unique gene in Panther online classification system (http://www.pantherdb.org). **Functional categories presented in italic correspond to hypothetical functions that were identified manually in Swiss-Prot. These functions were not used in the PANTHER analysis.*

TABLE S2

List of differentially expressed genes between Domestic (D) and Rupert (R) populations

Gene names	#clones	Pvalue (FDR)	Fold-change	Functional categories
Over-transcribed in D				
ATP synthase D chain, mitochondrial	1	0.0052	1.24	B.P.U
ATP synthase lipid-binding protein, mitochondrial precursor	3	0.0024	1.26	B.P.U
ATP synthase subunit alpha, mitochondrial precursor	2	0.0086	1.22	B.P.U
ATP synthase subunit beta, mitochondrial precursor	1	0.0147	1.21	B.P.U
Brain protein 44-like protein	1	0.0049	1.27	B.P.U
Diamine acetyltransferase 1	2	0.0027	1.26	**Cell cycle regulation*
Protein kinase C eta type	1	0.0046	1.27	Intracellular signaling cascade/ calcium mediated signaling/ cell proliferation and differentiation/ signal transduction
Periostin precursor	1	0.0072	1.29	Cell adhesion
Anterior gradient protein 2 homolog precursor	1	0.0163	1.46	Developmental process
Cytochrome c oxidase polypeptide VIa, mitochondrial precursor	1	0.0146	1.19	Electron transport
Cathepsin L2 precursor	1	0.0101	1.36	**Enzyme activity*
Heat shock protein HSP 90-beta	1	0.0320	1.17	Immunity and defense
Immune-related Hdd11	1	0.0027	1.41	**Immunity*
ADP/ATP translocase 2	3	0.0060	1.25	Intracellular transport
Myelin expression factor 2	1	0.0048	1.31	Intracellular transport
Vacuolar ATP synthase 16 kDa proteolipid subunit	2	0.0428	1.14	Intracellular transport

Tubulin alpha-1C chain	1	0.0421	1.17	**Intracellular transport / Energy metabolism*
Calpain-1 catalytic subunit	1	0.0092	1.27	**Ion binding*
Hemoglobin subunit beta-2	1	0.0000	1.83	**Ion transport / Oxygen transport*
Phospholemman-like protein precursor	1	0.0177	1.15	**Ion transport*
Translation machinery-associated protein 46	1	0.0254	1.19	**Ion transport*
Apolipoprotein B-100 precursor	2	0.0074	1.19	L.F.A. metabolism
Apolipoprotein Eb precursor	1	0.0254	1.31	**L.F.A metabolism*
Lysosomal acid lipase/cholesteryl ester hydrolase precursor	1	0.0054	1.38	**L.F.A metabolism*
Myosin heavy chain, fast skeletal muscle	1	0.0497	1.15	**Muscle contraction*
CTP synthase 1	1	0.0098	1.46	**Nucleotide binding*
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	1	0.0046	1.20	Oxydative phosphorilation
Cathepsin L precursor	3	0.0012	1.51	**Peptidase activity*
Heterogeneous nuclear ribonucleoprotein A0	1	0.0130	1.22	Pre-mRNA processing
Heterogeneous nuclear ribonucleoprotein A3 homolog 1	1	0.0029	1.41	Pre-mRNA processing
Kelch-like protein 6	1	0.0116	1.89	**Protein binding*
Polyadenylate-binding protein 1-A	1	0.0218	1.16	**Protein binding/RNA binding*
40S ribosomal protein S2	1	0.0278	1.17	Protein metabolism and modification
40S ribosomal protein S27	1	0.0074	1.19	Protein metabolism and modification
Elongation factor 1-delta	1	0.0380	1.12	Protein metabolism and modification
Eukaryotic translation initiation factor 1A, X-chromosomal	1	0.0136	1.17	Protein metabolism and modification
Eukaryotic translation initiation factor 1b	3	0.0027	1.28	Protein metabolism and modification
Eukaryotic translation initiation factor 2 subunit 2	2	0.0113	1.20	Protein metabolism and modification
Protein disulfide-isomerase precursor	3	0.0370	1.35	Protein metabolism and modification

T-complex protein 1 subunit epsilon	1	0.0071	1.24	Protein metabolism and modification
FK506-binding protein 1A	1	0.0150	1.19	**Protein metabolism and modification*
Nucleoside diphosphate kinase A	5	0.0146	1.27	Pyrimidin metabolism
Nucleoside diphosphate kinase B	4	0.0046	1.31	Pyrimidin metabolism
Cold-inducible RNA-binding protein	2	0.0025	1.25	RNA binding
Nucleophosmin	1	0.0242	1.29	**RNA binding*
Transcription factor BTF3 homolog 4	1	0.0042	1.25	**mRNA metabolism*
NHP2-like protein 1	3	0.0203	1.29	rRNA metabolism
Nucleolar protein 5	1	0.0058	1.19	rRNA metabolism
Mu-crystallin homolog	1	0.0027	1.28	Sensitive receptor
CD63 antigen	1	0.0021	1.23	Signal transduction
Collagen alpha-1(X) chain precursor	4	0.0027	1.37	Signal transduction/cell adhesion
Actin, cytoplasmic 1	1	0.0034	1.26	Strutural molecule activity
Dynein light chain 1, cytoplasmic	1	0.0484	1.19	**Strutural molecule activity*
T-complex protein 1 subunit gamma	1	0.0147	1.17	**Strutural molecule activity*
Acyl-CoA-binding protein	1	0.0219	1.24	None
Apolipoprotein A-I precursor	7	0.0003	1.67	None
Apolipoprotein A-I-2 precursor	1	0.0033	1.53	None
Apolipoprotein A-IV precursor	2	0.0002	2.06	None
Chymotrypsin A precursor	1	0.0176	2.20	None
Chymotrypsin B	3	0.0150	2.04	None
Complement C3-1	2	0.0043	1.28	None
Cystatin-B	1	0.0379	1.16	None

Cytochrome c	1	0.0471	1.22	None
Danio rerio SET translocation B	1	0.0380	1.24	None
Ependymin-1 precursor	4	0.0012	1.48	None
Ependymin-2 precursor	1	0.0106	1.45	None
Exocyst complex component 7	1	0.0156	1.21	None
Fatty acid-binding protein, liver	3	0.0155	1.43	None
Homo sapiens splicing factor, arginine/serine-rich 2 (SFRS2), mRNA	1	0.0010	1.27	None
Nucleolin	1	0.0208	1.27	None
Oncorhynchus mykiss mRNA for type II keratin E1 (E1 gene)	1	0.0407	1.20	None
Oncorhynchus mykiss SYPG1 (SYPG1), PHF1 (PHF1), and RGL2 (RGL2) genes	1	0.0264	1.24	None
Oncorhynchus nerka connective tissue growth factor (CTGF) gene	1	0.0051	1.30	None
Osteopontin-like protein	1	0.0060	1.20	None
Pachymedusa dacnicolor partial mRNA for ribosomal protein S16 (rps16 gene)	1	0.0164	1.27	None
Pleiotrophic factor-alpha-2 precursor	1	0.0322	1.17	None
PREDICTED: Danio rerio similar to type V collagen (LOC799369), mRNA	1	0.0176	1.20	None
Retinol-binding protein II, cellular	1	0.0150	1.24	None
rRNA 2'-O-methyltransferase fibrillarin	1	0.0380	1.14	None
Tropomyosin-1 alpha chain	1	0.0060	1.19	None
Trypsin-1 precursor	4	0.0074	3.04	None
Tubulin beta-1 chain	1	0.0200	1.24	None
Type-4 ice-structuring protein precursor	3	0.0389	1.49	None

Under-transcribed in D				
Cystathionine gamma-lyase	1	0.0062	0.78	A.a metabolism
ES1 protein homolog, mitochondrial precursor	2	0.0027	0.71	B.P.U
WD repeat protein 23	1	0.0003	0.65	B.P.U
Acidic mammalian chitinase precursor	1	0.0003	0.37	Carbohydrate metabolism
Transaldolase	1	0.0052	0.84	Carbohydrate metabolism
Asialoglycoprotein receptor 2	1	0.0130	0.81	Cell adhesion
Epithelial-cadherin precursor	1	0.0046	0.82	Cell adhesion
Glutathione peroxidase 2	2	0.0078	0.85	**Cell cycle regulation / response to stress*
Fatty acid-binding protein, brain	1	0.0209	0.84	**Cell proliferation / L.F.A metabolism*
Tetraodon nigroviridis partial BEL-like LTR retrotransposon	1	0.0360	0.75	**DNA mediated*
Cytochrome c oxidase polypeptide VIIa-liver/heart, mitochondrial precursor	1	0.0114	0.73	Electron transport
Betaine aldehyde dehydrogenase	1	0.0215	0.79	**Energy metabolism*
Zymogen granule membrane protein 16 precursor	3	0.0000	0.27	**Extracellular transport*
BOLA class I histocompatibility antigen, alpha chain BL3-7 precursor	1	0.0387	0.86	**Immunity*
Hemoglobin subunit alpha-4	1	0.0000	0.30	**Ion transport / Oxygen transport*
Ubiquinol-cytochrome c reductase complex 11 kDa protein, mitochondrial precursor	1	0.0407	0.86	**Ion transport*
Peroxisomal multifunctional enzyme type 2	1	0.0012	0.74	L.F.A. metabolism
H-2 class II histocompatibility antigen gamma chain	7	0.0000	0.59	MHCII-mediated immunity
Proteasome activator complex subunit 2	1	0.0000	0.38	**Proteasome activity*
Elongation factor 1-beta	1	0.0001	0.54	Protein metabolism and modification

Maleylacetoacetate isomerase	1	0.0164	0.83	Protein metabolism and modification
Nuclease sensitive element-binding protein 1	2	0.0259	0.86	**Regulation of transcription*
Peroxiredoxin-5, mitochondrial precursor	3	0.0254	0.85	**Response to oxidative stress*
Cofilin-2	1	0.0004	0.73	**Strutural molecule activity*
Intermediate filament protein ON3	1	0.0439	0.82	**Strutural molecule activity*
Oncorhynchus mykiss invariant chain S25-7 mRNA, complete cds	1	0.0003	0.69	None
60S acidic ribosomal protein P2	1	0.0000	0.35	None
60S ribosomal protein L19	1	0.0364	0.82	None
Actin-related protein 2/3 complex subunit 5	1	0.0391	0.87	None
Beta-2-microglobulin precursor	16	0.0001	0.70	None
Cytochrome c oxidase subunit 1	1	0.0175	0.82	None
Differentially regulated trout protein 1 [Oncorhynchus mykiss]	3	0.0001	0.52	None
Glutathione S-transferase P	3	0.0046	0.81	None
Glutathione S-transferase P 1	1	0.0254	0.82	None
Histone H1.0	1	0.0364	0.79	None
Keratin, type I cytoskeletal 13	1	0.0035	0.70	None
Lysozyme g	1	0.0110	0.81	None
NADH-ubiquinone oxidoreductase chain 3	2	0.0002	0.68	None
Nonhistone chromosomal protein H6	2	0.0196	0.79	None
Oncorhynchus masou gene for alpha-glycoprotein subunit 1, 5' flanking region	1	0.0052	0.77	None
Oncorhynchus mykiss mRNA for carbonic anhydrase 1, complete cds	1	0.0364	0.82	None
Oncorhynchus mykiss mRNA for Keratin 13 (k13 gene)	1	0.0037	0.70	None

Oncorhynchus mykiss partial mRNA for Keratin 12 (k12 gene)	1	0.0017	0.66	None
PREDICTED: Danio rerio hypothetical LOC556254 (LOC556254), mRNA	1	0.0273	0.78	None
Salmo salar clone BE7 beta-2 microglobulin (B2m) mRNA, complete cds	1	0.0117	0.82	None
Salmo salar MHC class I (UBA) mRNA, UBA*1401 allele, complete cds	1	0.0059	0.75	None
Salvelinus fontinalis differentially regulated trout protein 1 mRNA, complete cds	1	0.0004	0.49	None
Sensor protein dcuS	1	0.0207	0.82	None
Serotransferrin precursor	2	0.0007	0.71	None
Serotransferrin-1 precursor	2	0.0094	0.67	None
Serotransferrin-2 precursor	1	0.0012	0.69	None
Serum albumin 1 precursor	1	0.0254	0.75	None

Fold-change represents the fold changes of average ratio; p-value (FDR) corresponds to the FDR-corrected (1000 permutations) p-value of the ANOVA test. #clones: number of clones. Abbreviation for the functional categories: A.A: Amino acid; B.P.U.: Biological process unclassified; L.F.A.: Lipid and fatty acid; None: no functional category defined for unique gene in Panther online classification system (http://www.pantherdb.org). ***Functional categories presented in italic correspond to hypothetical functions that were identified manually in Swiss-Prot. These functions were not used in the PANTHER analysis.*

TABLE S3

List of differentially expressed genes between Laval (L) and Domestic (D) populations

Gene names	#clones	Pvalue(FDR)	Fold-change	Functional categories
Over-transcribed in D				
ATP synthase subunit alpha, mitochondrial precursor	1	0.0264	1.18	B.P.U
Calmodulin	1	0.0253	1.16	Calcium mediated signaling/cell proliferation and differentiation/ signal transduction/intracellular signaling cascade
Protein kinase C eta type	1	0.0013	1.32	Calcium mediated signaling/cell proliferation and differentiation/signal transduction/intracellular signaling cascade
Tubulin alpha-1A chain	2	0.0026	1.30	Cell motility/intracellular protein trafic/chromosome segregation
Tubulin beta-2B chain	2	0.0043	1.25	Cell motility/intracellular protein trafic/chromosome segregation
Tubulin beta-2C chain	1	0.0170	1.24	Cell motility/intracellular protein trafic/chromosome segregation
Tubulin alpha-1C chain	3	0.0495	1.20	**Cell motility/intracellular protein trafic/chromosome segregation*
Nucleophosmin	1	0.0490	1.24	**Cell proliferation and differentiation / Intracellular transport*
T-complex protein 1 subunit zeta	1	0.0488	1.18	**DNA binding / Protein binding*
UV excision repair protein RAD23 homolog B	1	0.0215	1.24	DNA repair
Cytochrome c oxidase polypeptide VIa, mitochondrial precursor	1	0.0261	1.21	Electron transport
DnaJ homolog subfamily A member 2	1	0.0028	1.20	Immunity and defense
Heat shock protein HSP 90-beta	6	0.0017	1.28	Immunity and defense
ADP/ATP translocase 2	3	0.0077	1.19	Intracellular transport

ATP-binding cassette sub-family F member 2	1	0.0095	1.21	Intracellular transport
THO complex subunit 4	1	0.0329	1.14	Intracellular transport
Vacuolar ATP synthase 16 kDa proteolipid subunit	2	0.0238	1.25	Intracellular transport
60S ribosomal protein L10a	2	0.0011	1.22	**Intracellular transport / RNA binding*
Protein transport protein Sec61 subunit beta	2	0.0058	1.19	**Intracellular transport*
Purpurin precursor	3	0.0026	1.51	**Extracellular transport*
Hemoglobin subunit beta-2	1	0.0002	1.63	**Ion transport / oxygen transport*
Sepiapterin reductase	1	0.0368	1.13	**Ion transport / Energy metabolism*
Apolipoprotein B-100 precursor	3	0.0005	1.27	L.F.A. metabolism
Phospholipase D4	1	0.0133	1.18	L.F.A. metabolism
Apolipoprotein A-IV precursor	2	0.0171	1.46	**L.F.A metabolism*
Core histone macro-H2A.2	1	0.0015	1.25	Nucleotid metabolism
Transcription factor BTF3 homolog 4	1	0.0225	1.18	**mRNA metabolism*
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	1	0.0308	1.14	Oxydative phosphorilation
Heterogeneous nuclear ribonucleoprotein A/B	1	0.0171	1.17	Pre-mRNA processing
Heterogeneous nuclear ribonucleoprotein A0	1	0.0431	1.17	Pre-mRNA processing
Heterogeneous nuclear ribonucleoprotein L	1	0.0131	1.17	Pre-mRNA processing
Polyadenylate-binding protein 1	1	0.0024	1.23	Pre-mRNA processing
Pre-mRNA-processing-splicing factor 8	1	0.0088	1.17	Pre-mRNA processing
Endoplasmin precursor	1	0.0305	1.21	**Protein binding / DNA binding / Response to stress*
Endoplasmin precursor	1	0.0305	1.21	**Protein binding / DNA binding / Response to stress*
Polyadenylate-binding protein 1-A	1	0.0022	1.24	**Protein binding/RNA binding*
Trafficking protein particle complex subunit 5	1	0.0493	1.15	**Protein binding / Extracellular transport*

40S ribosomal protein S2	1	0.0108	1.20	Protein metabolism and modification
78 kDa glucose-regulated protein precursor	1	0.0086	1.30	Protein metabolism and modification
Elongation factor 1-delta	1	0.0375	1.12	Protein metabolism and modification
Eukaryotic translation initiation factor 3 subunit 4	1	0.0376	1.15	Protein metabolism and modification
Protein disulfide-isomerase precursor	2	0.0375	1.35	Protein metabolism and modification
T-complex protein 1 subunit epsilon	1	0.0152	1.20	Protein metabolism and modification
FK506-binding protein 1A	1	0.0015	1.28	**Protein metabolism and modification*
Glucose-regulated protein 94 [Paralichthys olivaceus]	1	0.0236	1.19	**Protein metabolism and modification*
Membrane-bound transcription factor site-1 protease precursor	1	0.0095	1.19	Proteolysis
Activated RNA polymerase II transcriptional coactivator p15	1	0.0346	1.16	**Regulation of transcription / DNA binding*
Cold-inducible RNA-binding protein	3	0.0010	1.40	RNA binding
NHP2-like protein 1	3	0.0093	1.29	rRNA metabolism
Nucleolar protein 5	1	0.0004	1.28	rRNA metabolism
Guanine nucleotide-binding protein G(I)/G(S)/G(O) gamma-5 subunit precursor	1	0.0093	1.30	Signal transduction
Intraflagellar transport 52 homolog	1	0.0490	1.15	Signal transduction
Regulator of telomere elongation helicase 1	1	0.0173	1.19	Signal transduction
Stathmin	2	0.0431	1.28	Signal transduction
Collagen alpha-1(X) chain precursor	1	0.0138	1.24	Signal transduction/cell adhesion
Fatty acid-binding protein, brain	6	0.0170	1.33	Signal transduction/L.F.A. metabolism
T-complex protein 1 subunit gamma	1	0.0056	1.20	**Strutural molecule activity*
60S ribosomal protein L4-A	1	0.0492	1.15	None
Apolipoprotein A-I precursor	4	0.0069	1.53	None
Apolipoprotein A-I-2 precursor	1	0.0254	1.35	None

Apolipoprotein CII [Oncorhynchus mykiss]	1	0.0446	1.20	None
Collagen alpha-2(I) chain precursor		0.0338	1.25	None
Ependymin-1 precursor	4	0.0010	1.43	None
Ependymin-2 precursor	1	0.0090	1.46	None
Exocyst complex component 7	1	0.0342	1.18	None
Ferritin, heavy subunit	1	0.0431	1.15	None
Glutamine synthetase	1	0.0368	1.22	None
HDCME31P-like protein [Ictalurus punctatus]	1	0.0171	1.22	None
Homo sapiens splicing factor, arginine/serine-rich 2 (SFRS2), mRNA	1	0.0002	1.35	None
Metallothionein B	4	0.0010	1.28	None
Oncorhynchus mykiss SYPG1 (SYPG1), PHF1 (PHF1), and RGL2 (RGL2) genes, complete cds;	1	0.0073	1.21	None
Oncorhynchus nerka connective tissue growth factor (CTGF) gene, partial sequence	1	0.0024	1.34	None
Oocyte protease inhibitor-2 [Oncorhynchus mykiss]	1	0.0250	1.19	None
Pleiotrophic factor-alpha-2 precursor	2	0.0068	1.19	None
Probable ribosome biogenesis protein RLP24	1	0.0072	1.28	None
rRNA 2'-O-methyltransferase fibrillarin	1	0.0126	1.18	None
Salvelinus alpinus metallothionein B gene, introns 1 and 2 and partial cds	1	0.0140	1.21	None
Serum albumin 2 precursor	1	0.0152	1.31	None
T-complex protein 1 subunit delta	1	0.0026	1.26	None
Tropomyosin-1 alpha chain	1	0.0049	1.20	None
Tubulin alpha chain	10	0.0069	1.25	None
Tubulin beta-1 chain	4	0.0090	1.26	None

Tubulin beta-4 chain	1	0.0026	1.28	None
Type-4 ice-structuring protein precursor	2	0.0206	1.28	None
Uncharacterized protein C6orf58 homolog precursor	1	0.0142	1.27	None
Under-transcribed in D				
Peroxiredoxin-5, mitochondrial precursor	1	0.0209	0.85	**Antioxydation*
Phosphoribosyl pyrophosphate synthetase-associated protein 1	1	0.0057	0.71	B.P.U
SH3 domain-binding glutamic acid-rich protein	1	0.0184	0.76	B.P.U
Transmembrane 4 L6 family member 4	1	0.0068	0.70	B.P.U
WD repeat protein 23	1	0.0046	0.74	B.P.U
6-phosphofructokinase, muscle type	1	0.0084	0.74	**Energy metabolism*
Cytochrome c oxidase polypeptide VIIa-liver/heart, mitochondrial precursor	1	0.0193	0.75	**Energy metabolism*
Selenoprotein Pa precursor	1	0.0111	0.64	**Extracellular transport*
BOLA class I histocompatibility antigen, alpha chain BL3-7 precursor	1	0.0138	0.83	**Immunity*
Gamma-aminobutyric acid receptor-associated protein-like 2	1	0.0376	0.82	**Intracellular transport*
Nuclear transport factor 2	1	0.0262	0.77	**Intracellular protein trafic*
Hemoglobin subunit alpha	1	0.0362	0.72	**Ion transport / Oxygen transport*
Hemoglobin subunit alpha-4	1	0.0002	0.38	**Ion transport / Oxygen transport*
Parvalbumin-2	1	0.0495	0.77	**Ion transport / muscle contraction*
Parvalbumin-7	1	0.0073	0.75	**Ion transport / muscle contraction*
Peroxisomal multifunctional enzyme type 2	1	0.0016	0.74	L.F.A. metabolism
H-2 class II histocompatibility antigen gamma chain	8	0.0020	0.72	MHCII-mediated immunity
26S proteasome non-ATPase regulatory subunit 12	1	0.0068	0.74	**Proteasome activity / Protein binding*
Myosin light chain 1, cardiac muscle	3	0.0052	0.78	**Muscle contraction*

Proteasome activator complex subunit 2	1	0.0000	0.41	**Proteasome activity*
DNA polymerase subunit delta 4	1	0.0195	0.78	**Protein binding*
L-xylulose reductase	1	0.0261	0.83	**Protein binding / Energy metabolism*
26S proteasome non-ATPase regulatory subunit 14	1	0.0089	0.71	Protein metabolism and modification
Elongation factor 1-beta	1	0.0007	0.62	Protein metabolism and modification
Peptidyl-prolyl cis-trans isomerase, mitochondrial precursor	1	0.0308	0.80	**Protein synthesis*
Transcriptional adapter 3-like	1	0.0264	0.80	**Regulation of transcription*
Annexin A1	1	0.0362	0.82	Signal transduction/cell motility/L.F.A. metabolism
Asialoglycoprotein receptor 2	1	0.0493	0.85	Signal transduction
Cofilin-2	1	0.0002	0.68	**Strutural molecule activity*
F-actin capping protein subunit alpha-1	1	0.0121	0.80	**Strutural molecule activity*
Gelsolin precursor	1	0.0254	0.80	**Strutural molecule activity / Intracellular transport*
Tropomodulin-4	1	0.0446	0.76	**Strutural molecule activity*
Troponin I, fast skeletal muscle	3	0.0012	0.66	**Strutural molecule activity / Intracellular transport*
14 kDa phosphohistidine phosphatase	1	0.0236	0.81	None
60S acidic ribosomal protein P2	1	0.0001	0.43	None
Beta-2-microglobulin precursor	14	0.0000	0.63	None
Collagen alpha-2(I) chain precursor	2	0.0462	0.82	None
cyclin-dependent kinase inhibitor 1C	1	0.0344	0.81	None
Differentially regulated trout protein 1 [Oncorhynchus mykiss]	2	0.0092	0.62	None
Fructose-1,6-bisphosphatase isozyme 2	1	0.0332	0.76	None
Gamma crystallin M2	1	0.0051	0.74	None
Glutathione S-transferase A	1	0.0337	0.81	None

Glycine amidinotransferase, mitochondrial precursor	1	0.0404	0.79	None
Keratin, type I cytoskeletal 13	1	0.0095	0.74	None
Lipocalin precursor	1	0.0404	0.86	None
Lysozyme g	1	0.0141	0.82	None
Myosin heavy chain, fast skeletal muscle	1	0.0495	0.81	None
Oncorhynchus mykiss invariant chain S25-7 mRNA, complete cds	1	0.0046	0.77	None
Oncorhynchus mykiss mRNA for Keratin 13 (k13 gene)	1	0.0446	0.80	None
Oncorhynchus mykiss mRNA for type II keratin E1 (E1 gene)	1	0.0446	0.78	None
Oncorhynchus mykiss partial mRNA for Keratin 12 (k12 gene)	1	0.0344	0.78	None
Parvalbumin beta 1	1	0.0105	0.78	None
Parvalbumin beta 2	1	0.0046	0.75	None
PREDICTED: similar to expressed sequence AV312086 [Canis familiaris]	1	0.0493	0.81	None
Salmo salar clone BE7 beta-2 microglobulin (B2m) mRNA, complete cds	1	0.0026	0.78	None
Salmo salar MHC class I (UBA) mRNA, UBA*1401 allele, complete cds	1	0.0026	0.73	None
Salvelinus fontinalis differentially regulated trout protein 1 mRNA, complete cds	1	0.0303	0.68	None
Serotransferrin precursor	2	0.0039	0.71	None
Serotransferrin-1 precursor	2	0.0024	0.77	None
Serotransferrin-2 precursor	1	0.0010	0.68	None

Fold-change represents the fold changes of average ratio; p-value (FDR) corresponds to the FDR-corrected (1000 permutations) p-value of the ANOVA test. #clones: number of clones. Abbreviation for the functional categories: A.A: Amino acid; B.P.U.: Biological process unclassified; L.F.A.: Lipid and fatty acid; None: no functional category defined for unique gene in Panther online classification system (http://www.pantherdb.org). ***Functional categories presented in italic correspond to hypothetical functions that were identified manually in Swiss-Prot. These functions were not used in the PANTHER analysis.*

TABLE S4

List of over-dominant or under-dominant genes among DR, LD, and RL hybrids

Gene names	#clones	p value	NP fold change	Functional categories
DR hybrids				
Over-dominant genes				
ATP synthase O subunit, mitochondrial precursor	1	0.0088	1.21	Coenzyme metabolism
Cytochrome c oxidase polypeptide VIIa-liver/heart, mitochondrial precursor	1	0.0055	1.33	Electon transport
Breakpoint cluster region protein	1	0.0159	1.14	Intracellular signaling cascade
3-oxo-5-beta-steroid 4-dehydrogenase	1	0.0120	1.18	Energy metabolism
Succinyl-CoA ligase [GDP-forming] subunit alpha, mitochondrial precursor	1	0.0159	1.15	**Energy metabolism*
Tetraspanin-3	1	0.0159	1.17	**Membrane component*
Cytochrome c oxidase subunit 3	1	0.0277	1.15	None
Cytochrome c oxidase subunit VIb isoform 1	1	0.0186	1.17	None
PREDICTED: similar to thioredoxin domain containing 14	1	0.0159	1.22	None
Thymidine phosphorylase precursor	1	0.0125	1.18	None
Under-dominant genes				
GDP-L-fucose synthetase	1	0.0250	0.81	Carbohydrate metabolism
Ferritin, heavy subunit	3	0.0088	0.86	None
Metallothionein A	1	0.0221	0.80	None
Metallothionein B	1	0.0159	0.79	None
Type-4 ice-structuring protein precursor	1	0.0159	0.81	None
LD hybrids				
Over-dominant genes				

Midasin	1	0.0000	1.68	B.P.U
Tumor protein D52	1	0.0005	1.98	**Calcium mediated signaling / Ion binding*
Ubiquitin carboxyl-terminal hydrolase isozyme L1	1	0.0024	1.63	**Cell proliferation / Response to stress*
Transketolase	1	0.0038	1.51	**Ion binding / Regulation of growth*
Hemoglobin subunit alpha	11	0.0002	2.19	**Ion transport / oxygen transport*
Hemoglobin subunit alpha-1	1	0.0005	2.28	**Ion transport / oxygen transport*
Hemoglobin subunit alpha-4	4	0.0005	1.58	**Ion transport / oxygen transport*
Hemoglobin subunit beta	5	0.0022	1.53	**Ion transport / oxygen transport*
Hemoglobin subunit beta-1	9	0.0002	2.41	**Ion transport / oxygen transport*
Hemoglobin subunit beta-4	3	0.0058	1.52	**Ion transport / oxygen transport*
40 kDa peptidyl-prolyl cis-trans isomerase	1	0.0002	1.60	Nuclear transport
U6 snRNA-associated Sm-like protein LSm4	1	0.0002	1.61	Pre-mRNA processing
Eukaryotic translation initiation factor 3 subunit 7	2	0.0005	2.21	Protein metabolism and modification
Thymidine phosphorylase precursor	1	0.0033	1.15	**Pyrimidin metabolism*
Ubiquitin-conjugating enzyme E2 G1	1	0.0000	2.03	**Regualtion of protein metabolism*
Caspase-8 precursor	1	0.0002	1.64	None
DNA-directed RNA polymerase II subunit RPB1	1	0.0004	1.48	None
Glyceraldehyde-3-phosphate dehydrogenase	1	0.0038	1.54	None
Histone H1	1	0.0002	1.35	None
Homo sapiens ARP1 actin-related protein 1 homolog B	1	0.0058	1.51	None
Microsomal glutathione S-transferase 3	1	0.0076	1.34	None
Proteasome subunit alpha type 1	1	0.0005	1.24	None
Transforming growth factor-beta-inducible early growth response protein 3	1	0.0040	1.57	None

Under-dominant genes				
Peroxiredoxin-5, mitochondrial precursor	2	0.0074	0.86	Antioxydation
Band 4.1-like protein 3	1	0.0005	0.85	B.P.U
Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	1	0.0027	0.82	**Calcium mediated signaling / Muscle contraction*
Protein BCCIP homolog	1	0.0044	0.82	**DNA repair*
Electron transfer flavoprotein subunit alpha, mitochondrial precursor	1	0.0010	0.86	**Electron transport*
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4	1	0.0056	0.85	**Electron transport*
Vacuolar ATP synthase 16 kDa proteolipid subunit	1	0.0005	0.83	**Electron transport*
Creatine kinase M-type	1	0.0036	0.76	**Energy metabolism / RNA binding*
Fructose-bisphosphate aldolase A	1	0.0110	0.82	**Energy metabolism*
Nucleolar GTP-binding protein 1	1	0.0026	0.80	**Energy metabolism*
Ornithine decarboxylase antizyme 1	1	0.0004	0.81	**Enzyme activity*
Immune-related Hdd11 [Hyphantria cunea]	1	0.0050	0.79	**Immunity*
Acyl-CoA-binding protein	1	0.0006	0.82	Intracellular transport
ATP synthase coupling factor 6, mitochondrial precursor	1	0.0078	0.87	Intracellular transport
THO complex subunit 1	1	0.0022	0.81	Intracellular transport
Vacuolar ATP synthase subunit G 1	1	0.0007	0.83	Intracellular ransport
Tubulin beta-6 chain	1	0.0006	0.85	**Intracellular transport / Energy metabolism*
Isocitrate dehydrogenase [NADP], mitochondrial precursor	3	0.0006	0.81	**Ion binding*
Zinc finger CCHC domain-containing protein 10	1	0.0005	0.81	**Ion binding / Nucleic acid binding*
Parvalbumin-2	2	0.0005	0.82	**Ion transport / muscle contraction*
Tetraspanin-3	1	0.0004	0.82	**Membrane component*
Creatine kinase B-type	1	0.0007	0.72	Muscle contraction

BET1 homolog	1	0.0019	0.80	**Protein binding / Intracellular transport*
Erythrocyte band 7 integral membrane protein	1	0.0041	0.78	**Protein binding*
Nucleolar RNA helicase 2	1	0.0018	0.83	**Protein binding / RNA binding / Energy metabolism*
40S ribosomal protein S10	1	0.0009	0.84	Protein metabolism and modification
40S ribosomal protein S6	1	0.0005	0.85	Protein metabolism and modification
Eukaryotic translation initiation factor 3 subunit 5	1	0.0005	0.85	Protein metabolism and modification
Reticulon-3	1	0.0015	0.86	**Protein binding / Extracellular transport*
Actin, cytoplasmic 1	1	0.0022	0.79	**Strutural molecule activity*
Macrophage erythroblast attacher	1	0.0010	0.83	**Strutural molecule activity / Cell division*
Guanidinoacetate N-methyltransferase	1	0.0019	0.78	**Transferase activity*
60S ribosomal protein L18	1	0.0005	0.82	None
60S ribosomal protein L4-A	2	0.0010	0.84	None
60S ribosomal protein L4-B	1	0.0005	0.80	None
ATP synthase subunit g, mitochondrial	4	0.0002	0.80	None
Cyclin-G1	1	0.0042	0.78	None
Danio rerio Y box binding protein 1, mRNA (cDNA clone MGC:158477 IMAGE:6972160), complete cds	1	0.0058	0.88	None
Glutamine synthetase	1	0.0006	0.75	None
Paralichthys olivaceus ornithine decarboxylase antizyme ORF1	1	0.0006	0.84	None
Plasma retinol-binding protein I	1	0.0032	0.64	None
RL hybrids				
Over-dominant genes				
Peroxiredoxin-5, mitochondrial precursor	1	0.0038	1.14	**Antioxydation*
Hepatocellular carcinoma-associated antigen 127	1	0.0003	1.24	B.P.U

CCAAT/enhancer-binding protein delta	1	0.0003	1.60	Cell proliferation and differentiation
Barrier-to-autointegration factor	1	0.0061	1.38	**DNA binding*
Cathepsin L2 precursor	1	0.0005	1.42	**Enzyme activity*
Complement factor D precursor	1	0.0061	1.24	**Enzyme activity*
Ornithine decarboxylase antizyme 1	1	0.0019	1.17	**Enzyme activity*
Apolipoprotein B-100 precursor	2	0.0003	1.20	L.F.A metabolism/transport
Protein kinase C eta type	1	0.0043	1.59	Intracellular signaling cascade/calcium mediated signaling/cell proliferation and differentiation/signal transduction
Rab GDP dissociation inhibitor beta	1	0.0003	1.36	Intracellular signaling cascade/neurotransmitter release/ligand-mediated signaling/signal transduction/intracellular protein trafic
ADP/ATP translocase 2	1	0.0050	1.19	**Intracellular transport / Energy metabolism*
Gamma-aminobutyric acid receptor-associated protein-like 2	1	0.0066	1.28	**Intracellular transport*
Iron(III)-zinc(II) purple acid phosphatase precursor	1	0.0012	1.17	**Ion binding*
Metalloproteinase inhibitor 2 precursor	1	0.0042	1.42	**Ion binding / Enzyme activity*
NF-kappa-B-repressing factor	1	0.0003	1.22	mRNA transcription
28S ribosomal protein S17, mitochondrial precursor	1	0.0003	1.47	Signal transduction
Leukocyte cell-derived chemotaxin 2 precursor	1	0.0005	1.22	Signal transduction/ligand-mediated signaling
T-complex protein 1 subunit gamma	1	0.0028	1.15	**Strutural molecule activity*
Nonspecific lipid-transfer protein	1	0.0006	1.16	**Transferase activity / L.F.A metabolism*
Serine incorporator 1	1	0.0028	1.33	**Protein binding / Intracellular transport / L.F.A. metabolism*
Elongation factor 1-delta	1	0.0035	1.12	Protein metabolism and modification
Eukaryotic translation initiation factor 3 subunit 3	1	0.0043	1.14	Protein metabolism and modification
125 kDa kinesin-related protein	1	0.0033	1.38	None
40S ribosomal protein S13	1	0.0003	1.38	None

60S ribosomal protein L23	1	0.0039	1.14	None
Acyl-CoA-binding protein	1	0.0017	1.25	None
Adenylyl cyclase-associated protein 1	1	0.0016	1.24	None
Cystatin-B	3	0.0011	1.19	None
Ependymin precursor	3	0.0017	1.40	None
FAM128B protein [Homo sapiens]	1	0.0004	1.35	None
Ferritin middle subunit [Salmo salar=Atlantic salmon, liver, mRNA, 1010 nt]	1	0.0029	1.32	None
Ferritin, middle subunit	19	0.0003	1.40	None
Glutathione S-transferase P	4	0.0017	1.24	None
Glutathione S-transferase P 1	1	0.0017	1.24	None
Histone H1.0	4	0.0011	1.35	None
Metallothionein B	4	0.0003	1.39	None
Oncorhynchus mykiss histone H1-0 (H1f0) mRNA, partial cds	1	0.0003	1.51	None
Pfam06077, LR8, LR8 protein	2	0.0088	1.19	None
Plasma retinol-binding protein I	3	0.0028	1.60	None
Plasma retinol-binding protein II	1	0.0029	1.62	None
Receptor expression-enhancing protein 5	2	0.0029	1.19	None
Salvelinus alpinus metallothionein B gene, introns 1 and 2 and partial cds	1	0.0003	1.29	None
Uncharacterized protein KIAA1279 homolog	1	0.0004	1.40	None
Zona pellucida sperm-binding protein 3 precursor	1	0.0003	1.47	None
Under-dominant genes				
Tubulin alpha-1A chain	1	0.0030	0.79	Cell motility/intracellular protein trafic/chromosome segregation
Tubulin alpha-1C chain	1	0.0011	0.83	Cell motility/intracellular protein trafic/chromosome segregation

Tubulin beta-2C chain	1	0.0004	0.76	Cell motility/intracellular protein trafic/chromosome segregation
Chromobox protein homolog 3	1	0.0046	0.85	**Chromosome segregation*
High mobility group protein B2	1	0.0039	0.77	**Chromosome segregation / Regulation of transcription*
Histone H2A.Z	1	0.0044	0.78	**Chromosome segregation / DNA binding*
Histone H3.3	3	0.0058	0.86	**Chromosome segregation / DNA binding / Response to hormone stimulus*
Protein S100-A1	4	0.0062	0.75	Intracellular signaling cascade/signal transduction
Syntaxin-binding protein 3	1	0.0060	0.74	Neurotransmitter release/intracellular protein trafic
Thymidine phosphorylase precursor	1	0.0003	0.81	**Pyrimidin metabolism / Cell proliferation and differentiation*
Cold-inducible RNA-binding protein	1	0.0018	0.79	RNA binding
Calmodulin	2	0.0005	0.83	Signal transduction/intracellular signaling cascade/calcium mediated signaling/cell proliferation and differentiation
Collagen alpha-1(I) chain precursor	1	0.0039	0.72	Signal transduction/cell adhesion
Acyl-CoA-binding domain-containing protein 7	1	0.0078	0.76	None
Collagen alpha-2(I) chain precursor	3	0.0011	0.78	None
Histone H2A.x	1	0.0119	0.84	None
NADH-ubiquinone oxidoreductase chain 1	1	0.0050	0.82	None
Nonhistone chromosomal protein H6	5	0.0030	0.76	None
Oncorhynchus mykiss Onmy-LDA gene for MHC class I antigen, allele: Onmy-LDA*0101, and other genes, complete cds	1	0.0035	0.69	None
Oryzias latipes hox gene cluster, complete cds, contains hoxCa	1	0.0035	0.83	None
Parvalbumin beta 1	1	0.0020	0.88	None
Peptidyl-prolyl cis-trans isomerase B precursor	1	0.0060	0.71	None
rRNA 2'-O-methyltransferase fibrillarin	1	0.0034	0.88	None
Salmo salar hyperosmotic glycine rich protein mRNA, complete cds	1	0.0083	0.78	None
Tubulin alpha chain	9	0.0004	0.80	None

Tubulin alpha-4A chain	1	0.0038	0.81	None
Tubulin beta-1 chain	1	0.0024	0.80	None
Tubulin beta-4 chain	1	0.0003	0.78	None

P values refer to equality of means test between hybrids and parental strains. NP (near parent) fold-change represents the fold changes of average ratio between hybrids and parental strains. Abbreviation for the functional categories: A.A: Amino acid; B.P.U.: Biological process unclassified; L.F.A.: Lipid and fatty acid; None: no functional category defined for unique gene in Panther online classification system (http://www.pantherdb.org). ***Functional categories presented in italic correspond to hypothetical functions that were identified manually in Swiss-Prot. These functions were not used in the PANTHER analysis.*

TABLE S5

List of genes exhibiting an additive mode of transcription regulation in one hybrid cross and a non-additive mode in one or two of the other hybrid crosses.

Genes, for which ontology annotation was not found, are presented here.

Gene names	#clones	DR	LD	RL	Functional categories
60S acidic ribosomal protein P2	1	A	N-A	*	None
60S ribosomal protein L4-A	1	*	N-A	A	None
Beta-2-microglobulin precursor	10	A	N-A	*	None
BOLA class I histocompatibility antigen, alpha chain BL3-7 precursor	1	A	N-A	*	**Immunity*
Cathepsin L precursor	2	A	*	N-A	**Peptidase activity*
Cathepsin L2 precursor	1	A	*	N-A	**Peptidase activity*
Collagen alpha-2(I) chain precursor	1	*	A	N-A*	None
Complement C3-1	1	A	*	N-A	None
Diamine acetyltransferase 1	1	A	*	N-A	**Cell cycle regulation*
Endoplasmin precursor	1	*	A	N-A	**Intracellular transport / response to stress*
Ependymin-1 precursor	4	A	N-A	*	None
Ependymin-2 precursor	1	A	N-A	*	None
Fatty acid-binding protein, liver	1	N-A	A	*	None
glucose-regulated protein 94	1	*	A	N-A	**Protein process*
Glutathione peroxidase 2	1	A	*	N-A	**Cell cycle regulation / response to stress*

Glutathione S-transferase P	3	A	*	N-A*	None
Glutathione S-transferase P 1	1	A	*	N-A*	None
Guanidinoacetate N-methyltransferase	1	*	N-A*	A	**Transferease activity*
Hemoglobin subunit alpha-4	1	A	N-A	*	**Ion transport / oxygen transport*
Hemoglobin subunit beta-2	1	A	N-A	*	**Ion transport / oxygen transport*
Immune-related Hdd11	1	A	N-A*	N-A	**Immunity*
Membrane-bound transcription factor site-1 protease precursor	1	*	N-A	A	*L.F.A metabolism*
Oncorhynchus nerka connective tissue growth factor (*CTGF*) gene	1	A	N-A	*	None
Pachymedusa dacnicolor partial mRNA for ribosomal protein S16	1	A	*	N-A	None
Pleiotrophic factor-alpha-2 precursor	1	A	N-A	*	None
Polyadenylate-binding protein 1-A	1	A	N-A	*	**Protein binding*
Proteasome activator complex subunit 2	1	A	N-A	*	**Proteasome activity*
rRNA 2'-O-methyltransferase fibrillarin	1	A	N-A	N-A*	None
Salmo salar clone BE7 beta-2 microglobulin	1	A	N-A	*	None
Salmo salar MHC class I (UBA) mRNA	1	A	N-A	*	None
Serotransferrin precursor	2	A	N-A	*	None
Serotransferrin-1 precursor	2	A	N-A	*	None
Serotransferrin-2 precursor	1	A	N-A	*	None
Transcription factor BTF3 homolog 4	1	A	N-A	*	**mRNA metabolism*
Tubulin alpha chain	7	*	A	N-A*	None

Tubulin alpha chain	1	*	A	N-A	None
Tubulin beta-1 chain	1	A	A	N-A*	None
Tubulin beta-6 chain	1	*	N-A	A	**Intracellular transport / Energy metabolism*
Zymogen granule membrane protein 16 precursor	2	A	N-A	*	**Extracellular transport*

#clones: number of clones; DR: Domestic-Rupert hybrids; LD: Laval-Domestic hybrids; RL: Rupert-Laval hybrids; N-A: non-additive mode; A: additive mode; *: transcripts did not show significant difference between their parental strains. Abbreviation for the functional categories: L.F.A.: Lipid and fatty acid; None: no functional category defined for unique gene in Panther online classification system (http://www.pantherdb.org). **Functional categories presented in italic correspond to hypothetical functions that were identified manually in Swiss-Prot. These functions were not used in the PANTHER analysis.*