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Comparative transcriptomics of anadromous and resident brook charr *Salvelinus fontinalis* before their first salt water transition

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Abstract Most salmonid taxa have an anadromous life history strategy, whereby fish migrate to saltwater habitats for a growth period before returning to freshwater habitats for spawning. Moreover, several species are characterized by different life history tactics whereby resident and anadromous forms may occur in genetically differentiated populations within a same species, as well as polymorphism within a population. The molecular mechanisms underlying the physiological differences between anadromous and resident forms during the first transition from freshwater to saltwater environments are only partially understood. Insofar research has typically focused on species of the genus *Salmo*. Here, using a 16,000 cDNA array, we tested the hypothesis that anadromous brook char*r Salvelinus fontinalis* are characterized by differences in their transcriptome relative to resident brook charr before the anadromous fish migration. Families originating from parapatric populations of anadromous and resident charr were reared in controlled environments mimicking natural temperature and photoperiod, and sampled in spring, while still in fresh water. While anadromous and resident charr showed similar transcriptome profiles in white muscle, they were characterized by striking differences in their gill transcriptome profiles. Genes that were upregulated in the gills of anadromous charr were principally involved in metabolism (mitochondrial electron transport chain, glucose metabolism, and protein synthesis), development (tissue differentiation) and innate immunity. We discuss the nature of these transcriptomic differences in relation to molecular mechanisms underlying the expression of anadromous and resident life history tactics and suggest that the anadromous charr express some of the molecular processes present in other migratory salmonids [*Current Zoology* 58 (1): 155–167, 2012].

Keywords Transcriptome, Life history tactics, Anadromy, Migration to salt water, Ecotypes, Salmonids

Salmonidae demonstrate a tremendous diversity in terms of phenotypic traits both at the interspecific and intraspecific levels (Hendry and Stearns, 2004). In this family typically present in lakes and rivers of the Northern Hemisphere, variation in phenotypic traits generally relates to reproductive strategies, mobility associated with foraging tactics, habitat selection within lakes, and tendency for anadromy (Hendry and Stearns, 2004). Anadromy refers to the directed and period-specific movements undertaken by fish between freshwater and saltwater environments. With respect to anadromy, two main ecotypes are observed: the anadromous ecotype, which undertakes migration from freshwater lakes and rivers to estuaries or oceans and eventually return to freshwater habitats for spawning, and the resident (non-anadromous) ecotype, which permanently resides in freshwater (Hendry et al., 2004; Harris and Milner, 2006). Although potentially increasing mortality risks, migration to saltwater habitats can be advantageous because juvenile anadromous fish may access more abundant food resources in saltwater habitats and experience enhanced growth rates (Hendry et al., 2004). The expression of the anadromous or resident life tactics is probably determined by complex interactions involving the endocrine system, environmental parameters (e.g., temperature, photoperiod, feeding resources), individual characteristics (e.g., sex, age, or size), and the genome (Høgåsen, 1998; McCormick, 2001; Hendry et al., 2004; Harris and Milner, 2006; McCormick, 2009). Thus, understanding the determinants of these life history tactics is particularly challenging.

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Through the recent application of molecular tools to non-model organisms, the complex genomic mechanisms linked to migration have begun to be unveiled (Liedvogel et al., 2011). In salmonids in particular, using cDNA microarrays, Giger et al. (2006) compared the transcriptome of anadromous and resident ecotypes of brown trout *Salmo trutta* from various European populations. They found that the life history trait anadromy was a better predictor of expression profiles than phylogenetic realtionships: phylogenetically remote populations of the same ecotype had more similar transcriptome profiles than those of more phylogenetically related populations belonging to different ecotypes. Subsequently, Giger et al. (2008) identified a set of candidate genes potentially involved in preparatory adaptations to saltwater migration in brown trout and Atlantic salmon *Salmo salar*. Insofar, most studies examining gene expression regulation and its role in anadromy have focused primarily on the genus *Salmo* (e.g., brown trout: Giger et al., 2006; Giger et al., 2008 and Atlantic salmon: Hagen-Larsen et al., 2005; Seear et al., 2010). As the expression of the anadromous behavior varies interspecifically and intraspecifically (Rounsefell, 1958; Hendry et al., 2004; Quinn and Myers, 2004; Curry et al., 2010), additional studies, focusing on other salmonids (e.g., *Salvelinus*, *Oncorhynchus*, *Coregonus*) are required to assess the generality and diversity of molecular mechanisms that are linked to anadromy.

Brook charr is typically found in cold, pristine freshwater lakes and streams of eastern North America where the resident ecotype prevails. Where brook charr has access to marine environments, the anadromous ecotype may be present (Curry et al., 2010), although it is considered less anadromous than *Salmo* species (Rounsefell, 1958; Quinn and Myers, 2004; Curry et al., 2010). In some rivers, the anadromous and resident ecotypes co-occur despite the absence of physical barriers preventing gene flow between these divergent forms (Curry et al., 2010). The ecotypes may live in sympatry either as alternative life history tactics within a single gene pool (Thériault et al., 2007) or as locally adapted and genetically distinct populations (Perry et al., 2005). In both situations, the anadromous "adult" charr (usually defined as > 20 cm in fork length) undertake feeding migration to their home river's estuary. Notorious differences in anadromy tendency have been reported between river systems (Curry et al., 2010). For instance, in the Kennebecasis River (southern NB), adult charr rarely enter the estuary (Curry et al., 2002). In the Petite Cascapédia River (Gaspésie, QC), adult charr move to the estuary in the spring and reside in this saltwater environment for a few weeks (Van de Sande, 2004). Finally, adult charr of the Laval River (Côte Nord, QC) migrate to estuaries and reside in near full salinity environments (i.e., up to 27 ppt) all summer (Curry et al., 2006). Juvenile brook charr (defined as > 12 month old; < 20 cm in fork length) may also express the anadromous behavior (Lenormand et al., 2004; St-Laurent, 2007). Therefore, as observed in other salmonids (Quinn and Myers, 2004), some brook charr populations appear behaviorally "more anadromous" than others. Curry et al. (2010) recently proposed that anadromy was the ancestral trait in this species and also developed a model to explain the occurrence of the migratory behavior. Accordingly, anadromy would be the result of key factors including the species' propensity to move and to disperse, over-production of juveniles, retained ability to reside in salt water, and persistence of key habitats. When spatial and physical environmental conditions are met, the migratory behavior would be expressed.

Here, we use cDNA micrarrays to test whether the anadromous and resident brook charr can be differentiated through their transcriptome before the first transition to salt water. Our goal is to identify general molecular processes and potential footprints of transcriptional differences between ecotypes. Microarrays are particularly suited for studying complex traits such as anadromy because they can quantify the relative expression of multiple genes simultaneously (Rise et al., 2007; Miller and Maclean, 2008; Larsen et al., 2011; Liedvogel et al., 2011). Thus, using a 16,000 cDNA-feature microarray chip (cGRASP project, Von Schalburg et al., 2005) previously applied to brook charr (Mavarez et al., 2009; Bougas et al., 2010; Sauvage et al., 2010), we compared the transcription profiles of anadromous and resident brook charr. We focused on the specific life stage when the anadromous form is still in fresh water but would very soon undertake its first transition to salt water (or the estuary). The first transition to salt water was selected in order to avoid biases related to differential acclimation between experienced and non-experienced fish in full salinity. The transcriptome analysis focused on two types of tissues: 1) the white muscle, involved in locomotion, activity that should be enhanced during migration; 2) gills, because of their specific role in osmo-iono-regulation. The anadromous and resident charr originate from wild populations living in parapatry in the same river system (Boula et al., 2002; Perry et al., 2005). Anadromous and resident fish had the same age

and were reared in identical conditions from egg to juvenile stages. The controlled conditions in the fish facility mimicked the temperature and photoperiod observed in the wild in the spring time.

1 Materials and Methods

1.1 Fish families

As previously described in Perry et al. (2005), anadromous and resident fish were collected in the Laval River drainage: the Adams Brook and the Laval River mainstream. The Adams Brook system runs approximately 20 km from the Laval Bay inlet to Lake Jacques (roughly 3 km in length), then continues for an additional 10 km to the Adams Brook branch. Adams brook charr are predominantly resident types while fish in the Laval River main system are mostly anadromous (see Boula et al., 2002). The two populations are parapatric, but have very limited geographic separation (see Castric and Bernatchez, 2003; Castric and Bernatchez, 2004). Perry et al. (2005) also showed pronounced genetic divergence between resident and anadromous brook charr in the Laval River drainage (Fst = 0.15) based on allele frequencies at nine microsatellite loci, confirming that they compose genetically distinct populations.

Six pure anadromous and six pure resident fullsib families were produced at the Station aquicole de l'UQAR-ISMER (Rimouski, QC). The anadromous families came from the third generation produced at UQAR-ISMER and the resident families from the first generation produced at the same site. The anadromous and resident breeders were reared under natural temperature, salinity and photoperiod conditions, according to their respective life history patterns. Fertilized eggs from both anadromous and resident families were incubated in the dark within individual trays and at controlled temperature (4°C) until hatching. At hatching, anadromous and resident fry were gradually acclimated to 8°C (rate = 1°C / week) and maintained under these conditions until early June, when the average outside temperature was also 8°C.

Juveniles used for this study were reared in freshwater and under natural photoperiod and temperature conditions from hatching through their first year (minimal temperature [2.0°C] reached in February; maximal temperature [15°C] reached in September). Each family was raised in a separate basin until fish reached approximately 4.0 g. Once fish reached the exogenous feeding stage, the number of fish was standardized to 700 individuals / family and regular random culls were done within families to maintain similar densities and optimal stocking conditions (< 30 kg fish per m^3). Fish were fed with commercial pellets, based on needs at specific ages and water temperature. In August-September, fish were fin clipped for family identification and families were randomly pooled in rearing tanks. Fin markings were verified every 3–4 months and fish without clear markings were removed from tanks. Fish were healthy with no sign of disease at any time during the rearing experiment.

1.2 Fish sampling

Sampling was made during the first week of June, when fish where 17-month old, this time of the year being characterized by rapid acclimation to salt water (Claireaux and Audet, 2000). Before sampling, charr were anaesthetized using a 3-aminobensoic acid ethyl ester solution (0.12 g/L, pH adjusted with NaOH 0.1 N) and weighted. For each sampled fish, we collected a piece of white muscle and one gill arch. All samples were immediately frozen in dry ice and then stored at -80 ºC. Sex of individuals was determined by examining gonad development during sampling procedures. Mean weight and length for anadromous fish were 27.14 g (± 13.81 g *SD*) and 13.8 cm (± 2.1 cm *SD*), whereas mean weight and length for resident fish were 32.43 g (± 11.8 g *SD*) and 14.2 cm (± 1.7 cm *SD*), respectively. All the fish used in this study were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

1.3 Microarray experiment

Two fish per family were analyzed, for a total of 12 anadromous and 12 resident fish. We extracted total RNA from muscle and gill tissues using 2–4 mL of TRIZOL ® Reagent (Invitrogen Life Technologies, Burlington, ON), as detailed in Roberge et al. (2006). The RNA integrity was confirmed using a spectrometer and a 2100 Bioanalyser (Agilent Technologies, Mississauga, ON). We used 15 µg (gill) or 20 µg (muscle) of RNA for the reverse-transcription reaction with the enzyme SuperScript II (Invitrogen Life Technologies). The cDNA obtained from the reaction was labeled using Genisphere 3DNA Array 50 kit (Genisphere Inc., Hatfield, PA) and hybridized on a 16,000 gene microarray chip specifically designed for salmonids (Consortium for Genomics Research on All Salmonids Project [cGRASP], University of Victoria, BC; Von Schalburg et al. 2005; http://web.uvic.ca/cbr/grasp/; GEO accession number: GPL2989). The cGRASP microarray chip contains 13,421 Atlantic salmon and 2,576 rainbow trout cDNA features. The array shows similar hybridization levels across several salmonid species (Rise et al.,

2004b; Von Schalburg et al., 2005; Rise et al., 2007) and has previously been applied successfully to brook charr (Mavarez et al., 2009, Bougas et al., 2010). The Genisphere protocol adopted by cGRASP (http://web.uvic.ca/cbr/grasp/enisphere_Array50_Protocol_v.4.pdf) was followed with minor modifications, as detailed in Roberge et al. (2006). We used Cy 3 and Alexa 647 dyes to differently label samples from anadromous and resident fish. Samples of anadromous and resident fish were directly hybridized against each other to the same array (12 arrays / tissue) and all hybridization experiments included individuals of the same sex. Fluorescence levels of dyes were measured using a ScanArray Express scanner (Packard Bioscience, Meriden, CT). Clone spots (thereafter referred to as features) were referenced and quantified using QUANTARRAY software (Packard Bioscience). The quality of features was verified manually and those showing hybridization artifacts were eliminated. The local background signals were subtracted from raw signals and overall array intensity was normalized for each dye channel (Drăghici, 2003). Features with expression levels lower than mean expression levels of negative controls (e.g., blank spots, *Arabidopsis thaliana* genes) + 2 standard deviations were discarded, resulting in 5710 (white muscle) and 5534 (gills) analyzed features.

1.4 Statistical analyses

Following Drăghici (2003), microarray data were subjected to transformations before statistical analyses: imputation of missing values using K nearest neighbor algorithm, LOWESS curve fitting correction between Cy 3and Alexa 647 fluorescence levels, and finally log2 transformation (St-Cyr et al., 2008; Bougas et al., 2010; Sauvage et al., 2010). These calculations were computed using the R/MAANOVA package (Kerr et al., 2000). To identify genes differentially expressed between ecotypes, we applied a mixed-effect model and carried out, in R/MAANOVA, series of Fs tests (Cui et al., 2005) based on 1000 permutations. The mixed-effect model included "array" (12 arrays for each tissue) as a random term, as well as "form" (anadromous or resident) and "dye" (Cy 3 or Alexa 647) as fixed terms. Dye-swaps were applied on biological replicates within fish families. The QValue R package (Storey, 2002) was used to determine a false discovery rate (FDR), which is the expected proportion of false positives among the tests found to be significant. For each test, the package provides a *P* value that is associated with a *Q* value. This *Q* value represents the FDR of the list of tests obtained by including all the tests with identical or lower *P* values. Whenever applicable, the *Q* values calculated by this program were reported next to classic *P* values. When reporting specific transcripts as differentially expressed between ecotypes, a stringent FDR of 0.05 was applied; this FDR corresponded to a maximum *P* value of 0.00053. Otherwise, a FDR of 0.10 (corresponding to a maximum *P* value of 0.00583) was used. For each differentially expressed feature, the expression data were recovered by reversing the logarithm transformation on the normalized data (St-Cyr et al., 2008; Bougas et al., 2010; Sauvage et al., 2010). The expression levels are then reported as A/R ratios, where the mean transcription level of a gene in anadromous fish (A) was divided by the mean transcription level of the same gene in resident fish (R). Therefore, ratios > 1 identified transcripts that were upregulated anadromous charr and ratios < 1 identified transcripts that were downregulated in this same ecotype. Permutation tests were conducted to determine if there were differences in the patterns of fold changes observed among upregulated and downregulated genes (PERM program, Duchesne et al., 2006).

1.5 Biological processes

To identify gill-specific biological processes that may be linked with adaptation to salt water, a test list containing all the genes differentially expressed in gills was created and was compared against a reference list containing all genes expressed in gills. For most features, gene identity and gene ontology annotations were obtained from cGRASP. When annotations were missing, they were completed using the Uniprot database (http://www.uniprot.org/). To create the test list, a unigene code was assigned to the 214 transcripts that were significant at an FDR level of 0.10. A total of 95 distinct genes (or unigenes) were thus identified. Each gene was represented by a maximum of one expressed transcript. A unigene code was also assigned to the 5534 gill transcripts (i.e, those with expression levels above background) and 1643 unigenes were obtained for the reference list. The proportional representation of the biological processes was compared between the test and reference lists under a random sampling hypothesis using the Panther web tool (http://www.pantherdb.org/tools/compareToRefListForm.jsp, see also Thomas et al., 2003). This analysis could not be applied to the muscle tissue because too few transcripts were significantly expressed between ecotypes for that tissue (see section 2.1).

1.6 Clustering analysis

To identify features (genes) and fish samples that ex-

hibit similar expression patterns, a hierarchical clustering analysis (Eisen et al., 1998) was performed using MultiExperiment Viewer version 4.5.1 (Saeed et al., 2003). The clustering procedure was applied only to the features that were differently regulated between resident and anadromous charr at an FDR of 0.05. Using the gene expression data of these selected features, a matrix of pairwise Pearson correlation was created and the 2-dimentional cluster tree was built via an average linking method. The topology of the gene tree and sample tree were bootstrapped 10,000 times to obtain statistical support for the tree nodes. Following the approached detailed in St-Cyr et al. (2008), a normalization procedure on LOWESS-transformed data was applied after applying a reverse logarithm to minimize potential biases pertaining to differential array fluorescence. Briefly, each clone expression value of a given fish was divided by the clone expression value of the matching fish on the array, (e.g., for array #1 and clone #1: expression value anadromous #1 / expression value of resident #1 and expression value resident #1 / expression value anadromous charr #1).

2 Results

2.1 Comparison of transcriptome profiles between tissues

In gills, among the 5534 features detected over background, 214 were differently expressed when using a FDR = 0.10. At a FDR of 0.05, we found that 38 features over 5534 were differently expressed. In contrast, in white muscles, only 2 of the 5710 features detected over background showed differential expression between ecotypes (FDR = 0.10). Using a FDR of 0.05, no feature was differently expressed and consequently, no further analyses were pursued for the muscle tissue. In summary, under controlled experimental conditions, the transcriptomics differences between anadromous and resident fish were much more pronounced in gills than in white muscles (for FDR of 0.10, Chi-square = 219.0, $P < 0.0001$; for FDR = 0.05, Chi-square = 39.3, $P < 0.0001$).

2.2 Transcriptomic profiles in gills

A general upregulation of the gill transcriptome was observed for anadromous charr: at FDR = 0.05, upregulated features accounted for 68% (26/38, Table 1) of the significant transcripts. Moreover, the absolute fold change was higher for upregulated transcripts (30.8 % ± 18.1 %, mean ± *SD*) than for downregulated transcripts (18.4% ± 4.5%) (permutation test, $P = 0.03$).

Relative to random expectations, gills had a significant enrichment of transcripts belonging to the protein biosynthesis and protein metabolism-modification functional categories (Fisher exact tests, $P = 0.03$ and $P = 0.04$, respectively). They also showed a tendency to be deficient in transcripts involved in cell proliferation and cell differentiation ($P = 0.06$) but enriched in transcripts involved in apoptotic processes, DNA degradation, and angiogenesis ($P = 0.06$ in all cases).

As resident and anadromous brook charr showed differences in several major biological processes (Fisher exact test on transcripts significant at a FDR of 0.10, $P = 0.003$, Fig. 1), the transcription profiles of features meeting the stringent criterion (P value ≤ 0.00053 or a Q value ≤ 0.05; Table 1) were examined more closely. Among these, 26 were significantly upregulated in the gills of anadromous fish (19 with identified names, 7 unknown, Table 1). These included elements of the innate immune system, such as the C-type lectin gene, which encodes for a calcium-dependent protein that binds to carbohydrates exposed on the membrane of invading pathogens, as well as the hemagglutinin/amebocyte aggregation factor precursor, which encodes for another lectin that aggregates amebocytes and agglutinates erythrocytes. Upregulation in anadromous fish also involved grancalcin, a gene coding for a calcium-binding protein promoting neutrophil adhesion, and the thioredoxin gene, which encodes for an enzyme acting as a chemoattractant for various immune cells. Genes involved in various metabolic functions were upregulated, such as a mitochondrial precursor of NADH dehydrogenase [ubiquinone] flavoprotein 3, which is involved in oxidative phosphorylation chain, as well as glyceraldehyde-3-phosphate dehydrogenase and transketolase, which are both involved in carbohydrate metabolism. There was also an upregulation of transcripts playing a specific role in protein metabolism, such as various forms of 40S and 60S ribosomal protein genes and the nascent polypeptide-associated complex subunit alpha gene. Upregulation was also observed in annexin A2, a gene coding for a calcium-dependant phospholipid-binding protein.

Twelve transcripts were significantly downregulated in anadromous charr (all P value ≤ 0.00053; all Q value ≤ 0.05; Table 1). Some of these transcripts were involved in energetic metabolism, including three cytochrome oxidase c subunits, an ATP synthase alpha chain, and a pyruvate kinase. Downregulation was also observed in transcripts playing a role in cellular processes, such as cell division cycle 5-like protein and two actin forms (cytoplasmic 1 and alpha cardiac muscle 1).

Table 1 Transcripts significantly and differentially expressed within the gills of anadromous and resident charr (at a FDR of 0.05)

Transcript	A/R ratios	Permutated *P* values	Corresponding *Q* values	Main category
Upregulated in anadromous charr				
40S ribosomal protein S29 (BU965653)	1.15	0.000131	0.026	Met
40S ribosomal protein S15a (CA042050)	1.15	0.000140	0.026	Met
40S ribosomal protein S15a (CA053546)	1.12	0.000221	0.037	Met
40S ribosomal protein S5 (CB496891)	1.27	0.000316	0.043	Met
40S ribosomal protein S19 (CB505812)	1.15	0.000359	0.045	Met
60S ribosomal protein L8 (CB495575)	1.15	0.000422	0.048	Met
60S acidic ribosomal protein P2 (CB510301)	1.24	0.000508	0.049	Met
Annexin A2 (CA057408)	1.18	0.000039	0.013	Mul
C-type lectin 2 (CB506101)	1.71	0.000021	0.010	Imm
C-type lectin 2 (CB506151)	1.46	0.000027	0.010	Imm
Glyceraldehyde-3-phosphate dehydrogenase (BU965890)	1.52	0.000054	0.016	Met
Grancalcin (CK990820)	1.24	< 0.000001	< 0.001	Cel
HD domain-containing protein 3 (CB492794)	1.25	0.000475	0.048	-
Hemagglutinin/amebocyte aggregation factor precursor (CA044621)	1.67	0.000074	0.018	-
Myosin light polypeptide 6 (CA036755)	1.25	0.000240	0.039	Cel
NADH dehydrogenase [ubiquinone] flavoprotein 3, mitochondrial precursor (CB497043)	1.58	0.000012	0.009	Met
Nascent polypeptide-associated complex subunit alpha (CA057781)	1.24	0.000385	0.047	Cel
Unknown (CK990488)	1.64	< 0.000001	< 0.001	-
Unknown (CB501598)	1.40	0.000016	0.010	-
Unknown (CA043183)	1.34	0.000022	0.010	-
Unknown (CK991328)	1.09	0.000072	0.018	-
Unknown (CB507979)	1.21	0.000278	0.041	-
Unknown (CA037319)	1.25	0.000285	0.041	-
Unknown (CB500659)	1.25	0.000001	0.002	-
Thioredoxin (CB498297)	1.25	0.000507	0.049	Cel
Transketolase (CB498658)	1.34	0.000214	0.037	Gro, Met
Downregulated in anadromous charr				
40S ribosomal protein S20 (CA043722)	0.85	0.000114	0.024	Met
Actin, alpha cardiac muscle 1 (CB496584)	0.72	0.000448	0.048	Cel
Actin, cytoplasmic 1 (CB514461)	0.82	0.000470	0.048	Cel
ATP synthase a chain (CB493612)	0.81	0.000040	0.013	Met
Cathepsin B precursor (CB496605)	0.82	0.000071	0.018	Met
CDC5L, Cell division cycle 5-like protein (CB497475)	0.84	0.000089	0.020	Cel
Cytochrome c oxidase subunit 3 (CB501432)	0.75	0.000012	0.009	Met
Cytochrome c (CB510558)	0.87	0.000348	0.045	Met
Cytochrome c oxidase subunit 3 (CK991224)	0.80	0.000530	0.050	Met
Protein SET (CA041701)	0.86	0.000424	0.048	Met
Pyruvate kinase muscle isozyme (CA054312)	0.84	0.000288	0.041	Met
Unknown (CA052125)	0.81	0.000469	0.048	-

Ratios were calculated as mean transcription value in anadromous (A) fish / mean transcription value in resident (R) fish. *P* values were derived from 1000 permutations. The accession number of each gene is indicated in parentheses. Abbreviations: Cel: cellular process, Gro: growth, Imm: immune system process, Met: metabolic process, Mul: multicellular organismal process.

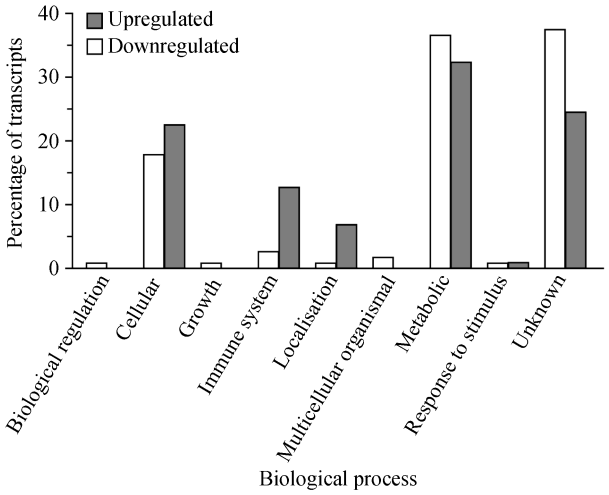

Fig. 1 Percentage of upregulated and downregulated transcripts (at a FDR of 0.10) in gills of anadromous charr relative to gills of resident fish by main biological processes

2.3 Clustering of individual fish and cDNA features

The hierarchical clustering analysis classified individuals by their ecotype with very strong support (100% bootstrap support for the node, top dendrogram, Fig. 2). Within ecotype, however, individuals of the same sex or family did not systematically cluster together. The clustering analysis defined four gene clusters with 100% bootstrap support: clusters 1a and 1b were composed of features upregulated in anadromous charr, whereas clusters 2a and 2b were composed of features downregulated in anadromous charr (left dendrogram, Fig. 2). Cluster 1a included genes encoding 60S ribosomal proteins, lectins, and proteins of the glucose pathways, whereas cluster 1b contained genes involved in protein metabolism (40S ribosomal proteins and nascent polypeptide-associated complex), two genes encoding binding proteins (e.g., grancalcin, annexin) and a gene encoding for a structural protein (a myosin subunit). Cluster 2a included genes with diverse functions whereas cluster 2b was dominated by mitochondrial genes (cytochrome c and ATP synthase subunits).

3 Discussion

In this study, we used cDNA micrarrays to test whether the transcriptome of anadromous and resident brook is differentiated during the period just prior anadromous fish would migrate to salt water. In doing so, our main goal was to identify general molecular processes and potential transcriptomic footprints of transcriptional differences between these ecotypes. Our results revealed that anadromous and resident brook charr originating from wild populations and reared in common environmental conditions strongly differ in their transcriptome in the period that shortly precedes the migration of the anadromous form to the estuary. This finding corroborates results obtained in anadromous and resident brown trout captured in the wild (Giger et al., 2008). Here, the transcriptomic differences were exclusively observed in gills, which are a pivotal organ of the osmoregulatory function and thus important for a successful transition to salt water (Høgåsen, 1998). Below, we discuss the factors that can contribute to these transcriptomic differences.

3.1 Divergent life history tactics

Although causal phenotypic effects cannot be rigorously associated with the transcriptional differences we observed, some of these differences could nevertheless play a role in adaptation associated with alternative resident and anadromous life history tactics. This could include various functions including innate immunity, metabolism, and development. For example, in gills of anadromous charr, there was an upregulation of three genes involved in innate immunity: C-type lectin (Arason, 1996), hemagglutinin/amebocyte aggregation factor (Iwagana and Kawabata, 1998), and grancalcin (Xu et al., 2006). In salmonids, the C-type lectin gene is upregulated in various tissues or immune cells infected by bacteria or parasites (Bayne et al., 2001; Tsoi et al., 2004; Rise et al., 2004a; Ewart et al., 2005; Martin et al., 2006; Morrison et al., 2006; Gerwick et al., 2007; Roberge et al., 2007; Young et al., 2008). It also plays a role in the acute phase response (Bayne et al., 2001; Martin et al., 2007). The hemagglutinin/amebocyte aggregation factor is highly expressed in salmonids infected by protists (Roberge et al., 2007) or bacteria (Rise et al., 2004a; Ewart et al., 2005; Martin et al., 2006; Gerwick et al., 2007). Finally, grancalcin possibly plays a role in murine host defense against bacterial infection (Liu et al., 2004) and is expressed in salmonid blood cells after heat stress (Lewis et al., 2010). As anadromous and resident fish looked healthy during daily observations and no infections were detected during the time course of sampling, it is unlikely that the upregulation of these genes was triggered by differential pathogenic infection. A more parsimonious explanation would be that this upregulation of elements of the innate immune function relates to the expression of anadromous life history tactic. The expression of genes such as C-type lectins could be increased in preparation to contact with new pathogens specific to saltwater habitats. For instance, Seear et al. (2010) showed in anadromous

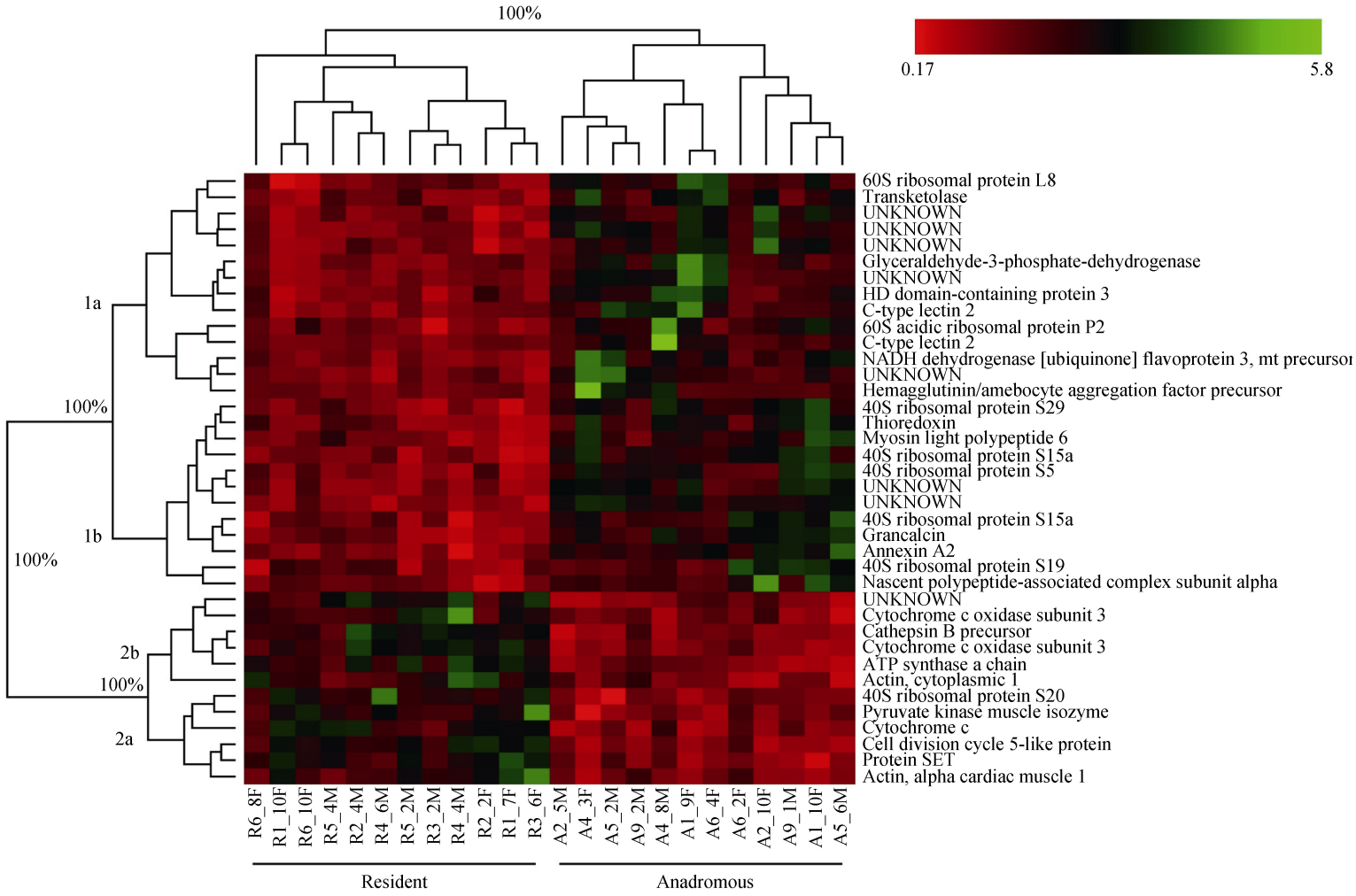

Fig. 2 Two dimensional hierarchical clustering of 38 features showing differential transcription profiles between brook charr ecotypes at a FDR level of 0.05

The trees were built using a matrix of Pearson correlation coefficients and an average linking method. The tree topology was bootstrapped 10,000 times to obtain statistical support for the nodes. The features shown in green are upregulated in anadromous brook charr whereas those in red are downregulated. The top dendrogram illustrates the global differences in transcription profiles between 12 anadromous (A) and 12 resident (R) charr (100% support). Families are identified by the number following the ecotype type (anadromous families: A1, A2, A5, A6 and A9; resident families: R1, R2, R3, R4, and R5), individuals are referred to by the number after the underscore sign and sex is identified by the letter "M" (males) and "F" (females). The left dendrogram regroups features with similar transcription profiles across samples: the clusters 1a, 1b, 2a, and 2b were all defined by nodes with 100% support.

Atlantic salmon that C-type lectin was more expressed in gills of smolt than in gills of parr. In addition, Evans et al. (2011) demonstrated that during the spawning migration of sockeye salmon *Oncorhynchus nerka*, C-type lectin is differently regulated between ocean and freshwater habitats. Clearly, the role of these immunity genes in fish migration deserves further investigation.

Other transcriptomic differences between resident and anadromous brook charr involve various metabolic functions, including oxidative, protein and carbohydrate metabolism. As previously reported in gills of Atlantic salmon smolts (Seear et al., 2010), the precursor gene of NADH dehydrogenase flavoprotein 3 was upregulated in anadromous brook charr. This flavoprotein is an accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (hereafter, Complex I). Seear et al. (2010) also reported that the expression of two subunits of the Complex I (NADH dehydrogenase subunits 1 and 4) was higher in smolt than in parr. In the present study however, the expression of the gene cytochrome c oxidase subunit 3 was downregulated in anadromous brook charr (Table 1). In contrast, Seear et al. (2010) reported an upregulation in two types of cytochrome genes (cytochrome b, cytochrome c oxidase polypeptide Via) in smolt. Although this must be more rigorously tested, these results suggest that metabolic strategies for adapting to freshwater-saltwater transition comprise both elements of similarities and differences among species.

Other transcriptomic differences between resident and anadromous charr could be involved in differential structural development in gill tissues and be related to the expression of these different life history tactics. Gills adapted to salt water possess cellular and structural adaptations to facilitate osmoregulatory function in high salinity environments (McCormick, 2001), implying that some pathways must be activated to initiate and complete the remodeling of the gills. In Atlantic salmon, Seear et al. (2010) observed differences in the expression of structural elements between smolt and parr and they suggested that the gills of smolt were undergoing a reorganization associated with the smoltification process. Here, the gill transcriptome was enriched in genes involved in apoptotic processes, DNA degradation and angiogenesis. Of particular relevance to tissue reorganization, the gene annexin A2 was overexpressed in gills of anadromous charr. The protein is involved in cellular differentiation (Gilmore et al., 2004). This gene is expressed in gills of fish that have migratory forms (rice field eel *Monopterus albus*, Shang et al., 2007; rainbow trout, Martin et al., 2007) and is up-regulated in testes and ovaries of maturing rainbow trouts (Von Schalburg et al., 2008).

3.2 Genetic background and selection effects

Differences in the genetic background – independent of the divergent life history tactics – may also contribute to the transcriptomic differences observed between laboratory-reared anadromous and resident brook charr. Indeed, the wild populations showed significant divergence at both neutral markers and quantitative genetic traits (Boula et al., 2002; Perry et al., 2005) and differed in the number of generations since introduction to the Station aquicole de l'UQAR-ISMER. Yet, the much more pronounced difference of gene expression in the gills relative to muscle tissue is a strong indication that such expression differences relate to divergent life-history tactics as opposed to mere genetic background effects. Indeed, differences primarily driven by genetic background should have resulted in similar expression differences in both tissues. Moreover, in a previous study comparing the same anadromous and resident brook charr populations and applying the same microarray methodology, but at the much younger fish-feeding stage, Mavarez et al., (2009) reported, that only 14 transcripts were differentially expressed between ecotypes, whereas we found 38 significant transcripts for a single tissue. Only three significant transcripts were common to both studies (NADH dehydrogenase flavoprotein 3 mitochondrial precursor, annexin A2, and pyruvate kinase muscle isozyme). None of these transcripts were regulated in the same fashion in the two studies. The anadromous and resident charr then appear more distinct at the stage corresponding to the first spring migration to salt water (Fig. 2) than at the first feeding stage despite the fact that many more tissues were included in the analysis of the later. While this comparison does not entirely rule out the effect of genetic background on the differential pattern of gill transcription profiles, it indicates that as they develop, resident and anadromous brook charr increasingly express differences in a tissue specific manner (pronounced in gills and absent in muscle) that may relate to the expression of divergent life history tactics.

Domestication of fish through selective breeding can evolve over a few generations and influence gene transcription profiles, as reported for Atlantic salmon (Roberge et al., 2006), coho salmon *O. kisutch* (Devlin et al., 2009), rainbow trout (Tymchuk et al., 2009) and brook charr (Sauvage et al., 2010). As anadromous charr were from the third generation produced at

UQAR-ISMER and the resident, from the first generation, one may suggest that the difference in the number of generations – or domestication – may contribute to the transcriptomic differences between anadromous and resident charr. Although the potential contribution of this factor cannot be excluded, it was probably minimized via common laboratory rearing conditions. Moreover, the anadromous group used in the present study corresponds to the "control line" that was compared to a "selected line for rapid growth" in the transcriptome study done by Sauvage et al. (2010). Of relevance to this issue, Tymchuk et al. (2009) examined the transcriptomic differences between juvenile rainbow trout from "fast-growing domestic" and "slow-growing wild" strains. They reported that the expression differences were much more prevalent in white muscle (398 significant features) than in liver (269 significant features) or brain (201 significant features), suggesting that during the domestication process for rapid growth, white muscle tissues appears to be under more intense selection than some other tissues. In contrast, anadromous and resident brook showed very minor differences in their muscle transcriptome (2 features at a FDR of 0.10; no feature at FDR of 0.05).

3.3 Reliability of microarray results

The correlation between microarray and qRT-PCR is often considered to be high (Dallas et al., 2005). In previous studies using the 16K cGRASP microarray including qRT-PCR validation, the expression profiles of key genes identified by this array were generally confirmed via concordant significance or concordance in the direction of a non-significant trend. In some instances, concordance between approaches reached ≥ 90% (Baerwald et al., 2008; Roberge et al., 2008; Tymchuk et al., 2009; Evans et al., 2011; Xu et al., 2011) and the coefficient of correlation between data from the two approaches was ≥ 0.90 (Søfteland et al., 2011). However, it may happen that microarray results do not concur with qRT-PCR results and a number of studies reported discrepancies between approaches for specific genes (e.g., Von Shalburg et al., 2008: SPARC and procollagen C-proteinase enhancer; Richards et al., 2010: peroxisomal multifunctional enzyme type 2; Jeukens et al. 2009: parvalbumin; LeBlanc et al., 2010 with the 32K cGRASP array: tensin, hemoglobin subunit alpha; Sutherland et al., 2011 also with the 32K cGRASP array: titin). Factors that were invoked for explaining the discrepancies included cross-hybridisation, alternative splicing, or presence of paralogs (LeBlanc et al., 2010).

In the present study, the results highlighted major differences between muscle and gill responses and we were more interested by overall differences at many genes related to numerous functional groups than focusing on specific expression level for specific genes. In such a context, and given the previously demonstrated reliability of the Atlantic salmon microarray for studies of other salmonid species, qRT-PCR analysis of a few specific genes, as routinely performed as a standard validation step, would not be very relevant and informative here. Alternatively, proteomic approaches such as 2D-DIGE/MALDI/-TOF/TOF (Søfteland et al., 2011) and LC MS/MS (Easy and Ross, 2009) have begun to be applied to the study of salmonids (namely Atlantic salmon) and this could be seen as a more useful step in future studies of resident and anadromous fish from the Laval River.

To conclude, brook charr has been described as a salmonid that has some of the interrelated traits that characterize anadromy (Rounsefell, 1958; Curry et al., 2010). Here, parapatric anadromous and resident charr exhibited very distinct transcriptomic differences during the preparatory period preceding transition to salt water. Differences were associated with only one of the two tissues analyzed and were related to many biological functions. Also, anadromous charr express at least a partial set of the molecular processes exhibited by other migratory salmonids, such as the genus *Salmo*. Additional studies on other taxa are required to circumscribe the spectrum of molecular adaptations underpinning the expression of anadromy in salmonids. In particular, it would be pertinent to perform a comparative study of various salmonids genera known to represent different levels of physiological capacity for anadromy.

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