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Differences in transcription levels among wild, domesticated, and hybrid Atlantic salmon (*Salmo salar*) from two environments

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Abstract

Escaped domesticated individuals can introduce disadvantageous traits into wild populations due to both adaptive differences between population ancestors and human-induced changes during domestication. In contrast to their domesticated counterparts, some endangered wild Atlantic salmon populations encounter during their marine stage large amounts of suspended sediments, which may act as a selective agent. We used microarrays to elucidate quantitative transcriptional differences between a domesticated salmon strain, a wild population and their first-generation hybrids during their marine life stage, to describe transcriptional responses to natural suspended sediments, and to test for adaptive genetic variation in plasticity relating to a history of natural exposure or nonexposure to suspended sediments. We identified 67 genes differing in transcription level among salmon groups. Among these genes, processes related to energy metabolism and ion homoeostasis were over-represented, while genes contributing to immunity and actin-/myosin-related processes were also involved in strain differentiation. Domestic–wild hybrids exhibited intermediate transcription patterns relative to their parents for two-thirds of all genes that differed between their parents; however, genes deviating from additivity tended to have similar levels to those expressed by the wild parent. Sediments induced increases in transcription levels of eight genes, some of which are known to contribute to external or intracellular damage mitigation. Although genetic variation in plasticity did not differ significantly between groups after correcting for multiple comparisons, two genes (metallothionein and glutathione reductase) tended to be more plastic in response to suspended sediments in wild and hybrid salmon, and merit further examination as candidate genes under natural selection.

Keywords: domestication, gene transcription, outbreeding depression, plasticity, suspended sediments

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Introduction

Gene flow from domesticated individuals can change local wild phenotypes by introducing domestication-induced traits and traits adapted only to the natural ancestral environment from which the domesticated individuals were once taken (Rhymer & Simberloff 1996; Hutchings & Fraser 2008). Consequently, when domesticated individuals escape and interbreed with wild populations, a major conservation concern is that a lower fitness of hybrids may lead to a decline of wild populations (outbreeding depression), particularly of those populations already at heightened risk of extinction (Hutchings 1991; McGinnity *et al*. 2003).

Genetically based differences between wild and domesticated individuals can arise in three principle

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ways. First, domesticated individuals may differ from wild individuals because the wild ancestor of the domesticated individuals experienced different natural selection pressures (Hutchings & Fraser 2008). Second, differences can arise via domestication through intentional selection (e.g. improving traits beneficial for production; Rauw *et al.* 1998), inadvertent selection (e.g. the amount and quality of space or contact with humans favours some individuals over others; Kohane & Parsons 1988) or the relaxation of natural selective pressures in the captive environment (Price & King 1968; Mignon-Grasteau *et al.* 2005). Finally, genetic drift and inbreeding can also alter the genetic makeup of domesticated strains (Price & King 1968; Taberlet *et al.* 2011). The probability and magnitude of fitness reduction in hybrids may depend on how divergent their wild and domesticated parents are and on the source of these differences. For example, as a by-product of selection for productivity, domestication can lead to changes in metabolism, reproduction and health that can be disadvantageous *per se* (Rauw *et al.* 1998). However, some domestication-induced behavioural changes may disappear in the wild because the required genetic variation required to revert may still be present in domesticated populations (Mignon-Grasteau *et al.* 2005). In contrast, when domesticated and wild populations differ due to ancestral adaptations to their respective environments, these differences are likely to be maintained in the wild, and therefore may play a major role in environment-specific hybrid fitness.

Adaptive differences between wild populations are traditionally seen as genetic-based phenotypic differences selected for by environmental variables. However, individuals from different populations might also differ in their genetic variation for phenotypic plasticity, which may be adaptive in variable environments (Schlichting & Pigliucci 1998), even under high gene flow (Conner & Hartl 2004). This phenotypic plasticity of a trait may depend on differential gene expression (Khaitovich *et al.* 2006; Gibson 2008). Although some authors have pointed out that it is largely unknown to what extent gene expression regulation reflects local adaptation (Staubach *et al.* 2010), others have suggested that it has important evolutionary consequences (reviewed by Wray *et al.* 2003; Aubin-Horth & Renn 2009).

Fishes are useful systems in which to study the effects of domestication because the relatively recent onset of their domestication enables a meaningful comparison with their wild counterparts, unlike studies of animals with unknown, uncertain or extinct ancestors (Mignon-Grasteau *et al.* 2005). Although several studies have examined the effects of domestication on fish gene transcription in general (Roberge *et al.* 2006, 2008; Devlin *et al.* 2009; Normandeau *et al.* 2009; Tymchuk *et al.* 2009a,b; Bougas *et al.* 2010; Sauvage *et al.* 2010), none have tested for differences in gene transcription between wild and domesticated individuals relevant to a direct response to environmental variables or in regard to genetic variation in plasticity.

Wild Atlantic salmon (*Salmo salar*) may be especially affected by outbreeding with domesticated individuals because of the high frequency and magnitude of aquaculture-related escape events (Morris *et al.* 2008). Of the many fish that escape, some spawn in rivers where wild fish cooccur (Carr *et al.* 1997), which can result in hybrid offspring (Crozier 1993; Skaala *et al.* 2006; Bourret *et al.* 2011) that have been shown to have lower lifetime reproductive success in nature than their wild counterparts (McGinnity *et al.* 2003). Compounding the effects of this outbreeding depression is the observation that wild Atlantic salmon are in decline throughout their natural range in the north Atlantic (ICES 2010).

The Canadian inner Bay of Fundy (iBoF) salmon populations are listed as 'endangered' under the Canadian Species at Risk Act (COSEWIC 2011). This group of several river populations has been at critically low numbers since the late 1980s, which is most likely associated with low survival during the marine phase (DFO 2010). Interestingly, many iBoF populations have been hypothesized to exhibit an unusual localized marine migration pattern that appears to be restricted to the Bay of Fundy (Huntsman 1931; Jessop 1976), rather than undertaking a long-distance migration to the waters east of Newfoundland, as favoured by most North American Atlantic salmon (Ritter 1989; COSEWIC 2006). In this bay, extreme tidal movement, erosion of surrounding red beds and riverine input cause high amounts of suspended sediments (Yeo & Risk 1981), which can result in physiological changes, a stress-induced immune response, and mortality in salmonids (Bash *et al.* 2001). Domesticated–wild outbreeding has the potential to negatively affect remaining iBoF populations and accelerate their extirpation because domesticated salmon may have lowered general fitness in the wild and lack adaptation to locally important selective factors. Suspended sediments from the iBoF may be one of these selective factors because sediments likely affect fitness and are naturally experienced by wild salmon but not by current domesticated salmon nor by their ancestors.

Using common garden experiments in combination with microarray technology, we compared gene transcription profiles of individuals from an iBoF Atlantic salmon population with individuals of the major eastern Canadian domesticated strain and their first-generation hybrids. This allowed us to: (i) investigate general differences in transcription patterns during the marine stage among groups (strains) and describe the transcriptional consequences of outbreeding in hybrids; (ii) investigate

the transcriptional response to naturally suspended sediments; and (iii) test for possible adaptive genetic variation in plasticity of gene transcription in response to suspended sediments in the wild strain.

Materials and methods

Salmon strains and tank experiment

The wild salmon used (Wild) are native to the Stewiacke River, a tributary of the Shubenacadie River on the Minas Basin, Nova Scotia, located at the head of the iBoF. Because of the confined migration of the iBoF populations and the location of the river, salmon from this river experience suspended sediments for prolonged periods repeatedly during their lives. Conversely, wild ancestors of the domesticated strain (Domesticated) were native to the Saint John River, New Brunswick, on the outer Bay of Fundy and would have undertaken a typical long-distance migration (Ritter 1989). Wild Saint John and wild Stewiacke River salmon also differ in several other life history traits (Huntsman 1931; COSEWIC 2006; Fraser *et al.* 2010), variation at neutral genetic markers, and gene transcription profiles (Tymchuk *et al.* 2010). The domesticated strain used in this study has undergone four generations of domestication, under artificial selection intended primarily to increase growth rate (Glebe 1998), which has also led to evolutionary changes in gene regulation (Roberge *et al.* 2006).

The fish used in this study were fertilized in 2005 (details in Fig. 1 and in Fraser *et al.* 2010); the parents of these fish were fertilized in 2001 (details in Lawlor *et al.* 2008). Fish of both generations were hatched and raised in the Aquatron facility at Dalhousie University under similar conditions (e.g. fish density, tank type, physical parameters and feed) each generation, thus minimizing differences attributable to parental environmental effects.

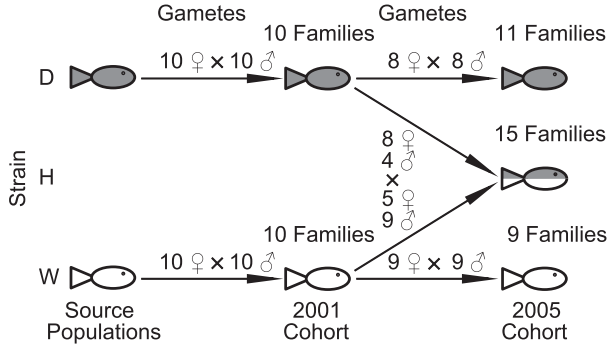

Fig. 1 Salmon crossing design. Indicated are numbers of dams and sires from each strain (wild: W, hybrid: H, domesticated: D) and each cohort crossed to generate the next cohort and numbers of families generated for each cohort.

In June 2008, smolts (the salmon developmental stage involving a migration from river to sea) from the 2005 generation were separated from parr (the salmon freshwater stage), using external criteria: silver body coloration and darkened fin edges in smolts. All fish were marked by strain with visible implant elastomer tags (Northwest Marine Technology) and in July 2008 distributed among four round tanks (1790 L, flow-through system with bottom drains). To do so, all fish were measured and grouped into 5-cm size classes. Fish from each size class within each strain were distributed evenly among tanks. When distributing fish, size differences between the strains could not be accounted for as fish of both parental strains differed in average length by 23%, although all fish were of the same age. In total, each tank contained 25 fish of each of the three strains, as well as 50 fish of two additional strains not used for this study (backcrosses and second-generation hybrids) for a total of 125 fish per tank. All tanks initially received dechlorinated municipal water (before the application of sediments). During October 2008, salinity in all tanks was slowly increased in steps of ~5 psu by adding ambient seawater every second week, to avoid a possible strong stress response due to exposing fish to the combination of sediments and salinity, until a level of ~20 psu was attained for the remaining duration of the experiment.

Intertidal sediments were collected during the summer from upper mudflats of the Minas Basin (Debert Beach, Lower Debert), air-dried (resulting in solid blocks) and manually ground to restore particle sizes, to allow for a standardized application (i.e. dry weight per litre) and facilitate resuspension. Starting in October 2008, 358 g of the sediment powder was resuspended in water and then poured into the middle of each of two sediment environment tanks daily. This resulted in a pulse of 200 mg/L of suspended sediments with a turbidity of 32 NTU (calibrated at $S = 18$ psu), mirroring the lower end of the natural sediment load range in the iBoF (Gordon 1994; 100–1000 mg/L). The lower end was chosen because salmonids may actively avoid high sediment concentrations in the wild, as demonstrated in the laboratory (Bisson & Bilby 1982). As a procedural control, clear water was poured into the middle of the two remaining tanks. The turbidity initially dropped from 32 to 21 NTU (120 mg/L) in the first half an hour, then decreased exponentially with a half-time of 1.8 h; all sediments had dissipated after 24 h, prior to the next application. It was technically not possible to mimic the natural tidal cycle of the Minas Basin, where strong currents resuspend sediments every 6 h (Yeo & Risk 1981).

Unexpectedly, some fish attained sexual maturity with a higher prevalence among wild (82%) than domesticated individuals (42%) on days 104 and 105 of

the experiment. To prevent a bias in gene transcription due to maturity status and sex, only mature males were used for the subsequent analysis because they could be identified with confidence (by gently pressing along the belly towards the tail, resulting in emergent milt) and were available in sufficient numbers in all strains. To facilitate synchronization of sampling, all excess fish (i.e. those not used in the microarray study, comprising females and immature males, backcrosses, and second-generation hybrids) were removed, leaving five mature males of each of the three strains for a total of 15 fish per tank. This was performed for one clear and one sediment environment tank after 104 days and for the remaining two tanks after 105 days. After an additional 16 days, fish from one clear and one sediment environment tank were sampled within 2 h (10–12 am); the sediment tank 1 h after the sediment application (to be able to also detect short-term effects). This was repeated the following day for the remaining two tanks in reverse order with respect to treatment. Fish were not fed for 2 days prior to sampling. For tissue sampling, all fish of a given tank were euthanized with tricaine methanesulphonate (250 mg/L) within 10 min of first disturbance. The left second gill arch of each fish individual was sampled in random order, wrapped in aluminium foil, flash-frozen in liquid nitrogen and kept at −80 °C until RNA was extracted. Five fish from each cross and tank were sampled (totalling 10 per cross and environment) but only randomly chosen four fish per cross from each of the four tanks were used for the subsequent analysis (eight per cross and environment). This sampling design allowed for the inference of effects caused by the environment that had not been confounded by tank effects.

Laboratory methods

Eight fish from each of the six experimental groups were analysed, totalling 48 fish. The filaments of each gill arch were cut from frozen tissue and disrupted in tubes with steel beads and PureLink lysis buffer (Invitrogen), using a commercial shaker (Quiagen TissueLyser). RNA was extracted following the manufacturer's manual for purifying RNA from animal tissue (PureLink, Invitrogen). Subsequently, 120 μg of each sample of total RNA was treated with DNase, as outlined in Normandeau *et al.* (2009). The quality and quantity of RNA was assessed by spectrometry, using Nanodrop (Thermo Scientific) after both the extraction and the DNase treatment. Samples were processed in blocks of randomly assigned arrays to exclude methodological bias.

Retrotranscription of 15 μg RNA per reaction was performed as outlined in Normandeau *et al.* (2009), but using Cy5 and Cy3 dyes with the Array50 kit (Genisphere). Because each sample was to appear on two different arrays (see next paragraph and Fig. 2), the RNA for both was retrotranscribed synchronously, unless arrays had to be repeated due to failures. Samples were stored at −20 °C until hybridization, which was performed as detailed in Normandeau *et al.* (2009) utilizing the Salmonid 32K microarray slide (GEO Platform GPL13225: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL13225). This microarray slide, which has been stepwise developed by the consortium of Genomic Research on Atlantic Salmon Project, also contains clones from Atlantic salmon gill tissue and is composed of 27 917 Atlantic salmon and 4065 rainbow trout (*Oncorhynchus mykiss*) cDNA features extracted from several cDNA libraries constructed from diverse tissue types and developmental stages of fish (Rise *et al.* 2004; von Schalburg *et al.* 2005; Koop *et al.* 2008). Of the total 31 982 features (constituting ~8800 different annotated genes), 10 482 constitute unknown genes.

The hybridization design consisted of loops contrasting the three strains (Strain) within each environment and swaps contrasting the two environments (Environ-

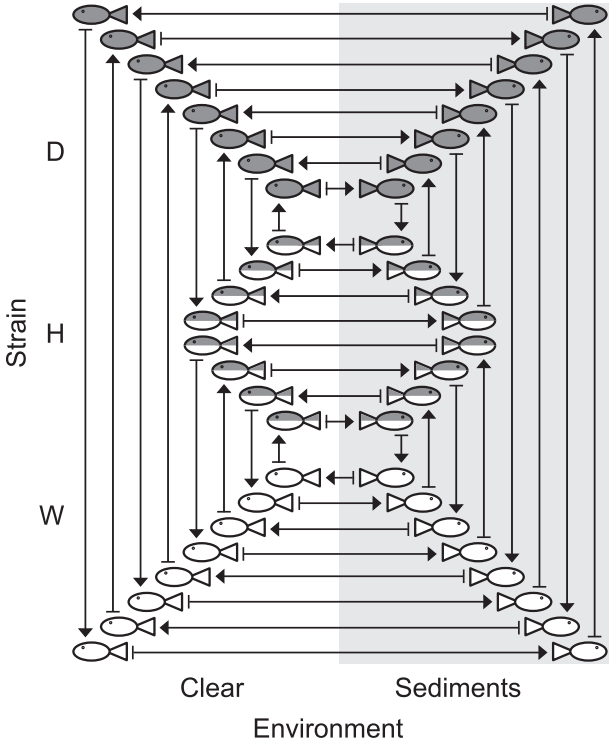

Fig. 2 Microarray hybridization design where every individual is represented by a fish symbol and each arrow represents one array with arrow-head and arrow-beginning for different dyes. Indicated are environments (no sediments: white background, suspended sediments: grey background) and strains (wild: W, hybrid: H, domesticated: D).

ment) within each strain (Fig. 2). Generally, this design is a compromise between the efficiency of detecting the main effects and their interaction (Landgrebe *et al.* 2006). Because each individual was compared once among Strain between Environment and once between Environment among Strain, dye swaps between comparisons of both main factors and for each individual were equilibrated. This resulted in a total of 48 individuals on 48 slides. A maximum of four slides were processed simultaneously and scanned directly after the dye hybridization step, using a ScanArray scanner (PerkinElmer) at a resolution of 10 μm and 90% laser power, while manually equilibrating intensity between dyes for each slide, using the photomultiplier tube settings. All scanned images were saved in TIF format and imported into the software QuantArray (Packard BioScience), which was used to manually adjust the grid, flag bad spots and extract the intensity data.

Statistical analyses

For each spot, local background signal was subtracted from the mean intensity value. For the subsequent analysis, only spots were used for which (i) intensity was found to be higher than the average intensity plus two standard deviations of negative control spots and (ii) spots of all eight individuals of at least one of the six experimental groups fulfilled the first criterion.

The data from this subset of transcripts were analysed using the R MAANOVA package (http://churchill.jax.org/software/rmaanova.shtml). Missing data of flagged spots were imputed using the K-nearest neighbour algorithm. Each array was corrected for spatial heterogeneity and intensity differences between dyes using the Joint Lowess correction, followed by centralization to correct for variations in array mean intensity. A mixed-model ANOVA was fitted to the normalized data (4483 features fulfilled the aforementioned criteria), using dye, Environment, Strain, and the interaction between Strain and Environment as fixed effects and array and sample (individual) as random effects, using the REML method. The statistical significance of Strain, Environment and their interaction was assessed using *F*-tests for each spot with 1000 permutations, shuffling over samples, to account for possible violations of non-normality and homogeneity of variances. All empirical *P*-values were corrected for the false discovery rate (FDR) using the Q-VALUE package (Storey & Tibshirani 2003).

The genes that differed for Strain or Environment were tested for over-represented gene ontology (GO) categories (biological processes, cellular components or molecular functions) using Blast2Go (Conesa *et al.* 2005). All transcribed features were blasted with default values of the programme using the accession numbers as given in the annotation file (available at: http://web.uvic.ca/grasp/microarray/) followed by the mapping and annotation steps. A reference list was created from features with available GO terms, which collapsed the 4483 transcribed features into 3056 because 803 had no gene annotation ('unknown') and GO terms could not be assigned to 624 known genes. Then, a test list was created of all features with GO annotations found to be different between strains at a FDR < 0.05. It was not possible to test for over-representation of GO terms for Environment at the same significance level because there were too few features. Hence, to enable this test, a test list was drawn from features with GO annotation different for Environment at FDR < 0.2 (choosing this value arbitrarily to compromise between type 1 and type 2 errors). Both test lists were compared to the common reference list using Fisher's exact test of the Gossip package (Blüthgen *et al.* 2005) implemented in Blast2Go. Results were reduced to the most specific GO terms (i.e. the lowest level). Furthermore, genes with different transcription levels among strains and between environments (at FDR < 0.05) were also manually compared in regard to common biological functions because no GO term could be assigned for many array features using Blast2Go. This was conducted based on results obtained after using each gene as keyword on the UniProt Knowledgebase website (http://www.uniprot.org) and/or searching for articles on the ISI Web of Knowledge website (http://apps.isiknowledge.com).

For the subsequent analysis and for heatmaps depicting the main effects, intensity values of spots were first averaged across arrays for each individual and then averaged across features if representing the same gene, using the centralized intensity log2 values corrected for technical variation (dye and array) as estimated by ANOVA.

Correlations between transcription level and logarithmic weight were tested to evaluate the possibility that differences between strains may be caused by differences in their weight. This was conducted by means of Pearson correlations separate for each strain to avoid confounding strain and weight because between the parental strains individual weights did show little overlap (Fig. 3).

To obtain some insight into the transcription pattern of domesticated–wild hybrids, genes differently transcribed among strains were analysed for their genetic architecture. For each gene, first pairwise contrasts depicting the additive parameter α [(wild−domesticated)/2] were calculated, representing the absolute difference in intensity values between the parents. This was followed by calculating contrasts for the dominance parameter δ [(wild+domesticated/2)−hybrid], representing the absolute difference of the averaged observed

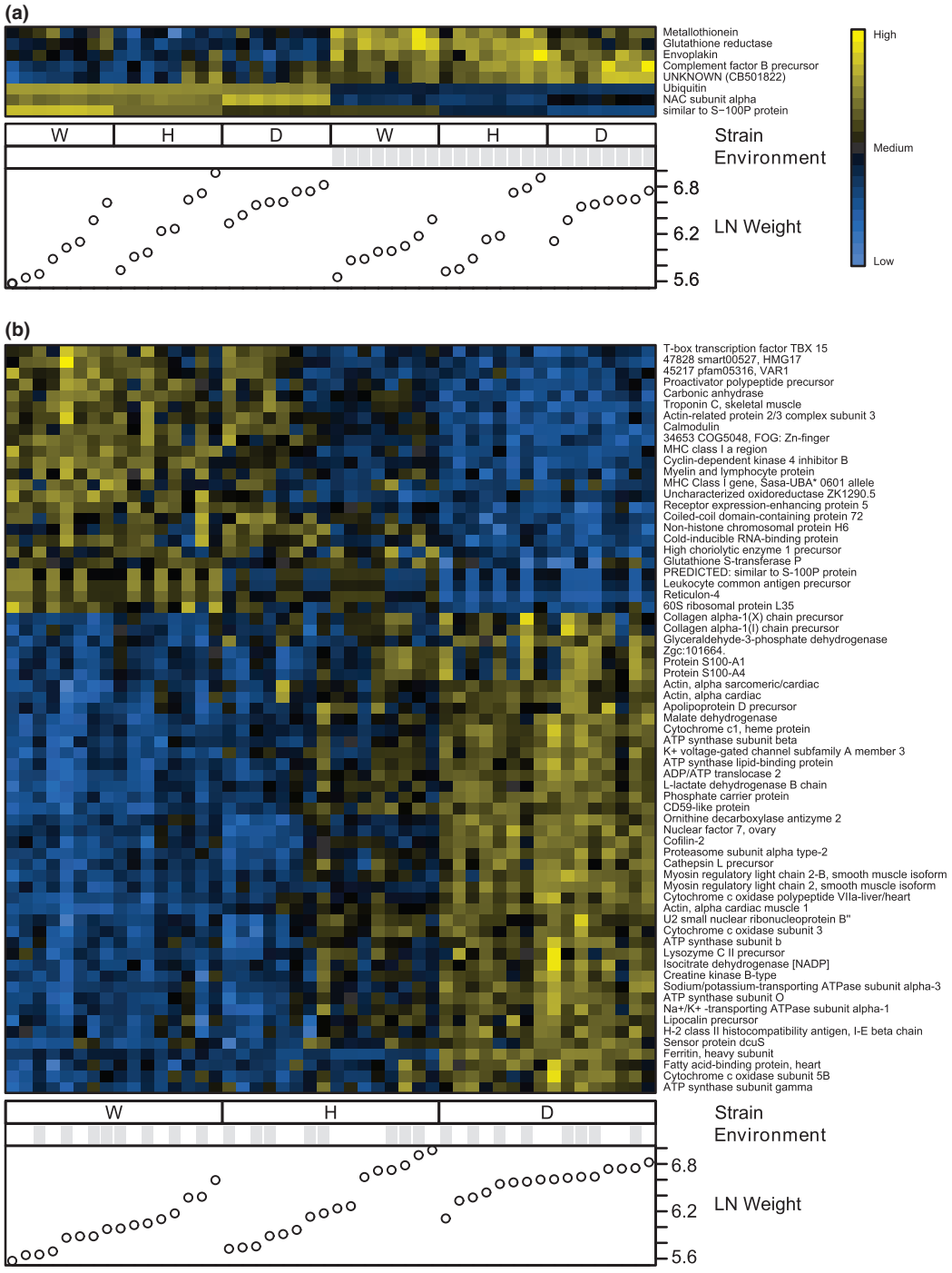

Fig. 3 Heatmap of relative transcription levels of annotated genes with different transcription levels (FDR < 0.05) for (a) environment and (b) strain. In addition to strain (wild: W, hybrid: H, domesticated: D) and environment (no sediments: white, suspended sediments: grey), the natural logarithms of weight (in grams) of individuals are indicated below each heatmap. Gene names have been shortened to fit the legend.

hybrid value from an expected mid-parent value under complete additivity. The ratios of δ/α with corresponding Fieller's 95% confidence intervals (CI) were calculated for features with $P < 0.05$ for both contrasts using the R-package MRATIOS (Dilba *et al.* 2006). This ratio reflects the relative difference of the hybrid value from a value under an additive genetic architecture. Values for δ/α including 95% CI greater or smaller than zero were interpreted as indicative of a nonadditive genetic architecture.

Results

Of a total of 32 280 features on the array, 4483 (~14%) were detected in gill tissue of Atlantic salmon. Among the three strains of wild, domesticated and first-generation hybrid salmon, transcription level was different for 104 features (maximum *P*-value of 0.0023 at FDR < 0.05; Table S1, Supporting information). Nine features showed different transcription levels among individuals from environments with or without suspended sediments (maximum *P*-value of 0.0001 at FDR < 0.05; Table S2, Supporting information). Only the S-100P protein transcript exhibited significant differences for both factors. No feature was significantly different for the interaction between Strain and Environment after the FDR correction (FDR = 1 for each feature). However, before FDR correction, 118 features were significant for this term (*P*-values between 0.0007 and 0.05; Table S3, Supporting information) of which two genes were also included in the set of genes that differed between the environments.

Differences between environments

The nine features that were transcribed at different levels between the two environments at FDR<0.05 were collapsed into eight putative genes (Fig. 3a). Fold changes for these genes ranged from 1.1 to 1.4 (Table S2, Supporting information). The transcription of three of the eight genes was downregulated in fish exposed to suspended sediments. The products of these three genes are either involved in a high number of processes (ubiquitin, NAC alpha) or have no known molecular or biological function that could be linked to specific processes induced by suspended sediments (S-100P protein: Ca^{2+}-binding). The five upregulated genes play a role in cell redox homoeostasis (glutathione reductase, metallothionein), the innate immune response (complement factor B precursor), and epithelial enforcement by keratinization and peptide cross-linking (envoplakin). The remaining feature (accession CB501822) had no significant Blast hit but showed a pattern among individuals similar to that of complement factor B and was, therefore, retained in further analyses (Fig. 3a).

Under a higher FDR of 0.2, 65 features were different between environments (maximum *P*-value of 0.0034) of which 40 could be assigned GO terms (Table S2, Supporting information). The comparison with the reference file resulted in no over-represented GO terms at a FDR < 0.05; however, the two most specific GO terms with the lowest FDR value (FDR = 0.19, maximum *P*-value of 0.00016; Table S4, Supporting information) resembled two of the processes that were inferred manually ('glutathione-disulphide reductase activity' and 'keratinization'). The genes for complement factor B and metallothionein did not yield any GO terms.

Differences between wild and domesticated salmon

Transcription fold changes among strains ranged from 1.1 to 3.0 (Table S1, Supporting information). The 104 features with different transcription levels among the three strains represented 86 different putative genes of which 19 were unknown. The 85 features with known functions represented, accordingly, 67 different genes (Fig. 3b), represented by up to five features (Table S1, Supporting information). Many of these genes contributed to three major processes: energy metabolism, the immune response, and actin-/myosin-related processes.

Many of the genes that differed between strains could be related to ATP production or its transport such as mitochondrial ATP formation via oxidative phosphorylation (11 genes: four types of ATP synthase subunits; ADP/ATP translocase 2; cytochrome *c* oxidase subunits 3 and 5b; cytochrome *c* oxidase polypeptide VIIa; cytochrome *c*1, heme protein; phosphate carrier protein) or via citric cycle (three genes: malate dehydrogenase; isocitrate dehydrogenase; L-lactate dehydrogenase). Further, glyceraldehyde-3-phosphate dehydrogenase and L-lactate dehydrogenase B chain, both involved in glycolysis, and creatine kinase that catalyses storage or availability of ATP via creatine, were also significantly different between strains. Some genes could be associated with the innate and adaptive immune response (eight genes: two types of MHC I; MHC II; lysozyme C; myelin and lymphocyte protein; leucocyte common antigen; CD59). The third group comprised some genes involved in muscle contraction or intracellular transport via actin filaments or networks (eight genes: three types of actin; myosin regulatory light chain 2 and 2b; troponin c; cofilin-2; actin-related protein 2/3 complex subunit 3).

The results of the analysis of over-represented GO categories at an FDR of 0.05 differed from manually inferred processes. Transcripts of proteins involved in biological processes such as 'ion transmembrane transport', 'proton transport' and 'ATP biosynthetic process' were among the top three most specific processes being over-represented. All three over-represented categories were represented by the same genes (several subunits of the ATP synthase and Na^+/K^+ transporting subunits alpha 1 and alpha 3; Table S4, Supporting information), except for calmodulin that only contributed to the last category. Additional meaningful over-represented GO categories included the process of 'regulation of pH' (represented by carbonic anhydrase and two Na^+/K^+ alpha polypeptides), the function 'monovalent inorganic cation transmembrane transporter activity' (mostly represented by ATP synthase subunits) and the cellular

component 'integral to plasma membrane' (Table S4, Supporting information). GO terms indicating relevance of the immune response (except for a contribution of the leucocyte common antigen and the myelin lymphocyte protein to the latter category) or actin–myosin-related structures or processes were not over-represented.

When transcription level was compared among the strains, 49 of the 86 putative genes were transcribed at a higher level in domesticated salmon (Table S7, Supporting information). Among those were all genes contributing to over-represented GO categories, except for calmodulin, myelin and lymphocyte protein, and leucocyte common antigen. Genes whose products were, based on manual inference, involved in the immune response or in actin–myosin-related structures, showed a less consistent transcription pattern. Higher transcription levels in domesticated relative to wild salmon were observed for MHC class II, lysozyme C and CD59, but lower transcription for MHC class I, leucocyte common antigen and the myelin lymphocyte protein. The proteins involved in actin–myosin-related structures (different types of actin, myosin regulatory light chain 2 and cofilin-2) were also upregulated in domesticated vs. wild fish. However, components regulating actin–myosin-related processes were downregulated, namely troponin C-regulating striated muscle contraction and actin-related protein 2/3 complex subunit 3 involved in cytoskeleton network formation regulation.

No correlation of transcription level with logarithmic weight was detected for domesticated salmon; however, 34 of the 86 putative genes tested correlated for hybrids and 13 for wild salmon, of which eight genes were common to both (Table S7, Supporting information). Remarkably, for these eight genes, the domesticated strain shows consistently higher transcription levels relative to the wild strain for positive correlations and lower transcription levels for negative correlations, each simultaneously found in the wild and the hybrid strains (Table S7, Supporting information).

Transcription pattern in domesticated–wild hybrids

An additive pattern of inheritance in hybrids was exhibited in 57 of the 86 differentially transcribed putative genes (δ/α not different from zero; Table S6, Supporting information). In hybrids, the levels of transcripts for some genes corresponded to predicted mid-parent values, i.e. they exhibited a 'true' additive pattern. However, many of the genes that exhibited an averaged-additive pattern did not show intermediate levels in every hybrid individual. Instead, hybrids exhibited a mosaic pattern of similarity to either parent across individuals and also across genes (Fig. 3b). The remaining one-third of all differentiated genes between wild and domesticated salmon deviated from an additive pattern of inheritance in hybrids with absolute values of δ/α ranging from 0.3 to 1.8 and CI not comprising zero (Fig. 4, Table S6, Supporting information). Of all 29 deviating genes, 23 showed mean values in hybrids that were more similar to wild than to domesticated salmon (i.e. $\delta/\alpha < 0$; Fig. 4).

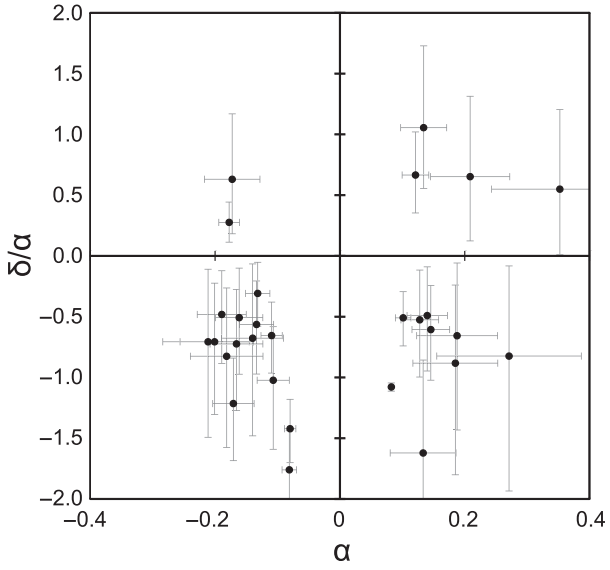

Fig. 4 Scatter plot of the ratio of dominance (δ) and additive parameter (α) vs. additive parameter for genes with different transcription levels among strains. Only genes with nonadditive transcription patterns in first-generation hybrids between wild and domesticated Atlantic salmon are shown. Error bars represent 95% confidence intervals and have been cut off for easier visualization. Values of α larger than zero indicate higher transcription in wild salmon, while values smaller than zero indicate higher transcription in domesticated salmon. Values of δ/α larger zero indicate hybrid values closer to domesticated salmon, and values smaller than zero indicate hybrid values closer to wild salmon.

Discussion

Our study examined (i) the degree to which gene transcription patterns differed between wild, domesticated Atlantic salmon, and their hybrids; (ii) how suspended sediments influence gene transcription; and (iii) whether wild salmon exhibit greater transcriptional plasticity. For the wild iBoF populations, it has been hypothesized that local adaptation in response to suspended sediments exists (COSEWIC 2006; Tymchuk *et al.* 2010). However, our data suggest that Minas Basin sediments did not result in significant differences in transcriptional plasticity among the salmon strains investigated. Nonetheless, it is still important to explore the data at hand in terms of its relevance to conservation, especially because statistical power to detect the

strain-by-environment interaction was low. The low power, caused by a small sample size and the stringent correction we applied for multiple comparisons, may have resulted in the FDR = 1 for all features. Among the 118 features that showed strain-by-environment interaction before correcting for multiple comparisons (Table S3, Supporting information) are two that exhibit differences in transcript levels between the environments (FDR < 0.05; see below): glutathione reductase and metallothionein. Both are often used as biochemical indicators in toxicological studies of fish (Atli & Canli 2008; Minghetti *et al.* 2008). Metallothionein has even shown indications of differences between fish populations in the plasticity of gene transcription response towards cadmium exposure (Knapen *et al.* 2004). Taking this into consideration, strain-specific regulation may underlie the transcription of metallothionein and glutathione reductase. For both of these genes, differences between strains become larger in the sediment environment with a higher transcript level in wild and hybrid salmon relative to the domesticated strain (Fig. 5e, h). This is concordant with the hypothesis of local adaptation to a temporarily experienced stressor through increased genetic plasticity only by the wild strain. Here, outbreeding by domesticated salmon did not negatively alter the genetically based plastic response in first-generation hybrids because they even show the steepest slopes between environments. It remains to be tested whether a reduction in plasticity may appear in further hybrid generations.

Differences between environments

Eight genes exhibited a response to suspended sediments in all salmon strains, and four of these genes may provide insight into the molecular consequences of suspended sediments. One of these genes is envoplakin, a protein found in stratified squamous epithelia. Envoplakin plays a structural role by reinforcing the plasma membrane and cell junctions (Kalinin *et al.* 2004). In fish, this epithelia type can be found enveloping the primary lamellae of the gills. Thus, the higher level of envoplakin transcript abundance in the environment containing sediments may indicate a regenerative or enforcing response to abrasive sediments, and this would be in agreement with observations of sediment-induced gill proliferation (i.e. hyperplasia and hypertrophy; Sutherland & Meyer 2006). The observed increase in the transcription of complement factor B could reflect an innate immune system response induced by the aforementioned mechanical stress or by the biological burden, such as bacteria, that sediments can carry. Pos-

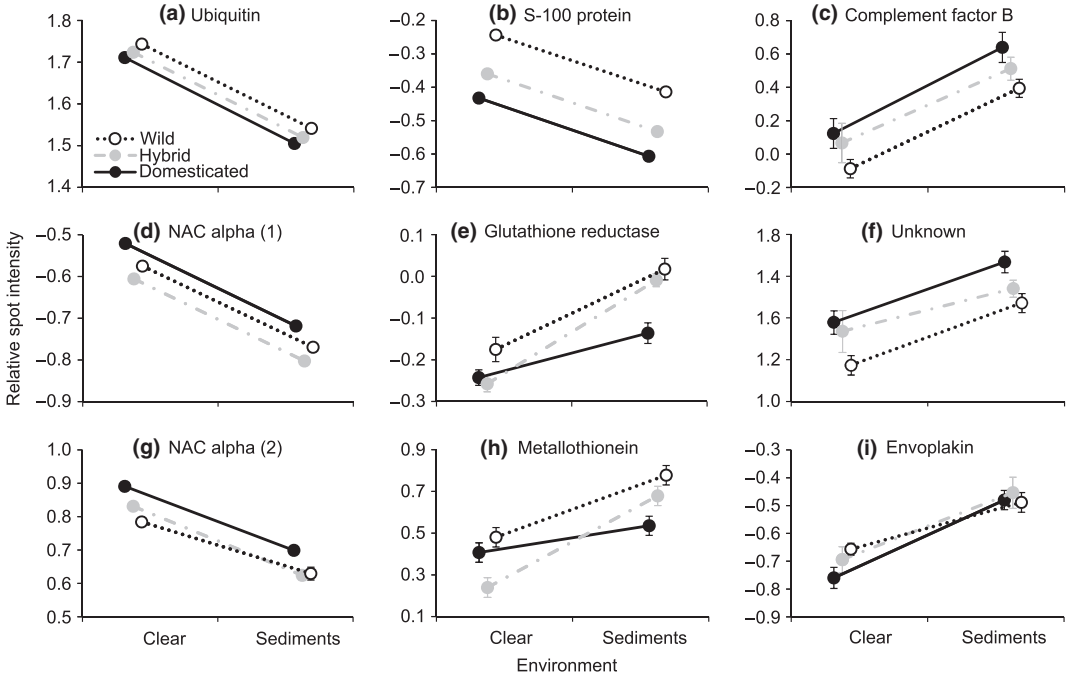

Fig. 5 Strain-by-environment interaction plots for features with transcript levels different between environments (FDR < 0.05) not containing (Clear) and containing suspended sediments (Sediments). Model corrected average relative transcription level of each experimental group is shown with 95% confidence intervals. The reaction norms in each plot are shown for three salmon strains (wild, hybrid, and domesticated). Known gene names are indicated above each plot. Only (e) and (h) yielded significance for the interaction of strain-by-environment (simple $P < 0.05$; none were significant after FDR correction).

sible reasons for an increase in the level of glutathione reductase and metallothionein transcripts are less clear. Both proteins are involved in protection from oxidative stress, which can have various causes including immunological challenge. However, metallothionein is also typically linked to protection from heavy metal toxicity (reviewed by Sato & Kondoh 2002). The iBoF sediments contain various metals with a higher concentration in finer particles (Loring 1979) that may be responsible for some of the differences observed.

Only eight genes responded to suspended sediments, but this modest response may have been caused by low statistical power, low sediment concentration or infrequent sediment application (once a day). Furthermore, sampling of only mature males may have biased the results, because mature males may differ in their response from immature males or females. The observed responses in transcript levels nevertheless indicate the presence of mechanisms to mitigate external mechanical as well as intracellular damage, so our results contribute to the growing field of 'ecological annotations of genes' (Landry & Aubin-Horth 2007).

Differences between wild and domesticated salmon

We identified quantitative transcriptional differences affecting 2.3% of all features detected in gill tissue between domesticated and wild salmon at the adult stage. In our study, the major manually inferred biological process differing between the strains was energy metabolism, in particular ATP production via oxidative phosphorylation, which was also over-represented by the analysis of GO terms. Because the gills are the major respiratory, ion-homoeostatic and excretory organ in bony fishes (Barton 2007), a higher local energy production may maximize capacities for those processes and contribute to faster growth. All genes associated with those processes were transcribed at a higher level in domesticated fish. A high representation of genes involved in oxidative phosphorylation among genes with high heritability for the level of transcription (Roberge *et al.* 2007) indicates that they have a strong potential to respond to selection. Accordingly, overtranscription of genes related to oxidative phosphorylation observed in the present study could partly be a result of selection, as this domesticated strain has been selected for fast growth (Glebe 1998). It is also possible that any observed difference between strains may have been caused by evolutionary factors pre-dating domestication. Aside from genetic drift, ecological differences such as migration distance or water temperature of the marine feeding grounds (cf. Locarnini *et al.* 2010) may be good candidates.

Admittedly, some differences observed in the present study may be the result of a cascade of gene transcription regulation initiated by only one or few pleiotropically acting genes not surveyed that are also responsible for differences in body size. For instance, a recent study on transgenic Pacific salmon revealed that quantitative changes in transcription at numerous genes originated from the insertion of a single gene coding for growth hormone (Devlin *et al.* 2009). Although it is possible to account for the confounding effects of strain and size by investigating both size- and age-matched individuals (e.g. Tymchuk *et al.* 2009b), this would have posed a large logistic and analytic challenge to our strain-by-environment approach that also incorporated hybrids. We argue that body size is not a predictor for all differences between strains as judged by visual (Fig. 3) and statistical (Table S7, Supporting information) inspection of the relationship of relative transcription level with weight. This did not result in a ubiquitous pattern of weight-transcription correlations across genes and strains. Nevertheless, those eight genes that correlated with size simultaneously in wild and hybrid salmon showed strong indications of size dependence, although it was impossible in this study to identify weight as a cause or effect of the transcription-level differences. Furthermore, the direction of the correlation of those eight genes corresponded to the direction of the transcription-level difference between wild and domesticated salmon. As a by-product of selection in domesticated salmon, transcription variation may be reduced, which would explain why correlations were nonsignificant in this group. The detection of more genes correlating with size in the hybrid strain may be a side effect of their wider size range increasing the probability to detect a correlation relative to the wild strain.

Many genes transcribed at different levels among strains are major players of the immune response. The analysis of GO terms did not corroborate this observation but may have been only of limited use because 30% of all the features transcribed in gills different between strains (at FDR < 0.05, Table S1, Supporting information) did not contribute any GO terms to the analysis, with MHC class I genes and the CD59-like protein among them. A trade-off between growth and immune response in fish has been suggested on the basis of comparisons among different types of fast-growing salmonids and their wild conspecifics (Tymchuk *et al.* 2009a). Under this scenario, fast growth comes at the cost of downregulation of the immune system, which is in agreement with general observations in livestock (Rauw *et al.* 1998). We found a mixed pattern with some up- and some downregulated genes involved in the immune response. Downregulation of MHC class I genes, leucocyte common antigen, as well as myelin and lymphocyte antigen in domesticated salmon corroborates the suggestions of Tymchuk *et al.* (2009a) and may indeed indicate a fitness reduction in

domesticated salmon (under the hypothesis that a reduced transcription translates into a reduced immune response) because the gill is a key tissue when encountering and dealing with pathogens (Haugarvoll *et al.* 2008). However, upregulation of CD59 and MHC class II in combination with higher lysozyme C transcript-levels in domesticated salmon opposes Tymchuk *et al.*'s (2009a) theory. It may even explain findings of domesticated fish displaying a higher resistance towards vibriosis, a common bacterial disease in aquaculture, when compared to wild fish (Lawlor *et al.* 2008), because both latter genes are involved in the immune response towards bacteria.

Differences in MHC transcript abundance between wild and domesticated salmon might have simply resulted from allele frequency differences between ancestral populations—which are common in salmon (Landry & Bernatchez 2001; Dionne *et al.* 2007; Evans *et al.* 2010). Allele-specific microarray-spot hybridization efficiencies may then have mimicked differences in transcription-level regulation. As another alternative explanation, a recent study found allele-specific transcription abundance of a MHC class II gene, linked to a putative *cis*–regulating DNA motif (Croisetière *et al.* 2010). Under the hypothesis that polymorphisms in allele-specific regulators are adaptive, outbreeding by domesticated fish could lead to outbreeding depression by reducing fitness of hybrid offspring via changed MHC protein quality and quantity.

Transcription pattern in domesticated–wild hybrids

For genes exhibiting an additive inheritance, we observed a mosaic pattern of transcriptional similarity to either parent, although some genes also exhibited a 'truly' intermediate transcription pattern. Interestingly, this mirrors phenotypic observations in interspecific hybrids where some morphological characters are exhibited as intermediate and some resemble either parental species across hybrid individuals (Campton 1987). This mosaic pattern may be caused by a combination of either the correlation of transcription level with size (confined mostly to hybrids; see above), individual-specific similarity to either parent (complex dominance patterns), or gene-specific effects of nonadditive transcription. Lastly, the unknown family identity of individuals, and hence parental effects, may have influenced the observed mosaic pattern. It is, however, impossible to infer consequences of this mosaic pattern on hybrid fitness in this study.

For the one-third of transcribed genes in hybrids deviating from an additive pattern, the hybrid transcription levels were often closer to the level in the wild strain (23 genes of 29). This can be seen as positive from a conservation perspective, as it would probably reduce a possible negative impact of domestic–wild hybridization in the wild. This observation is in contrast to findings in the liver of salmon juveniles reared only in fresh water, where the gene transcription level of hybrid backcrosses (F1 hybrid × wild) was closer to the domesticated strain, despite a higher genetic contribution from the same wild population used in this study (Normandeau *et al.* 2009). This likely indicates the presence of tissue-specific patterns of gene transcription, or salinity-specific influence on gene regulation as previously reported in the brook charr (*Salvelinus fontinalis*) (Côté *et al.* 2007). Alternatively, a biased parental contribution to hybrids different between this study and Normandeau *et al.*'s (2009) study may have caused the observed differences. The nonadditive transcription level in hybrids exceeding values of either parental strain has also been observed in other salmonids (Renaut *et al.* 2009; Bougas *et al.* 2010), is likely caused by a combination of allelic variants not found in either parental strain and may result from nonadditive effects within a locus (Rieseberg *et al.* 1999) or nonadditive effects among loci involved in regulatory networks (Landry *et al.* 2007). The latter explanation may be a more likely scenario for variation in gene transcription levels.

Conclusion

The present study contributed the first data on gene transcription in Atlantic salmon during the pivotal marine phase that is suspected to be critical for salmon conservation (DFO 2010). It identified genes related to energy metabolism and immunity as being the major differences in gene transcription in gill tissue between wild iBoF salmon and the major prevalent aquaculture strain. Transcription patterns in first-generation hybrids appeared unpredictable, and fitness consequences remain to be evaluated. Suspended sediments did induce a mostly similar change in the transcriptional patterns of all strains. However, two genes (glutathione reductase and metallothionein) did exhibit a strain-by-environment interaction with greater plasticity exhibited by the wild strain, a finding consistent with the hypothesis that this interaction represents an adaptive response to stressful environmental conditions during a critical developmental life stage. As such, these two genes merit further examination as candidate genes whose expression may be evolving under the effect of natural selection.

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References

Atli G, Canli M (2008) Responses of metallothionein and reduced glutathione in a freshwater fish *Oreochromis niloticus* following metal exposures. *Environmental Toxicology and Pharmacology*, **25**, 33–38.

Aubin-Horth N, Renn SC (2009) Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Molecular Ecology*, **18**, 3763–3780.

Barton M (2007) *Bond's Biology of Fishes*, 3rd edn. Thomson Brooks/Cole, Belmont, California.

Bash J, Berman C, Bolton S (2001) *Effects of Turbidity and Suspended Solids on Salmonids*. Washington State Transportation Center, Seattle, Washington.

Bisson PA, Bilby RE (1982) Avoidance of suspended sediment by Juvenile Coho Salmon. *North American Journal of Fisheries Management*, **2**, 371–374.

Blüthgen N, Brand K, Cajavec B *et al.* (2005) Biological profiling of gene groups utilizing gene ontology. *Genome Informatics. International Conference on Genome Informatics*, **16**, 106–115.

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

Bourret V, O'Reilly PT, Carr JW, Berg PR, Bernatchez L (2011) Temporal change in genetic integrity suggests loss of local adaptation in a wild Atlantic salmon (*Salmo salar*) population following introgression by farmed escapees. *Heredity*, **106**, 500–510.

Campton DE (1987) Natural hybridization and introgression in fishes: methods of detection and genetic interpretations. In: *Population Genetics & Fishery Management* (eds Utter F, Ryman N), pp. 161–192. University of Washington Press, Seattle, Washington.

Carr JW, Anderson JM, Whoriskey FG, Dilworth T (1997) The occurrence and spawning of cultured Atlantic salmon (*Salmo salar*) in a Canadian river. *ICES Journal of Marine Science*, **54**, 1064–1073.

Conesa A, Götz S, Garcia-Gómez JM *et al.* (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*, **21**, 3674–3676.

Conner JK, Hartl DL (2004) *A Primer of Ecological Genetics*. Sinauer Associates, Sunderland, Massachusetts.

COSEWIC (2006) *COSEWIC Assessment and Update Status Report on the Atlantic Salmon Salmo salar Inner Bay of Fundy Populations in Canada*. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario.

COSEWIC (2011) *COSEWIC Assessment and Status Report on the Atlantic Salmon Salmo salar in Canada*. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario.

Côté G, Perry G, Blier P, Bernatchez L (2007) The influence of gene-environment interactions on GHR and IGF-1 expression and their association with growth in brook charr, *Salvelinus fontinalis* (Mitchill). *BMC Genetics*, **8**, 87.

Croisetière S, Bernatchez L, Belhumeur P (2010) Temperature and length-dependent modulation of the MH class II beta gene expression in brook charr (*Salvelinus fontinalis*) by a *cis*-acting minisatellite. *Molecular Immunology*, **47**, 1817–1829.

Crozier WW (1993) Evidence of genetic interaction between escaped farmed Salmon and Wild Atlantic Salmon (*Salmo salar* L) in a Northern Irish River. *Aquaculture*, **113**, 19–29.

Devlin RH, Sakhrani D, Tymchuk WE, Rise ML, Goh B (2009) Domestication and growth hormone transgenesis cause similar changes in gene expression in coho salmon (*Oncorhynchus kisutch*). *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 3047–3052.

DFO (2010) *Recovery Strategy for the Atlantic Salmon (Salmo salar), Inner Bay of Fundy Populations [Final]*. Species at Risk Act Recovery Strategy Series, Fisheries and Oceans Canada, Ottawa, Ontario.

Dilba G, Bretz F, Guiard V (2006) Simultaneous confidence sets and confidence intervals for multiple ratios. *Journal of Statistical Planning and Inference*, **136**, 2640–2658.

Dionne M, Miller KM, Dodson JJ, Caron F, Bernatchez L (2007) Clinal variation in MHC diversity with temperature: evidence for the role of host-pathogen interaction on local adaptation in Atlantic salmon. *Evolution*, **61**, 2154–2164.

Evans ML, Neff BD, Heath DD (2010) MHC-mediated local adaptation in reciprocally translocated Chinook salmon. *Conservation Genetics*, **11**, 2333–2342.

Fraser DJ, Houde AL, Debes PV *et al.* (2010) Consequences of farmed-wild hybridization across divergent wild populations and multiple traits in salmon. *Ecological Applications*, **20**, 935–953.

Gibson G (2008) The environmental contribution to gene expression profiles. *Nature Reviews: Genetics*, **9**, 575–581.

Glebe BD (1998) East coast salmon aquaculture breeding programs: history and future. Canadian Stock Assessment Secretariat, Research Document 98/157,Fisheries and Oceans Canada, Ottawa, Ontario.

Gordon DC (1994) Intertidal ecology and potential power impacts, Bay of Fundy, Canada. *Biological Journal of the Linnean Society*, **51**, 17–23.

Haugarvoll E, Bjerkås I, Nowak BF, Hordvik I, Koppang EO (2008) Identification and characterization of a novel intraepithelial lymphoid tissue in the gills of Atlantic salmon. *Journal of Anatomy*, **213**, 202–209.

Huntsman AG (1931) *The Maritime Salmon of Canada*. The Biological Board of Canada, Bulletin No. XXI, Ottawa, Ontario.

Hutchings JA (1991) The threat of extinction to native populations experiencing spawning intrusions by cultured Atlantic salmon. *Aquaculture*, **98**, 119–132.

Hutchings JA, Fraser DJ (2008) The nature of fisheries- and farming-induced evolution. *Molecular Ecology*, **17**, 294–313.

ICES (2010) *Report of the ICES Advisory Committee, Book 10: North Atlantic Salmon Stocks*. International Council for the Exploration of the Sea, Copenhagen.

Jessop BM (1976) Distribution and timing of tag recoveries from native and non-native Atlantic Salmon (*Salmo salar*) released into Big Salmon River, New Brunswick. *Journal of the Fisheries Research Board of Canada*, **33**, 829–833.

Kalinin AE, Idler WW, Marekov LN *et al.* (2004) Co-assembly of envoplakin and periplakin into oligomers and Ca^{2+}-

dependent vesicle binding – implications for cornified cell envelope formation in stratified squamous epithelia. *Journal of Biological Chemistry*, **279**, 22773–22780.

Khaitovich P, Enard W, Lachmann M, Pääbo S (2006) Evolution of primate gene expression. *Nature Reviews: Genetics*, **7**, 693–702.

Knapen D, Bervoets L, Verheyen E, Blust R (2004) Resistance to water pollution in natural gudgeon (*Gobio gobio*) populations may be due to genetic adaptation. *Aquatic Toxicology*, **67**, 155–165.

Kohane MJ, Parsons PA (1988) Domestication: evolutionary change under stress. In: *Evolutionary Biology*, **Vol. 23** (eds Hecht MK, Wallace B), pp. 31–47. Plenum Press, New York.

Koop BF, von Schalburg KR, Leong J *et al.* (2008) A salmonid EST genomic study: genes, duplications, phylogeny and microarrays. *BMC Genomics*, **9**, 545.

Landgrebe J, Bretz F, Brunner E (2006) Efficient design and analysis of two colour factorial microarray experiments. *Computational Statistics & Data Analysis*, **50**, 499–517.

Landry CR, Aubin-Horth N (2007) Ecological annotation of genes and genomes through ecological genomics. *Molecular Ecology*, **16**, 4419–4421.

Landry C, Bernatchez L (2001) Comparative analysis of population structure across environments and geographical scales at major histocompatibility complex and microsatellite loci in Atlantic salmon (*Salmo salar*). *Molecular Ecology*, **10**, 2525–2539.

Landry CR, Hartl DL, Ranz JM (2007) Genome clashes in hybrids: insights from gene expression. *Heredity*, **99**, 483–493.

Lawlor JL, Dacanay A, Hutchings JA, Brown LL, Sperker SA (2008) Differences in pathogen resistance within and among cultured, conservation-dependent, and endangered populations of Atlantic salmon, *Salmo salar* L. *Environmental Biology of Fishes*, **84**, 69–78.

Locarnini RA, Mishonov AV, Antonov JI, *et al.* (2010) *World Ocean Atlas 2009, Volume 1: Temperature*. Levitus S, ed., NOAA Atlas NESDIS 68, U.S. Government Printing Office. Washington, DC, 184 pp.

Loring DH (1979) Baseline levels of transition and heavy metals in the bottom sediments of the Bay of Fundy. *Proceedings of the Nova Scotian Institute of Science*, **29**, 335–346.

McGinnity P, Prodohl P, Ferguson A *et al.* (2003) Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **270**, 2443–2450.

Mignon-Grasteau S, Boissy A, Bouix J *et al.* (2005) Genetics of adaptation and domestication in livestock. *Livestock Production Science*, **93**, 3–14.

Minghetti M, Leaver MJ, Carpene E, George SG (2008) Copper transporter 1, metallothionein and glutathione reductase genes are differentially expressed in tissues of sea bream (*Sparus aurata*) after exposure to dietary or waterborne copper. *Comparative Biochemistry and Physiology, Part C: Toxicology & Pharmacology*, **147**, 450–459.

Morris MRJ, Fraser DJ, Heggelin AJ *et al.* (2008) Prevalence and recurrence of escaped farmed Atlantic salmon (*Salmo salar*) in eastern North American rivers. *Canadian Journal of Fisheries and Aquatic Sciences*, **65**, 2807–2826.

Normandeau E, Hutchings JA, Fraser DJ, Bernatchez L (2009) Population-specific gene expression responses to hybridization between farm and wild Atlantic salmon. *Evolutionary Applications*, **2**, 489–503.

Price EO, King JA (1968) Domestication and adaptation. In: *Adaptation of Domestic Animals* (ed. Hafez ESE), pp. 34–45. Lea & Febiger, Philadelphia, Pennsylvania.

Rauw WM, Kanis E, Noordhuizen-Stassen EN, Grommers FJ (1998) Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livestock Production Science*, **56**, 15–33.

Renaut S, Nolte AW, Bernatchez L (2009) Gene expression divergence and hybrid misexpression between lake whitefish species pairs (*Coregonus* spp. Salmonidae). *Molecular Biology and Evolution*, **26**, 925–936.

Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics*, **27**, 83–109.

Rieseberg LH, Archer MA, Wayne RK (1999) Transgressive segregation, adaptation and speciation. *Heredity*, **83**, 363–372.

Rise ML, von Schalburg KR, Brown GD *et al.* (2004) Development and application of a salmonid EST database and cDNA microarray: data mining and interspecific hybridization characteristics. *Genome Research*, **14**, 478–490.

Ritter JA (1989) Marine migration and natural mortality of North American Atlantic salmon (*Salmo salar* L.). Canadian Manuscript Report of Fisheries and Aquatic Sciences No. 2041, Fisheries and Oceans Canada, Halifax, Nova Scotia.

Roberge C, Einum S, Guderley H, Bernatchez L (2006) Rapid parallel evolutionary changes of gene transcription profiles in farmed Atlantic salmon. *Molecular Ecology*, **15**, 9–20.

Roberge C, Guderley H, Bernatchez L (2007) Genomewide identification of genes under directional selection: gene transcription Q_{ST} scan in diverging Atlantic salmon subpopulations. *Genetics*, **177**, 1011–1022.

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

Sato M, Kondoh M (2002) Recent studies on metallothionein: protection against toxicity of heavy metals and oxygen free radicals. *Tohoku Journal of Experimental Medicine*, **196**, 9–22.

Sauvage C, Derôme N, Normandeau E *et al.* (2010) Fast transcriptional responses to domestication in the brook charr *Salvelinus fontinalis*. *Genetics*, **185**, 105–112.

von Schalburg KR, Rise ML, Cooper GA *et al.* (2005) Fish and chips: various methodologies demonstrate utility of a 16,006-gene salmonid microarray. *BMC Genomics*, **6**, 126.

Schlichting C, Pigliucci M (1998) *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer, Sunderland, Massachusetts.

Skaala Ø, Wennevik V, Glover KA (2006) Evidence of temporal genetic change in wild Atlantic salmon, *Salmo salar* L., populations affected by farm escapees. *ICES Journal of Marine Science*, **63**, 1224–1233.

Staubach F, Teschke M, Voolstra CR, Wolf JBW, Tautz D (2010) A test of the neutral model of expression change in natural populations of house mouse subspecies. *Evolution*, **64**, 549–560.

Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 9440–9445.

Sutherland AB, Meyer JL (2006) Effects of increased suspended sediment on growth rate and gill condition of two southern

Appalachian minnows. *Environmental Biology of Fishes*, **80**, 389–403.

Taberlet P, Coissac E, Pansu J, Pompanon F (2011) Conservation genetics of cattle, sheep, and goats. *Comptes Rendus Biologies*, **334**, 247–254.

Tymchuk W, Sakhrani D, Devlin R (2009a) Domestication causes large-scale effects on gene expression in rainbow trout: analysis of muscle, liver and brain transcriptomes. *General and Comparative Endocrinology*, **164**, 175–183.

Tymchuk WE, Beckman B, Devlin RH (2009b) Altered expression of growth hormone/insulin-like growth factor I axis hormones in domesticated fish. *Endocrinology*, **150**, 1809–1816.

Tymchuk WV, O'Reilly P, Bittman J, MacDonald D, Schulte P (2010) Conservation genomics of Atlantic salmon: variation in gene expression between and within regions of the Bay of Fundy. *Molecular Ecology*, **19**, 1842–1859.

Wray GA, Hahn MW, Abouheif E *et al.* (2003) The evolution of transcriptional regulation in eukaryotes. *Molecular Biology and Evolution*, **20**, 1377–1419.

Yeo RK, Risk MJ (1981) The sedimentology, stratigraphy, and preservation of inter-tidal deposits in the Minas Basin system, Bay of Fundy. *Journal of Sedimentary Petrology*, **51**, 245–260.

P.V.D. is interested in ecological and evolutionary concepts relevant to sustainable management and conservation of fishes. E.N's interests revolve around bioinformatics, especially the analysis of next generation sequencing and transcriptomics data. D.J.F's research program applies evolutionary principles towards biodiversity conservation and fisheries management. L.B's research focuses on understanding the patterns and processes of molecular and organismal evolution and their significance to conservation. J.H's research focuses on understanding the patterns and processes of organismal evolution in fishes and their significance to conservation.

Data accessibility

MIAME compliant data of the microarray experiment have been deposited at Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) with GEO accession number GSE30555.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Features transcribed at different levels among Atlantic salmon from suspended sediment and nonsediment environments (ANOVA permutation *F*-test, $P < 0.05$).

Table S2 Features transcribed at different levels among wild, domesticated, and hybrid Atlantic salmon (ANOVA permutation *F*-test, $P < 0.05$).

Table S3 Features with transcription levels different for the interaction of strain-by-environment (ANOVA permutation *F*-test, $P < 0.05$).

Table S4 Most specific GO terms over-represented (Fisher's exact test at FDR < 0.05) among the genes with different transcription levels among wild, domesticated, and hybrid Atlantic salmon (ANOVA permutation *F*-test, FDR < 0.05).

Table S5 Most specific GO terms over-represented (Fisher's exact test at FDR < 0.2) among the genes with different transcription levels between Atlantic salmon from suspended sediment and nonsediment environments (ANOVA permutation *F*-test, FDR < 0.2).

Table S6 Key variables of the analysis for nonadditive gene transcription patterns by means of the δ/α ratio in hybrid salmon for genes different between wild, domesticated, and hybrid Atlantic salmon (ANOVA permutation *F*-test, FDR < 0.05).

Table S7 *P*-values and Pearson's correlation coefficients obtained from the correlation between the relative transcription level and the natural logarithmic weight for each strain for genes different among strains.

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