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The transcriptomics of life-history trade-offs in whitefish species pairs (*Coregonus* sp.)

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Abstract

Despite the progress achieved in elucidating the ecological mechanisms of adaptive radiation, there has been little focus on documenting the extent of adaptive differentiation in physiological functions during this process. Moreover, a thorough understanding of the genomic basis underlying phenotypic adaptive divergence is still in its infancy. One important evolutionary process for which causal genetic mechanisms are largely unknown pertains to life-history trade-offs. We analysed patterns of gene transcription in liver tissue of sympatric dwarf and normal whitefish from two natural lakes, as well as from populations reared in controlled environments, using a 16 006-gene cDNA microarray in order to: (i) document the extent of physiological adaptive divergence between sympatric dwarf and normal species pairs, and (ii) explore the molecular mechanisms of differential life history trade-offs between growth and survival potentially involved in their adaptive divergence. In the two natural lakes, 6.45% of significantly transcribed genes showed regulation either in parallel fashion (2.39%) or in different directions (4.06%). Among genes showing parallelism in regulation patterns, we observed a higher proportion of over-expressed genes in dwarf relative to normal whitefish (70.6%). Patterns observed in controlled conditions were also generally congruent with those observed in natural populations. Dwarf whitefish consistently showed significant over-expression of genes potentially associated with survival through enhanced activity (energy metabolism, iron homeostasis, lipid metabolism, detoxification), whereas more genes associated with growth (protein synthesis, cell cycle, cell growth) were generally down-regulated in dwarf relative to normal whitefish. Overall, parallelism in patterns of gene transcription, as well as patterns of interindividual variation across controlled and natural environments, provide strong indirect evidence for the role of selection in the evolution of differential regulation of genes involving a vast array of potentially adaptive physiological processes between dwarf and normal whitefish. Our results also provide a first mechanistic, genomic basis for the observed trade-off in life-history traits distinguishing dwarf and normal whitefish species pairs, wherein enhanced survival via more active swimming, necessary for increased foraging and predator avoidance, engages energetic costs that translate into slower growth rate and reduced fecundity in dwarf relative to normal whitefish.

Keywords: adaptive radiation, fish, functional genomics, life history trade-offs, microarray, parallel evolution, speciation

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Introduction

Unravelling processes that underlie population divergence and speciation is a crucial step towards elucidating the origin and maintenance of biodiversity (Howard & Berlocher 1998; Coyne & Orr 2004). Over the last decades, much progress has been achieved in elucidating the ecological mechanisms responsible for phenotypic divergence, particularly in the context of an adaptive radiation (Schluter 2000). In contrast, there has been less focus on several issues that are also relevant to a full understanding of how divergent populations adapt to different environments under the effect of natural selection, and ultimately evolve

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into biological species. First, the vast majority of studies on adaptive radiation have focused on divergence in 'external phenotypes', putting most emphasis on adaptations in morphological (see Grant & Grant 2006; Gray & McKinnon 2006) and life-history traits (reviewed in Schluter 2000; but see also Roff 2002). Comparatively, there has been little focus on elucidating the extent of adaptive differentiation in physiological functions during the process of population adaptation and divergence (but see Whitehead & Crawford 2005; Schulte 2007). Yet, the paramount importance of physiological adaptations in coping with different environments has been amply documented by comparative physiologists (e.g. see Willmer *et al*. 2000), albeit very seldom in the context of adaptive radiation per se. This was recently pointed out by Hoekstra & Coyne (2007): '... it must be the case that many major evolutionary innovations and transitions involved changes that were not reflected in body forms'. Second, a thorough understanding of the genomic basis underlying phenotypic adaptive divergence is still in its infancy (MacCallum & Hill 2006; Mekel-Bobrov & Lahn 2006) although remarkable progress is being made in a few organisms, such as the threespine stickleback *Gasterosteus aculeatus* (Colosimo *et al*. 2005; Cresko *et al*. 2007), cichlid fishes (Terai *et al*. 2006), Darwin's finches (Schneider 2006), fruit flies (Matzkin *et al*. 2006; Laayouni *et al*. 2007), sunflowers (Edelist *et al*. 2006), monkeyflower (Streisfeld & Kohn 2005) and forest trees (Gonzàlez-Martinez *et al*. 2006).

One important evolutionary process for which causal genetic mechanisms is largely unknown pertains to life-history trade-offs, defined as negatively correlated responses to selection (Roff 2007). As expressed by Stearns & Magwene (2003), such trade-offs still represent '... black boxes located within theories that are much more explicit about mechanisms at the level of whole organisms that they are about mechanisms inside organisms'. Many species that originated from an adaptive radiation differ in life-history traits potentially involving trade-offs. In fish for instance, there are many cases of sympatric and parapatric occurrence of species pairs that strikingly differ in life-history traits. These generally involve a limnetic form, characterized by slower growth, shorter developmental time to reach maturity and shorter lifespan, and an alternative benthic form (Taylor 1999). In these species, fecundity increase with body size is achieved through a longer period of growth. On the other hand, the probability of surviving to reproduction will decrease with increased time-to-maturity (Roff 1992). For instance, Rennie *et al*. (2005) demonstrated that higher activity costs, accounting for a major proportion of fish energy budgets, traded-off against observed growth rate in wild populations of yellow perch (*Perca flavescens*). Although not a species pair, a parallel can nonetheless be drawn between this study on perch and the divergence between limnetic-benthic species pairs, which apparently involves differential trade-offs between fecundity and survival mediated through body size and developmental time. In other species, however, different trade-offs may be in question, and these processes might involve different physiological, behavioural, morphological or ecological factors (Roff 2007).

It has been proposed for more than 30 years that changes in gene regulation may play a crucial role in driving rapid evolutionary changes under the effect of selection (Britten & Davidson 1969; King & Wilson 1975; Schulte 2001; Saetre *et al*. 2004). This hypothesis was first supported by the pioneering work of Powers and colleagues on the physiological adaptation of *Fundulus heteroclitus* to different thermal environments (reviewed in Powers & Schulte 1998). Recently, Stearns & Magwene (2003) proposed that in a genomic context, 'trade-offs could be perceived as antagonistic pleiotropy representing conflicts between whole-organism function over the whole-genome patterns of gene expression which can be described by considering gene expression pattern in response to two physiological challenges' (e.g. growth vs. survival). Thus, studies that examine how quantitative variation in gene expression relates to phenotypic and quantitative variation in life-history traits would be of considerable interest (Roff 2007).

The development of microarray technologies, allowing the simultaneous detection of expression modulations at thousands of genes offers a powerful means of assessing the importance of evolutionary change in gene regulation involved in population divergence and adaptation (e.g. Olesiak *et al*. 2002, 2005; Bochdanovits *et al*. 2003; Singh 2003; Brodsky *et al*. 2005; Ranz & Machado 2005; Franchini & Egli 2006; Gilad & Borevitz 2006; Gonzàlez-Martinez *et al*. 2006; Matzkin *et al*. 2006; Street *et al*. 2006). Not only can gene transcription be considered a phenotypic trait (Gibson 2002; Gracey & Cossins 2003), but microarray studies also represent a unique way of documenting evolutionary change at specific genes occupying distinct molecular functions, relative to biological processes involved in morphological as well as in physiological adaptations to natural environments (Ouborg & Vriezen 2007). Furthermore, surveying simultaneous transcriptomic activity of thousands of genes offers the opportunity to conduct cluster analyses on their expression profiles which in turn allows testing for functional groupings of gene transcripts, thus giving new insights on the architecture of regulation pathways underlying phenotypic divergence (Eisen *et al*. 1998). This has been little explored in the specific context of an adaptive radiation. Moreover, microarray technology potentially represents a powerful means for exploring the molecular mechanisms of trade-offs and searching for genes that affect life-history traits in opposite ways (antagonistic pleiotropy). This has been investigated in a few systems to date (Oakeshott *et al*. 2003; Bochdanovits & de Jong 2004), although not in vertebrates to our knowledge.

The lake whitefish species complex (*Coregonus* sp., Salmonidae) has contributed in many ways to the understanding of the genetic basis of evolutionary change in the course of an adaptive radiation (Bernatchez 2004). Following ice-cap retreat after the Wisconsin glaciations 15 000 years ago, recurrent and independent sympatric divergence of two reproductively isolated whitefish phenotypic forms occurred in many lakes, so-called 'normal' and 'dwarf' ecotypes, adapted to occupying the benthic and limnetic niches, respectively (Pigeon *et al*. 1997; Lu & Bernatchez 1999). Previous studies revealed that divergent natural selection led to the evolution of many phenotypic differences between them including morphological, behavioural, ecological and life-history traits that most likely represent adaptations towards exploiting distinct trophic resources (Fenderson 1964; Bernatchez *et al*. 1999; Lu & Bernatchez 1999; Rogers, Gagnon & Bernatchez 2002). Linkage mapping has been used to document the number and effects of quantitative trait loci (QTL) involved in controlling the expression of these adaptive traits (Rogers *et al*. 2007), and genome scans performed in natural populations provided evidence that directional selection is maintaining genetic divergence between sympatric dwarf and normal whitefish by restricting gene flow at these QTL (Rogers & Bernatchez 2005, 2007). Recently, microarray studies provided further evidence for the role of directional selection in maintaining divergence between these sympatric ecotypes at the transcription level, mainly at genes involved in swimming activity and energy metabolism in the white muscle tissue (Derome & Bernatchez 2006; Derome *et al*. 2006).

Several lines of evidence indicate that the divergence observed in whitefish species pairs might involve differential trade-offs between fecundity and survival. Dwarf whitefish mature as early as 1 year old, seldom exceed 20 cm in length and 100 g in weight and rarely live more than 5 years, whereas normal whitefish mature at an older age (greater than 3 years old), commonly exceed 40 cm and 1000 g, and can live up to 20 years (Fenderson 1964). Experimental work on swimming behaviour performed in a controlled environment showed that dwarf whitefish are more active swimmers (Rogers *et al*. 2002; Rogers & Bernatchez 2007). Moreover, higher metabolic rate (partly associated with the cost of swimming activity) and lower bioenergetic conversion efficiency (defined as growth rate/consumption rate ratio) are associated with slower growth and younger age at sexual maturity in dwarf whitefish (Trudel *et al*. 2001). These observations raise the hypothesis that the adaptive divergence and the evolution of distinct life-history strategies between dwarf and normal whitefish might involve differential trade-offs between fecundity and survival that is mediated through the higher energetic cost of occupying the limnetic relative to the benthic trophic niche. Thus, higher metabolic rate, more active swimming and reduced bioenergetic conversion efficiency may constrain available energy for growth and reproduction at older ages in dwarf whitefish.

In this study, we analysed patterns of gene transcription in liver tissue using cDNA microarrays in order to: (i) further document the extent of physiological adaptive divergence between dwarf and normal whitefish, and (ii) explore the molecular mechanisms of differential life-history trade-offs potentially involved in the adaptive divergence of the two species. Rise *et al*. (2006) reviewed the multiples functions of the liver in growth regulation in salmonids, suggesting that this organ likely plays a central role in a large array of physiological processes for which dwarf and normal whitefish are known to show heritable divergence, namely energy metabolism (affecting survival via swimming activity devoted to foraging and predator avoidance) and protein synthesis (affecting growth and fecundity), and likely constitutes a major target of directional selection driving adaptive divergence between them. Moreover, the higher food consumption rate of dwarf relative to normal whitefish (Trudel *et al*. 2001) should also impact genes involved in blood filtration and waste management functions of the liver (Tortora & Grabowski 1993). More specifically, we predicted that differential trade-offs involving growth (and correlated fecundity) vs. survival between dwarf and normal whitefish should translate into differential patterns of gene transcription, with dwarf whitefish predominantly showing over-expression of genes associated with energy metabolism and under-expression at genes involved in protein synthesis and other functions associated with growth in normal whitefish.

Materials and methods

Sample collection

Sympatric dwarf and normal whitefish were collected in both Cliff Lake (46°23′59″N, 69°15′11″W) and Indian Pond (46°15′27″N, 69°17′29″W), located in the Allagash basin (St John River drainage), Maine, USA. Fish were sampled using gill nets during the growth season in late June 2003. Nets were pulled every 30 min, ensuring the fish were still alive prior to tissue collection, thus avoiding RNA degradation. Ten adult individuals [mean fork length for dwarf = 23.1 cm SD = 6.15 cm (Cliff Lake); 22.4 cm SD = 2.1 cm (Indian Pond); normal = 35.4 cm SD = 4.9 cm (Cliff Lake); 33.0 cm SD = 5.8 cm (Indian Pond)] were randomly collected in each population. Fish were euthanized with 0.001% Eugenol solution (Sigma-Aldrich) immediately prior to tissue collection. Liver tissue samples were frozen immediately in liquid nitrogen, and stored at –80 °C. Fish used in this study are the same individuals as those used for the transcriptomics analysis of white muscle by Derome *et al*. (2006).

Dwarf and normal whitefish reared in a controlled environment were originally sampled in 1998 in Témiscouata

Lake and Aylmer Lake (Québec), respectively, and reared in captivity at the LARSA facilities (Laboratoire Régional des Sciences Aquatiques, Université Laval, Québec). Family crosses were made in 2001 to generate pure F_1 breeds. Both normal and dwarf lines were kept at all times under the same environmental conditions (water temperature, photoperiod, diet). In September 2004, 12 individuals of comparable developmental stage for each population were randomly selected, measured (mean fork length = dwarf: 22.9 cm SD = 1.8 cm; normal: 28.9 cm SD = 2.2 cm) then euthanized with a 0.001% Eugenol solution just prior to tissue extraction. Liver tissue samples were immediately frozen on dry-ice and stored at –80 °C.

Microarray experiments

Total RNA extracts were obtained from 48 fish, including eight normal and eight dwarf individuals from Cliff Lake, Indian Pond and the controlled environment according to the TRIzol Reagent extraction Protocol (Gibco BRL) as described in Roberge *et al*. (2006). RNA integrity was verified with a 2100 Bioanalyser (Agilent). A total of 10 μg of RNA were retrotranscribed to cDNA using the Superscript-II protocol (Invitrogen Life Technologies) and probed on 16 006 gene cDNA microarrays developed for the Atlantic salmon (*Salmo salar*) by the cGRASP (consortium for Genomic Research on All Salmon Project; Rise *et al*. 2004; von Schalburg *et al*. 2005) following the Array 50 kit protocol (Genisphere; adapted as described in Roberge *et al*. 2006 and Derome *et al*. 2006). Efficiency of hybridization on the cDNA array was measured for lake whitefish (see Rise *et al*. 2007) and is comparable in both normal and dwarf whitefish (Derome & Bernatchez 2006). Two samples (one dwarf and one normal) were differentially labelled by fluorescence (Cy3 and Alexa647) and probed simultaneously on each array. Dye swaps were performed to minimize biases inherent to the dyes uneven fluorescence intensity (Churchill 2002). Gene expression levels were quantified using a ScanArray Express scanner (Packard Bioscience) and the QUANTARRAY software (Perkin Elmer).

Statistical analysis

A total of 3842 genes had significant levels of transcription (fluorescence intensity higher than the mean of controls plus twice their standard deviation; Roberge *et al*. 2006) for both dwarf and normal whitefish in all three experimental groups and were considered for further analyses. This percentage of significantly expressed genes is comparable with previous studies conducted on whitefish with the salmon cDNA array (Derome & Bernatchez 2006; Derome *et al*. 2006) and is also consistent with the calculated efficiency of heterologous hybridization conducted on the salmon cDNA array with various salmonid species, including the lake whitefish (Rise *et al*. 2007). Transcription data were corrected for intensity-related bias using a regional R-LOWESS algorithm and analysed by ANOVA using the R/MAANOVA software package (Kerr *et al*. 2000) under a mixed-effect model (sample and dye: fixed, array: random) using a permutation-based *F*-test (F3, 1000 sample permutations) in order to detect significant differences (P value < 0.05) in level of gene transcription between dwarf and normal whitefish (Cui & Churchill 2003). For each experimental group, eight biological replicates were used to insure sufficient statistical strength in the estimation of biological variance, leaving 6 degrees of freedom (d.f. = 8 biological samples, 2 treatments). The number of degrees of freedom for adequate microarray experimental designs should be 5 or more according to Churchill (2002). Changes in transcription levels were calculated as a D/N ratio using un-logged R-LOWESS normalized transcription data, where the mean transcription in dwarf individuals (D) was divided by mean transcription in normal individuals (N) for each gene. Genes showing at least 5% differences in transcription levels (D/N < 0.95 or > 1.05) and with a P value < 0.05 were considered differentially expressed and were kept for subsequent analyses.

The amount of false discoveries was accounted for by comparing samples from independent experimental groups (two lakes and a controlled environment group) for transcripts sharing patterns of regulation between dwarf and normal whitefish. Patterns of gene regulation were of two types: (i) 'parallel genes' applies to genes with D/N ratios either < 0.95 or > 1.05 in all compared groups, that is, either between the two lakes (when comparing natural populations only, see Results) or between the two lakes and the controlled environment (when comparing natural populations with a control group); (ii) 'nondirectional genes' applies to significantly expressed genes but regulated in opposed directions between two lakes when comparing natural populations only (see Results), or regulated in parallel in two of the three groups and in the opposed direction in the third experimental group when comparing natural populations with a control group. The probability to find the same false positive gene is thus decreased with the increasing number of independent experimental settings (see Cui & Churchill 2003).

The SAS software (SAS Institute) was used to test for normality of distribution (Shapiro-Wilk) for variance in transcription levels, and for nonparametric tests on mean variance of gene transcription, in parallel vs. nondirectional genes. Additional statistical analyses regarding proportions of functional groups represented in the parallel gene samples were carried out using the R Commander software package (http://www.r-project.org, http://socserv.socsci.mcmaster.ca/jfox/Misc/Rcmdr/), where a chi-squared (χ^2) test (exact binomial with 1 d.f.) was used to estimate the probability

of over-representation for each functional group, given their respective representation among the 3842 significantly transcribed genes. To avoid bias caused by redundancy of several repeated ESTs, DNA sequences of multiple clones of the same genes were considered as one single gene for every repeated gene with significant difference in transcription levels. An exception was the (CB491826) glyceraldehydes-3-phosphate dehydrogenase clone that failed to align with the other 6 GADPH spots (see Table 2) using the BIOEDIT sequence alignment editor software (Hall 1999), and was considered to be a second independent gene in this case. Similarly, multiple clones of the same gene were considered as one single gene when estimating the probability of over-representation of up-regulated genes found in each whitefish species in the relevant functional groups (χ^2-test, exact binomial with 1 d.f.), to test the hypothesis of a genomic basis underlying the phenotypic trade-offs in life-history traits.

Cluster analysis

Normal and dwarf whitefish individuals were initially profiled using transcription data for genes showing parallel patterns of transcription in the two lakes, and subsequently in all three experimental groups. Cluster trees were obtained by using the average-linking method (Sokal & Michener 1958; also described in Eisen *et al*. 1998) with pairwise distances estimated from the Pearson correlation coefficient (Qu & Xu 2006) using the GENESIGHT software (BioDiscovery). Given that bias introduced by differential array fluorescence could potentially cause individuals paired on the same array to systematically cluster together, we used a conservative approach whereby unlogged R-LOWESS data were further normalized by dividing expression values from one individual by that of the other and vice-versa (ex: array #1: D1 = dwarf #1 expression values/normal #1 expression values; N1 = normal #1 expression values/dwarf #1 expression values; for each gene). In this way, gene expression values in the cluster trees do not represent absolute, but rather relative gene transcription levels. This does not affect consistency in terms of directionality of regulation, nor does it affect the meaning of the clustering of genes or samples together. Moreover, repeated clones of the same gene were considered independent and were all included in this analysis, as listed in Tables 2 and 3.

Determination of gene functional groups

Spots on the cDNA array correspond to genes derived from EST library annotations using databases from GenBank (described in Rise *et al*. 2004; updates available on the cGRASP webpage: http://woodstock.ceh.uvic.ca/estproj/index.cgi). Gene clones showing parallel patterns of regulation between the three experimental groups or between the two natural populations (Cliff Lake and Indian Pond) were classified into 12 functional groups using information provided by the cGRASP website (http://web.uvic.ca/cbr/grasp/array.html), the SwissProt/TrEMBL database (http://c.expasy.org/sprot/), the NCBI browser (http://www.ncbi.nlm.nih.gov/), the KEGG Pathway database (www.genome.jp/kegg/pathway.html), the SOURCE Database (http://genome-www5.stanford.edu:80/cgi-bin/source/sourceSearch), the EMBL Bioinformatic harvester (http://harvester.embl.de/) and completed with references from the literature. Functional groups (see Tables 2 and 3) each include various biological processes (functions as annotated by cGRASP are in parenthesis) and are defined as follows: (i) blood and transport (plasma retinol-binding, transport, antifreeze protein); (ii) cell cycle regulation (intracellular signalling cascade, protein amino acid phosphorylation, response to oxidative stress, regulation of GTPase activity, phosphoenolpyruvate-dependent sugar phosphotransferase system, nucleotide metabolism, apoptosis signalling); (iii) cell structure (cytoskeleton organization and biogenesis); (iv) detoxification pathways (detoxification of xenobiotic agents and metabolic by-products); (v) energy metabolism (electron transport, glycolysis, tricarboxylic acid cycle intermediate metabolism, tricarboxylic acid cycle, malate metabolism, nucleotide-sugar metabolism, digestion, proteolysis and peptidolysis, tyrosine metabolism, tyrosine catabolism, L-phenylalanine catabolism); (vi) immunity (endocytosis, transport, antigen-binding, pathogen recognition, T-cell protease); (vii) germ-line formation (transport, sexual reproduction, ovulation (*sensu* Mammalia), progesterone metabolism); (viii) iron homeostasis (iron ion homeostasis, iron ion transport, negative regulation of cell proliferation); (ix) lipid metabolism (transport, lipid transport, lipoprotein metabolism, regulation of cholesterol absorption, bile acid metabolism, lipid biosynthesis, steroid biosynthesis, prostaglandin metabolism, prostaglandin biosynthesis, regulation of circadian sleep/wake cycle); (x) muscle contraction (regulation of muscle contraction, muscle development, hydrogen transport); (xi) protein catabolism (proteolysis and peptidolysis, protein metabolism, protein modification, ubiquitin-dependent protein catabolism); and (xii) protein synthesis (protein biosynthesis, electron transport, protein folding, ribosome biogenesis).

Sequences from ESTs previously matched with unknown gene loci were submitted to the BLASTN and BLASTX browsers from NCBI. Significant results were returned for only one unidentified, parallel gene spot (CK 990291) which partly matched the beta actin mRNA sequence (≥ 80% homology) for three species (*Dicentrarchus labrax*, *Epinephelus coioides* and *Onchorhynchus mykiss*). Accordingly, this gene clone was included in the cell structure functional group in the two lakes comparison (Table 3).

Table 1 Total number of gene clones showing significant differences in level of transcription between sympatric dwarf (D) and normal (N) whitefish in each experimental group, as well as detailed number of gene clones with parallel (D/N > 1, up-regulated in dwarf; D/N < 1, up-regulated in normal) and nondirectional patterns of transcription in comparisons among experimental groups

		Genes shared between two groups				Three groups
Groups	Total	Regulation pattern	Cliff Lake	Indian Pond	Control	Cliff Lake and Indian Pond
Cliff Lake	793	D/N > 1	354	65	84	
		D/N < 1	439	27	53	
		Nondirectional	—	156	50	
Indian Pond	502	D/N > 1		244	64	
		D/N < 1		258	36	
		Nondirectional		—	61	
Control	540	D/N > 1			298	28
		D/N < 1			242	6
		Nondirectional			—	42

Results

Differences in gene transcription by group

The number of differences in gene transcription levels between dwarf and normal whitefish varied among lakes and the control group at LARSA, but was significantly higher than expected by chance alone in all cases (expected: 5%; χ^2-test P value < 2.2e^{-16}) (Table 1). Fish from Cliff Lake showed the highest number of significant differences (P value < 2.2e^{-16}), with 793 gene clones showing at least a 5% increase or decrease in transcription level in dwarf vs. normal whitefish, whereas fish from Indian Pond and the control group had 502 and 540 differentially expressed gene clones, respectively. Thus, the proportion of genes out of 3842 that were significantly expressed showing differences in transcription level between dwarf and normal whitefish for each experimental group was 21% for Cliff Lake, 13% for Indian Pond, and 14% or the control group.

Parallel patterns of gene regulation between dwarf and normal whitefish

Table 1 provides details regarding the number of genes showing parallel patterns of regulation between dwarf and normal whitefish. When comparing groups for shared genes under regulation, we expected by chance alone to find $0.21 \times 0.13 \times 0.14 = 0.0038$ (0.38%) of genes showing patterns of regulation between dwarf and normal whitefish in all three experimental groups (Cliff Lake, Indian Pond and control at LARSA). Here, 1.98% of significantly expressed genes (76 gene clones) showed patterns of regulation between dwarf and normal whitefish in all three groups, which was significantly higher than expected by chance alone (χ^2-test P value < 2.2e^{-16}). Of those 76 gene clones, 34 showed parallel patterns of regulation, with six being down regulated in dwarf compared to normal whitefish, with an average 23.50% decrease in gene transcription level (min: 12.5%; max: 47.0%), whereas 28 were up-regulated in dwarf compared to normal samples, with an average 39.70% increase in gene transcription level (range: 10.1–152.3%). The other 42 genes (1.09%) showed nondirectional patterns of regulation between dwarf and normal whitefish among all three experimental groups (Table 1).

When comparing whitefish from the two natural lakes only, we expected to find a proportion of $0.21 \times 0.13 = 0.027$ (2.7%) of all significantly expressed genes showing regulation in both groups. We found that 6.45% of gene clones (248) showed patterns of regulation between dwarf and normal whitefish in both lakes, which was also significantly higher than expected by chance alone (χ^2-test P value < 2.2e^{-16}). A total of 92 genes (2.39%) showed parallel patterns of transcription, which included the aforementioned 34 parallel directional plus 58 others. Of these 92 genes, 65 were up-regulated in dwarf compared to normal whitefish, with an average 29.52% increase of gene transcription level (range, 9.84–177%) and 27 were down-regulated in dwarf compared to normal whitefish samples, with an average 18.7% decrease in transcription level (range, 5.42–65.62%). The other 156 genes showed nondirectional patterns of regulation between dwarf and normal whitefish. The total number of genes with significant differences of transcription between dwarf and normal whitefish in each group and shared among experimental groups are illustrated in Fig. 1. Details regarding the directionality of regulation are provided in Table 1. When considering each experimental group separately, proportions of up- and down-regulated genes between dwarf and normal whitefish were not different from a 50/50 distribution (χ^2-test on proportions: P value = 0.3269), whereas for genes with parallel patterns of regulation, the proportion of up-regulated genes in dwarf whitefish was significantly higher than 50% in all comparisons (P value < 0.001).

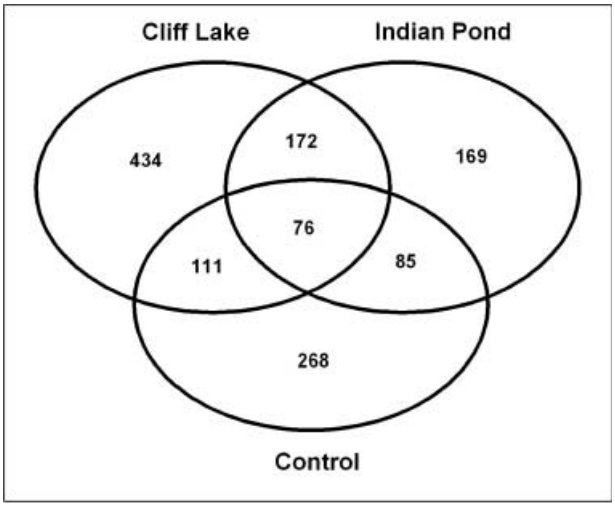

Fig. 1 Venn diagram showing the number of gene clones with significant differences in level of transcription between sympatric dwarf and normal whitefish in two natural lakes (Cliff Lake and Indian Pond) and controlled environment. Numbers are presented for each separate group, as well as for the number of genes shared between any of the two or all three groups. Details on directionality of transcription are presented in Table 1.

Figure 2 presents cluster trees obtained for 92 genes showing parallel patterns of regulation among the two natural lakes (2a), as well as for 34 genes showing parallel patterns of regulation between dwarf and normal whitefish among all three groups (2b). The cluster analysis performed on fish from the two natural lakes perfectly grouped dwarf and normal whitefish separately, whereas in the three group comparison, two major sample clusters stood out: one comprising a majority of normal individuals (91% normal; 20 normal and 2 dwarf whitefish), and the other being mostly composed of dwarf whitefish (84.6% dwarf; 22 dwarf and 4 normal samples). In contrast, cluster analysis performed on a data set including genes showing both parallel and nondirectional patterns of regulation between dwarf and normal whitefish were less discriminant, revealing four clusters with proportions of: 20% dwarf/80% normal, 25% dwarf/75% normal, 85% dwarf/15% normal, and 80% dwarf/20% normal (data not shown). Then, a cluster analysis performed using only nondirectional gene data from the three experimental groups failed to reveal any meaningful clustering (that is grouping either by form or origin), with three clusters in proportions varying between 45% dwarf/55% normal to 50%/50% (data not shown).

Functional groups associated with phenotypic divergence

Significant grouping patterns also appeared in both gene clusters, where a clear distinction emerged between genes that were up-regulated in dwarf from those that were up-regulated in normal whitefish (Fig. 2a, b). Moreover, different clones of the same genes tended to cluster together most of the time (e.g. brain protein 44-like protein clones, Fig. 2a; GAPDH clones, liver carboxylesterase 22 clones, malate dehydrogenase clones, anionic trypsin II clones, Fig. 2b). Genes with related functions also tended to cluster together, as illustrated by the five main gene clusters defined in Fig. 2(a). These consistent clustering patterns provide support for the reliability of the normalization method used to compare transcription profiles obtained from different experimental replicates together. Cluster 1 consisted of a group of 35 genes that were up-regulated in dwarf whitefish, 11 (31.4%) of which belonged to the energy metabolism group making it the best represented functional group in this cluster. Three out of four lipid metabolism genes also up-regulated in the dwarf whitefish grouped in cluster 2 as well as all three clones of malate dehydrogenase from the energy metabolism group. Cluster 3, 4 and 5 grouped genes up-regulated in the normal whitefish. Cluster 3 comprised nine gene clones, four of which pertained to protein synthesis and two to protein degradation functions. Cluster 4 contained seven genes from four different yet related functional groups (cell cycle, lipid metabolism, cell structure and energy metabolism; see Discussion for details); cluster 5 comprised six genes, three of which were classified in the immunity functions group, with another gene assigned to the blood and transport functional group (alpha-fetoprotein precursor) potentially involved in immune processes as well, by analogy of functions (see Discussion). Two additional small gene clusters included three proteases (elastase 2 and two anionic trypsin II gene clones) that were classified in the energy metabolism group, as well as three brain protein 44-like protein gene clones (cell cycle regulation) grouped with perforin and serum amyloid P-component precursor gene clones from the immunity functions group.

Table 2 provides full names and functional classifications of gene clones with parallel patterns of regulation among all three experimental groups, whereas Table 3 provides similar information for the remaining 58 gene clones showing parallel patterns of transcription in the two natural lakes only. A complete list of genes showing non-directional regulation is provided in Table S1, Supplementary material.

We compared the proportions of different functional groups represented in the data set, under the hypothesis that a significant fraction of genes under differential regulation should be functionally related to the phenotypic divergence observed between dwarf and normal whitefish. After regrouping repeated gene clones when required (see Materials and methods), all but one of the 12 functional groups of genes showing parallel patterns of transcription among the two natural lakes were over-represented in the

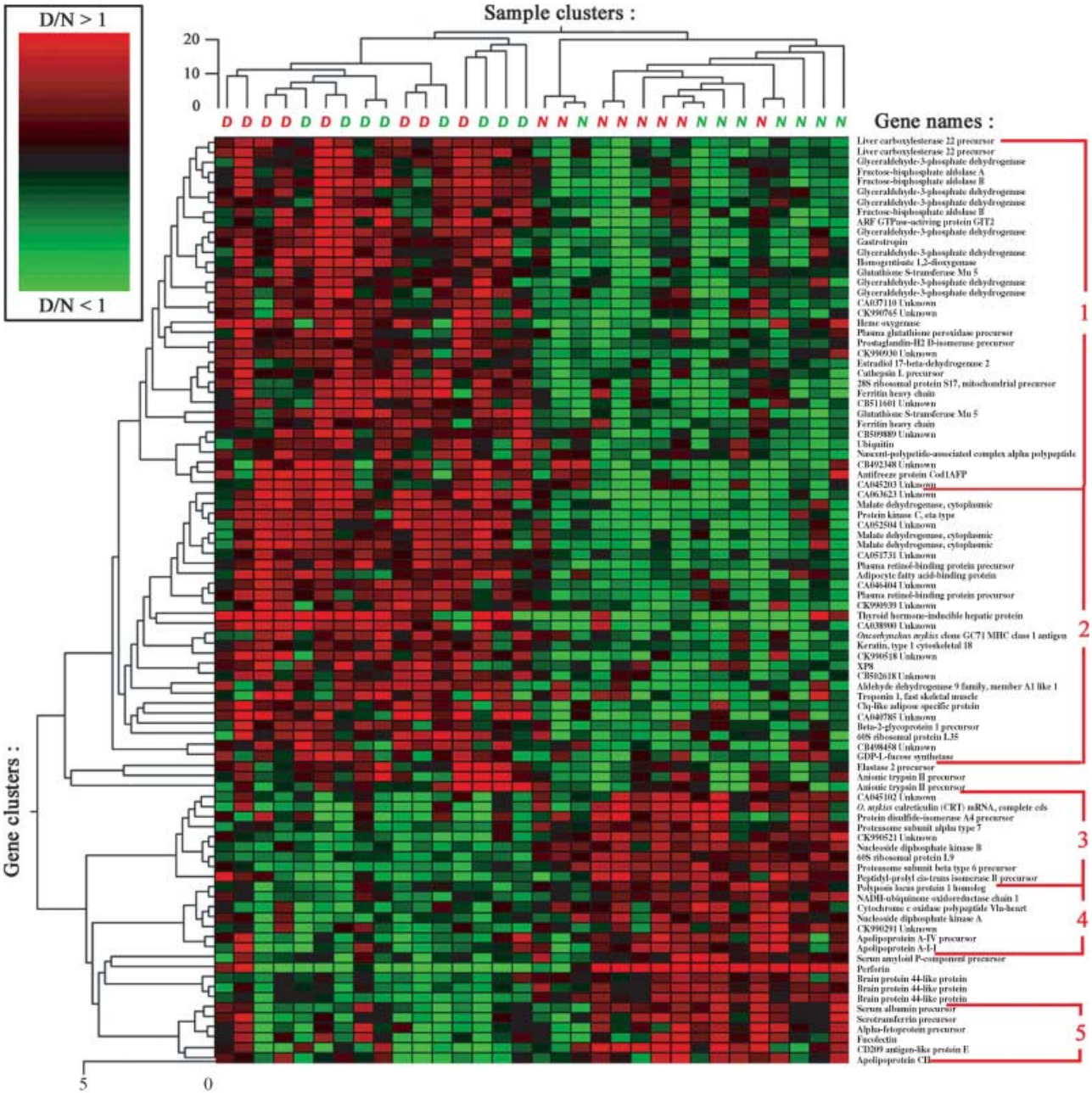

Fig. 2 (a) Cluster tree for 92 gene clones showing parallel patterns of regulation between sympatric dwarf and normal whitefish from the two natural lakes; (b) Cluster tree for 34 gene clones showing parallel patterns of transcription between sympatric dwarf and normal whitefish from two natural lakes and controlled conditions. Gene names are listed on the right of each tree. The dendrogram on top (Sample cluster) groups individuals based on similar patterns of transcription for genes with an average D/N value indicating parallel patterns of transcription in compared groups. Individuals are designated by the letters N and D for 'normal' and 'dwarf' whitefish (red, Cliff Lake; green, Indian Pond; grey, control). The dendrogram on the left (Gene cluster) groups genes with similar patterns of expression between individuals, where five broad functional clusters were defined (see Results). After normalization (see Material and methods), expression values shown in red (up-regulated in dwarf on average) are relatively higher in the experimental pairwise comparison and values shown in green (up-regulated in normal on average) are relatively lower in the experimental pairwise comparison. Hierarchical clustering analysis were performed using the average linking method (described in Eisen *et al*. 1998) with pairwise distances calculated by Pearson correlation coefficients (Qu & Xu 2006).

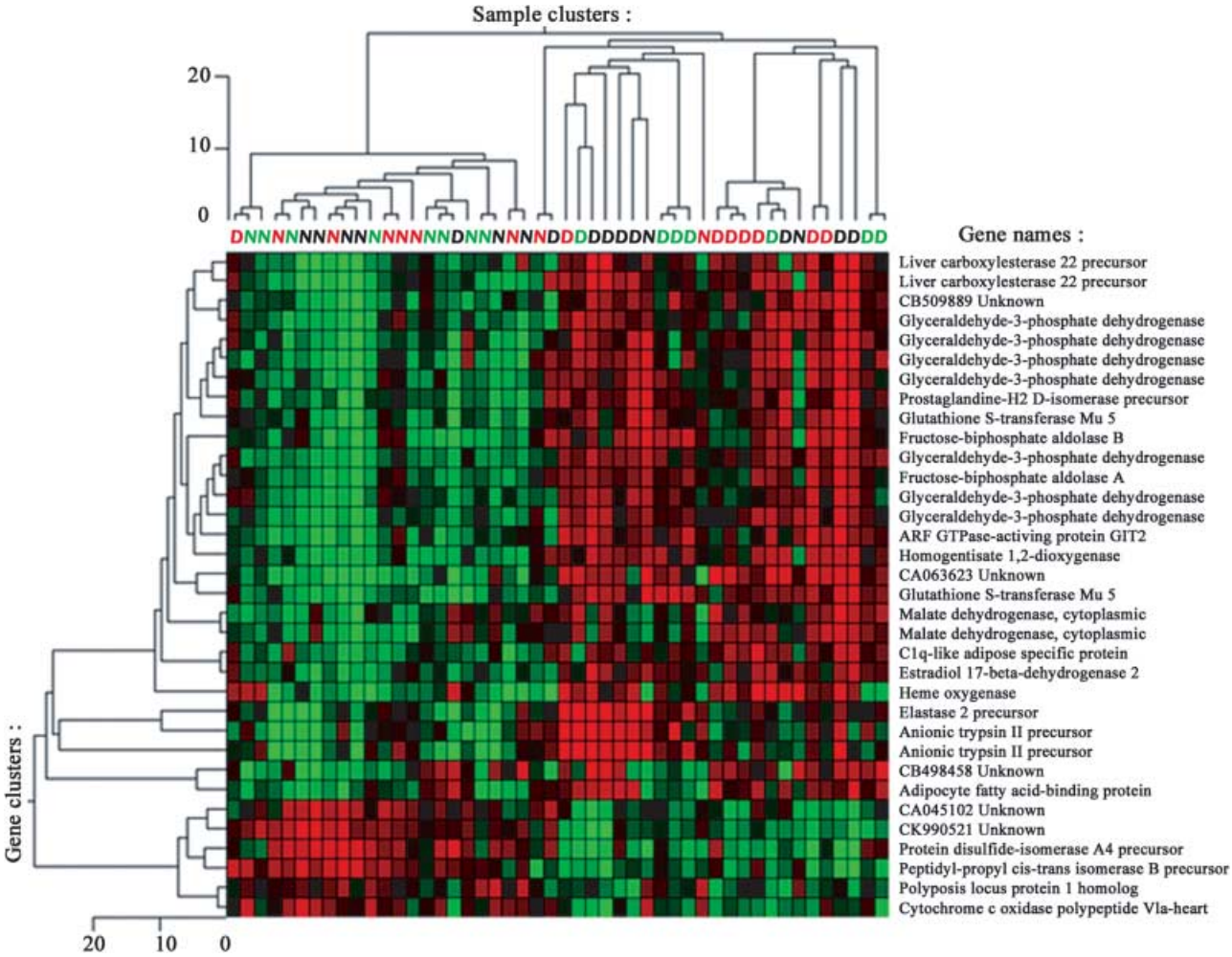

Figure 2 *Continued*

Table 2 Parallel changes in gene expression between dwarf and normal whitefish from two natural lakes and controlled environmental conditions

		Transcription ratio‡					
Functional group*	EST clone number and gene name†	Cliff Lake	Indian Pond	Control	*P*-CL§	*P*-IP§	*P*-ctl§
Cell cycle regulation	CB517934 [GO] [Q9JLQ2] ARF GTPase-activating protein GIT2	1.37	1.36	1.32	0.0038	0.0012	0.0149
	CB492176 [GO] [Q60870] polyposis locus protein 1 homologue	0.86	0.87	0.71	0.0220	0.0322	0.0146
Detoxification	CA057214 [GO] [Q64176] liver carboxylesterase 22 precursor	1.35	1.23	2.24	0.0019	0.0051	0.0001
	CB496876 [GO] [Q64176] liver carboxylesterase 22 precursor	1.33	1.17	2.16	0.0030	0.0174	0.0009
	CB496493 [GO] [P48774] glutathione S-transferase Mu 5	1.13	1.63	1.39	0.0258	0.0001	0.0415
	CB497579 [GO] [P48774] glutathione S-transferase Mu 5	1.10	1.16	1.73	0.0347	0.0182	0.0081

Table 2 *Continued*

Functional group*	EST clone number and gene name†	Transcription ratio‡					
		Cliff Lake	Indian Pond	Control	*P*-CL§	*P*-IP§	*P*-ctl§
Energy metabolism	CB516178 [GO] [P07146] anionic trypsin II precursor	1.55	2.52	2.25	0.0096	0.0000	0.0020
	CB515463 [GO] [P05208] elastase 2 precursor	1.23	2.24	1.56	0.0208	0.0001	0.0097
	CA045033 [GO] [P07146] anionic trypsin II precursor	1.24	2.14	1.45	0.0271	0.0001	0.0017
	CA062911 [GO] [P05064] fructose-bisphosphate aldolase A	1.21	1.18	1.28	0.0151	0.0085	0.0235
	CB502483 [GO] [Q91Y97] fructose-bisphosphate aldolase B	1.19	1.42	1.36	0.0094	0.0007	0.0205
	CB497681 [GO] [P16858] glyceraldehyde-3-phosphate dehydrogenase	1.13	1.46	1.71	0.0459	0.0027	0.0067
	CA768062 [GO] [P16858] glyceraldehyde-3-phosphate dehydrogenase	1.16	1.30	1.61	0.0080	0.0028	0.0019
	CB493574 [GO] [P16858] glyceraldehyde-3-phosphate dehydrogenase	1.23	1.18	1.47	0.0081	0.0101	0.0033
	BU965756 [GO] [P16858] glyceraldehyde-3-phosphate dehydrogenase	1.31	1.23	1.32	0.0076	0.0107	0.0128
	CB498361 [GO] [P16858] glyceraldehyde-3-phosphate dehydrogenase	1.23	1.28	1.33	0.0072	0.0032	0.0205
	CB514460 [GO] [P16858] glyceraldehyde-3-phosphate dehydrogenase	1.26	1.21	1.33	0.0073	0.0084	0.0223
	CB491826 [GO] [P16858] glyceraldehyde-3-phosphate dehydrogenase	1.18	1.11	1.39	0.0345	0.0421	0.0112
	CA055883 [GO] [O09173] homogentisate 1,2-dioxygenase	1.28	1.33	1.41	0.0051	0.0026	0.0044
	CB493498 [GO] [P14152] malate dehydrogenase, cytoplasmic	1.34	1.20	1.47	0.0013	0.0151	0.0422
	CB518115 [GO] [P14152] malate dehydrogenase, cytoplasmic	1.25	1.10	1.63	0.0074	0.0084	0.0195
	CA062141 [GO] [P43023] cytochrome *c* oxidase polypeptide VIa-heart	0.87	0.78	0.72	0.0339	0.0036	0.0100
Germ-line formation	CA062348 [GO] [P51658] estradiol 17-beta-dehydrogenase 2	1.12	1.20	1.28	0.0256	0.0146	0.0341
	CB496948 [GO] [O09114] prostaglandin-H2 D-isomerase precursor	1.20	1.13	1.71	0.0132	0.0348	0.0012
Immunity	CA037686 [NR] [AAM73701] C1q-like adipose specific protein	1.40	1.13	1.21	0.0011	0.0456	0.0270
Iron homeostasis	CB515893 [NR] [O73688] heme oxygenase	1.83	1.10	1.13	0.0001	0.0266	0.0103
Lipid metabolism	CK990220 [GO] [P04117] adipocyte Fatty acid-binding protein	1.28	1.11	1.70	0.0032	0.0186	0.0021
Protein synthesis	CA063352 [GO] [P24369] peptidyl-prolyl *cis*-trans isomerase B precursor	0.84	0.76	0.53	0.0244	0.0040	0.0030
	CA048973 [GO] [P08003] protein disulfide-isomerase A4 precursor	0.75	0.76	0.69	0.0036	0.0065	0.0042
Unknown	CA063623 Unknown	1.83	1.42	1.22	<0.0001	0.0005	0.0211
	CB509889 Unknown	1.23	1.24	1.83	0.0190	0.0056	0.0072
	CB498458 Unknown	1.28	1.20	1.60	0.0072	0.0180	0.0393
	CK990521 Unknown	0.79	0.75	0.66	0.0047	0.0024	0.0016
	CA045102 Unknown	0.80	0.83	0.79	0.0073	0.0124	0.0175

*Functional groups were defined as described in the Material and methods section.

†Each EST clone number represents a single EST sequence.

‡Transcription ratio corresponds to mean dwarf expression level divided by mean normal expression level (D/N).

§*P*-CL, *P*-IP and *P*-ctl correspond to permutated *P* values for Cliff Lake, Indian Pond and the control conditions, respectively, (ANOVA, *F*3 test, 1000 permutations).

Table 3 Parallel changes in gene transcription observed between dwarf and normal whitefish from two natural lakes only

Functional group*	Gene clone no. and name†	Transcription ratio‡			
		Cliff Lake	Indian Pond	*P*-CL§	*P*-IP§
Blood and transport	CB501248 [GO] [Q00724] plasma retinol-binding protein precursor	1.41	1.23	0.0015	0.0098
	CB509509 [GO] [Q00724] plasma retinol-binding protein precursor	1.34	1.12	0.0017	0.0429
	CB498410 [NR] [AAT95404] Cod1AFP antifreeze protein	1.34	1.15	0.0030	0.0162
	CB493984 [GO] [P02772] alpha-fetoprotein precursor	0.87	0.89	0.0211	0.0354
Cell cycle regulation	CA043387 [NR] [BAB33387] XP8	1.33	1.15	0.0022	0.0325
	CB510934 [GO] [P15532] nucleoside diphosphate kinase A	0.85	0.82	0.0254	0.0109
	CK991305 [GO] [Q01768] nucleoside diphosphate kinase B	0.84	0.83	0.0120	0.0098
	CA059018 [GO] [P63030] brain protein 44-like protein	0.83	0.78	0.0114	0.0035
	CB496707 [GO] [P63030] brain protein 44-like protein	0.84	0.87	0.0156	0.0255
	CA059976 [GO] [P63030] brain protein 44-like protein	0.88	0.88	0.0366	0.0324
Cell structure	CA052792 [GO] [P05784] keratin, type I cytoskeletal 18	1.15	1.13	0.0304	0.0319
	CK990291 unknown (possible beta actin)¶	0.87	0.87	0.0361	0.0165
Detoxification	CB494429 [NR] [AAH45932] aldehyde dehydrogenase 9 family, member A1 like 1	1.14	1.18	0.0245	0.0065
	CA052877 [GO] [P46412] plasma glutathione peroxidase precursor	1.19	1.15	0.0248	0.0203
Energy metabolism	CA062426 [GO] [Q91Y97] fructose-bisphosphate aldolase B	1.16	1.48	0.0040	0.0003
	CB497834 [GO] [P14152] malate dehydrogenase, cytoplasmic	1.33	1.27	0.0015	0.0041
	CB505763 [GO] [P23591] GDP-L-fucose synthetase	1.17	1.12	0.0355	0.0393
	CN442510 [GO] [P18929] NADH-ubiquinone oxidoreductase chain 1	0.87	0.90	0.0404	0.0326
Germ-line formation	CA061998 [GO] [P23298] protein kinase C, eta type	1.40	1.15	0.0009	0.0122
Immunity	CA040697 [NT] [AY071854] *Oncorhynchus mykiss* clone GC71 MHC class I antigen	1.18	1.26	0.0285	0.0050
	CB502941 [NR] [AAS89353] perforin	0.45	0.34	< 0.0001	< 0.0001
	CB496842 [GO] [Q91ZW7] CD209 antigen-like protein E	0.64	0.86	0.0001	0.0181
	CB497396 [GO] [P12246] serum amyloid P-component precursor	0.89	0.78	0.0460	0.0100
	CA037647 [GO] [P07724] serum albumin precursor	0.82	0.85	0.0299	0.0208
	CB504246 [NR] [AAU21486] fucolectin	0.86	0.89	0.0204	0.0410
Iron homeostasis	CA056696 [GO] [P09528] ferritin heavy chain	1.17	1.48	0.0277	0.0004
	CA768211 [GO] [P09528] ferritin heavy chain	1.11	1.16	0.0372	0.0227
	CA056544 [GO] [Q921I1] serotransferrin precursor	0.95	0.80	0.0381	0.0053
Lipid metabolism	CK990702 [GO] [Q62264] thyroid hormone-inducible hepatic protein	1.98	1.60	< 0.0001	< 0.0001
	CK990953 [GO] [P51162] gastrotropin	1.27	1.26	0.0061	0.0014
	CA038031 [GO] [Q01339] beta-2-glycoprotein I precursor	1.18	1.10	0.0241	0.0487
	CB497259 [NR] [AAB96972] apolipoprotein A-I-1	0.83	0.84	0.0135	0.0374

Table 3 *Continued*

Functional group*	Gene clone no. and name†	Transcription ratio‡			
		Cliff Lake	Indian Pond	*P*-CL§	*P*-IP§
	CB510585 [GO] [P06728] apolipoprotein A-IV precursor	0.86	0.84	0.0298	0.0259
	CB510956 [NR] [AAG11410] apolipoprotein CII	0.84	0.86	0.0280	0.0229
Muscle contraction	CB497373 [GO] [P13412] troponin I, fast skeletal muscle	1.12	1.25	0.0247	0.0031
Protein degradation	CK990562 [GO] [P06797] cathepsin L precursor	1.16	1.26	0.0204	0.0062
	CB510468 [GO] [P62991] ubiquitin	1.17	1.18	0.0423	0.0104
	CB511632 [GO] [Q60692] proteasome subunit beta type 6 precursor	0.78	0.80	0.0052	0.0052
	CA053701 [GO] [Q9Z2U0] proteasome subunit alpha type 7	0.89	0.85	0.0371	0.0160
Protein synthesis	CB504359 [GO] [Q9CQE3] 28S ribosomal protein S17	1.21	1.45	0.0124	0.0005
	CB500108 [NR] [AAM34649] 60S ribosomal protein L35	1.29	1.21	0.0022	0.0074
	CA050584 [NR] [AAQ97817] nascent-polypeptide-associated complex á-polypeptide	1.13	1.15	0.0417	0.0362
	CK991302 [NT] [AY372389] *Oncorhynchus mykiss* calreticulin	0.67	0.74	0.0005	0.0030
	CB496532 [GO] [P51410] 60S ribosomal protein L9	0.78	0.88	0.0032	0.0374
Unknown	CA040785 unknown	2.77	1.26	< 0.0001	0.0191
	CA038900 unknown	1.28	1.63	0.0013	0.0001
	CB492348 unknown	1.42	1.36	0.0030	0.0035
	CK990518 unknown	1.48	1.17	0.0006	0.0126
	CA052504 unknown	1.42	1.21	0.0007	0.0047
	CA045203 unknown	1.23	1.29	0.0126	0.0152
	CA046404 unknown	1.30	1.16	0.0044	0.0215
	CB511601 unknown	1.19	1.25	0.0224	0.0100
	CK990765 unknown	1.24	1.21	0.0110	0.0055
	CK990939 unknown	1.31	1.13	0.0028	0.0496
	CK990930 unknown	1.25	1.17	0.0049	0.0417
	CB502618 unknown	1.24	1.12	0.0071	0.0331
	CA037110 unknown	1.21	1.16	0.0471	0.0156
	CA051731 unknown	1.13	1.17	0.0374	0.0131

*Functional groups were defined as described in Material and methods.
†Each EST clone number represents a single EST sequence.
‡Transcription ratio corresponds to mean dwarf expression level divided by mean normal expression level (D/N).
§*P*-CL and *P*-IP correspond to permutated *P* values for Cliff Lake and Indian pond (ANOVA, F3 test, 1000 permutations).
¶BLASTN results partly match actin beta mRNA sequences for *D. labrax*, *E. coioides* and *O. mykiss*.

data set compared to proportions expected by chance (χ^2 *P* value < 0.05 except for the 'muscle contraction' functional group *P* value > 0.05; Table 4). When including comparison with the controlled environment condition, five out of eight functional groups present in nature and in the control group were also significantly over-represented in the data set compared to expectation by chance, based on their respective representation in the group of 3842 significantly expressed gene clones (Table 4).

Transcriptomic basis of trade-offs in life-history traits

A chi-squared test (χ^2) was conducted on the proportions of up-regulated genes to test if any of the different functional groups were significantly over-represented in the dwarf or normal whitefish, under the assumption that up-regulation of relevant transcripts would underlie the observed trade-offs in life-history traits between fecundity and survival via growth and activity (Table 5). Significant up-regulation

Table 4 Relative representation of functional groups for genes showing parallel patterns of regulation between sympatric dwarf and normal whitefish from two natural lakes (left), and from the two natural lakes plus the control group (right)

	Cliff Lake vs. Indian Pond		Cliff Lake vs. Indian Pond vs. control	
Functional groups*	Proportion (%)†	*P* value‡	Proportion (%)	*P* value
Blood and transport	3.9	0.03079	—	—
Cell cycle regulation	7.8	3.324e-05	8.0	0.0007494
Cell structure	2.6	0.03173	—	—
Detoxification	5.2	0.005477	8.0	0.00543
Energy metabolism	14.3	6.895e-05	36.0	8.764e-08
Germ-line formation	3.9	0.0007837	8.0	0.0009456
Immunity	9.1	0.002373	4.0	0.08622
Iron homeostasis	3.9	0.02045	4.0	0.1504
Lipid metabolism	9.1	1.699e-06	4.0	0.1778
Muscle contraction	1.3	0.1295	—	—
Protein catabolism	5.2	0.004873	—	—
Protein synthesis	9.1	0.02215	8.0	0.0143

*Functional groups are described in Material and methods.
†Proportion was calculated by dividing the number of genes in each functional group by the total number of nonredundant parallel genes × 100 (two lakes: $n = 77$; two lakes and control: $n = 25$).
‡*P* value from χ^2-test on proportions.

Table 5 Proportion of up-regulated genes in dwarf and normal whitefish in regards to their potential involvement in life history trade-offs observed between growth (increased fecundity) vs. survival. (a) Cliff Lake vs. Indian Pond; (b) Cliff Lake vs. Indian Pond vs. control group

	Dwarf		Normal	
	% of up-regulated genes	*P* value §	% of up-regulated genes	*P* value
(a) Functions potentially associated to survival				
Energy metabolism	81.8	0.03271	—	
Iron homeostasis	66.7	0.5	—	
Lipid metabolism	57.1	0.5	—	
Detoxification	100	< 0.0001	—	
All energy*	76.0	0.007317	—	
Germ-line formation†	100	< 0.0001	—	
Functions potentially associated to growth (increased fecundity)				
Cell cycle & cell growth	—		66.7	0.3438
Protein synthesis	—		57.1	0.5
All growth‡	—		61.5	0.2905
(b) Functions potentially associated to survival				
Energy metabolism	88.9	0.01953	—	
Iron homeostasis	100	< 0.0001	—	
Lipid metabolism	100	< 0.0001	—	
Detoxification	100	< 0.0001	—	
All energy	92.3	0.001709	—	
Germ-line formation	100	< 0.0001	—	
Functions potentially associated to growth (increased fecundity)				
Cell cycle and cell growth	—		50	0.75
Protein synthesis	—		100	< 0.0001
All growth	—		75	0.3125

*All energy includes the energy metabolism, iron homeostasis, lipid metabolism and detoxification functional groups taken together.
†This functional group does not relate to survival or growth but was differentially over-expressed as a whole in dwarf whitefish.
‡All growth includes the cell cycle/cell growth and protein synthesis functional groups taken together.
§*P* value from χ^2 test on proportions.

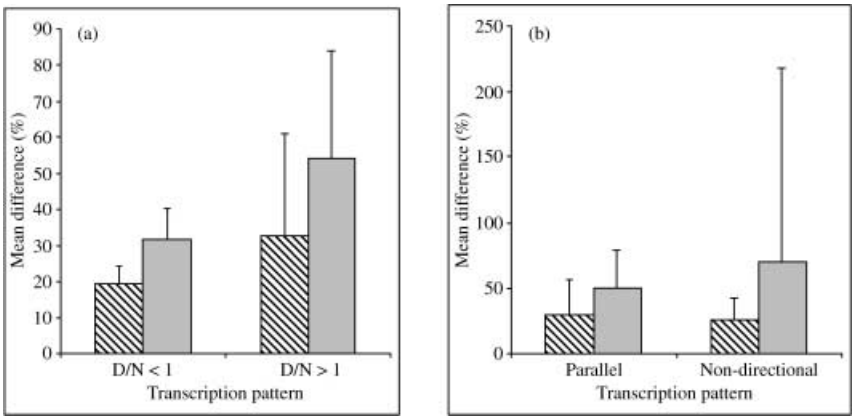

Fig. 3 (a) Mean differences (in percent) in transcription levels for gene clones showing parallel patterns of regulation (D/N > 1, up-regulation in dwarf; D/N < 1, up-regulated in normal) in whitefish from the two natural lakes (striped bars) and from the controlled environment (plain bars); (b) mean differences (%) in transcription levels for gene clones showing parallel (left) or nondirectional (right) patterns of regulation in fish from the two natural lakes (striped bars) or from the controlled environment (plain bars). Differences in expression were calculated using expression value in the normal ecotype as the reference; *t*-bars represent standard deviation for transcription values.

for a majority of genes involved in functions potentially associated with enhanced activity and survival, namely energy metabolism, lipid metabolism, iron homeostasis and detoxification, was observed in the dwarf whitefish, both in natural populations and all three groups comparisons (P value < 0.05; Table 5). Although less clear, a trend of up-regulation was observed for growth genes (protein synthesis, DNA synthesis, cell cycle) in the normal whitefish, but statistical significance was obtained solely for the protein synthesis group when comparing both natural populations and the control environment (P value < 0.0001; Table 5). Although not directly involved in growth-survival trade-off but possibly reflecting earlier sexual maturation in dwarf whitefish (see Discussion), up-regulation of genes involved in germ-line formation was also observed in dwarf whitefish (P value < 0.0001; Table 5), both in natural populations or when compared with the controlled conditions.

Differential variance in gene transcription between parallel and nondirectional genes

In natural populations, the variance in transcription for genes showing parallel patterns of regulation for both lakes (mean variance = 3.31) was significantly lower than the variance observed for nondirectional genes (mean variance = 5.78) (one-sided Wilcoxon test, P value = 0.0021). However, this reduced variance in parallel genes was not statistically significant when including genes from the control group. When compared with fish held in controlled conditions, variance in transcription levels was significantly lower in fish from the two lakes than in fish from the controlled environment for every pattern of regulation (up-regulated in dwarf: mean = 4.27 in lakes vs. 10.17 in control P value = 0.0028; up-regulated in normal: 0.55 in lakes vs. 2.10 in control P value = 0.0339; parallel (up and down-regulated): 3.62 in lakes vs. 8.75 in control P value = 0.0012; nondirectional: 2.10 in lakes vs. 13.07 in control P value < 0.0001; differently expressed in lakes mean = 2.79 vs. differently expressed in control mean = 11.11 P value < 0.0001; one-sided Wilcoxon test). Moreover, in genes showing parallel differences, the mean increase (D/N;%) or decrease (1–D/N;%) in transcription levels in dwarf individuals was significantly lower in natural populations than in the controlled environment, with an average decrease of 19.5% for down-regulated genes in Cliff Lake and Indian Pond vs. 31.6% in the control (P value = 0.0083, one-tailed *t*-test), and an average increase of 32.6% for up-regulated genes in Cliff Lake and Indian Pond vs. 54% in the control (P val = 0.0014, one-tailed *t*-test) (Fig. 3a). The more pronounced differences in level of transcription between dwarf and normal whitefish in the controlled environment relative to those from natural lakes held true for both parallel genes (up-regulated and down-regulated taken together) as well as for nondirectional genes (Fig. 3b).

Discussion

Patterns of gene regulation and parallel evolution of dwarf and normal whitefish

The first objective of this study was to investigate patterns of gene transcription in liver tissue using cDNA microarrays in order to further document the extent of physiological adaptive divergence between dwarf and normal whitefish. To this end, this study adds to that of Derome *et al.* (2006)

performed on white muscle of the same individuals in several ways. First, this study was based on the analysis of gene transcription by means of a 16 006 gene cDNA microarray (von Schalburg *et al*. 2005), which allowed coverage of a much broader set of genes and functions relative to the 3557 gene cDNA array that was available for the previous study. Of the 16 006 EST sequences printed on the array, a total of 3842 had significant levels of transcription in both dwarf and normal whitefish samples. Such a seemingly low number of significantly transcribed genes is expected, since this experiment consists in heterologous hybridization (see Renn *et al*. 2004) and since the chip includes transcripts derived from various tissues and developmental stages whereas we analysed one tissue at one life history stage only (von Schalburg *et al*. 2005).

Second, the use of the same individuals studied by Derome *et al*. (2006) allowed a rigorous comparison of the extent of parallelism in gene transcription of different tissues between dwarf and normal whitefish, and as such, to evaluate the possible role of directional selection in driving the evolution of transcription level of genes expressed in both tissues. The set of genes that we identified here, and for which transcription profiles are likely to have evolved under directional selection, adds to those identified in our previous study on white muscle tissue performed on dwarf and normal whitefish from the same natural populations (Derome *et al*. 2006). Overall, both studies yielded very comparable results, although the proportion of differentially expressed genes between dwarf and normal whitefish was higher in liver relative to the white muscle tissue. This corroborates the fact that the fractional rate of protein synthesis in the liver is 40 times that measured in white muscle (Mommsen 2001). When considering only the two natural lakes in this study, 6.45% of all significantly transcribed genes showed differences in transcription levels in the liver (248 gene clones), of which 2.39% showed parallel patterns of regulation (92 gene clones). For white muscle, and under the same statistical criteria, 4.3% (51 gene clones) of all significantly transcribed genes showed differences in level of transcription, with 1.35% (16 gene clones) showing parallel regulation between sympatric whitefish ecotypes in both lakes (Derome *et al*. 2006). Also, when considering parallel genes in both studies, we observed a higher proportion of over-expressed genes in dwarf relative to normal whitefish (70.6% of 92 genes) similar to what was observed for white muscle (62.3% of 16 genes). Moreover, the level of fold change for parallel genes between dwarf and normal whitefish was comparable for both tissues; we observed an average 29.5% increase of transcription level (range: 9.8–177%) for over-expressed genes in liver of dwarf whitefish, whereas Derome *et al*. (2006) reported an average 47% increase of transcription level (range 10–235%) for over-expressed genes in white muscle of dwarf whitefish. In both tissues, the level of expression in up-regulated genes in normal whitefish was lower than that observed in up-regulated genes in dwarf whitefish, being 18.7% for liver and 17% in white muscle.

Third, this study added the analysis of dwarf and normal whitefish maintained in identical, controlled environmental conditions. Although interindividual and interpopulational variation in gene expression has been reported to be heritable in fish (Whitehead & Crawford 2006; Roberge *et al*. 2007), differential regulation of genes is also certainly modulated by environmental variance, that may in turn influence phenotypic variation in response to different environmental conditions (Bochdanovits *et al*. 2003; West-Eberhart 2005; Alvarez & Nicieza 2006; Cossins *et al*. 2006). To our knowledge, however, there is no other published comparison of gene transcription analysed by microarrays between populations from both natural and controlled environments. Here, such comparison provided strong support to our interpretation regarding the predominant role of a genetic rather than environmental control on differential patterns of transcription observed at numerous genes between dwarf and normal whitefish in their natural environments. Thus, 76 gene clones (among which 34 were regulated in parallel) representing the same functional groups were differentially transcribed between dwarf and normal whitefish reared in controlled environment and sampled from natural lakes. This number of shared genes was much higher than expected by chance alone. Admittedly, there were also a number of genes that did not show parallelism in transcription between controlled and natural populations. However, this was anticipated since the number of genes remaining differentially expressed, in parallel or in a nondirectional fashion across different environments is expected to decrease with the number of groups being compared. Also, dwarf and normal whitefish reared in controlled conditions originated from different populations than those from the natural lakes we studied. Consequently, it was expected that these would also show population-specific transcription profiles at some genes (Derome *et al*. 2006). Finally, fish maintained in controlled conditions are most certainly exposed to different selective constraints than those found in natural environments. Despite all these differences, the fact that we detected a high number of genes under parallel regulation in fish from both natural and controlled environments provides very strong support for a predominant genetic control for the differential patterns of transcription shown by these genes between dwarf and normal whitefish. These results also provide strong indirect evidence for the role of directional selection in shaping these patterns in nature, since the independent evolution of divergent phenotypes among closely related lineages, as evidenced in previous studies of lake whitefish (e.g. Pigeon *et al*. 1997; Lu *et al*. 2001; Bernatchez 2004), is unlikely to be random, and is generally interpreted as being the consequence of natural selection (Schluter 2000).

Further evidence for the role of selection in shaping differential patterns of transcription comes from the comparison of variance between parallel and nondirectional genes. Stabilizing selection within population should exert a constraining action on the phenotypic response, which in the present case, is predicted to translate into reduced interindividual variance of transcription for genes that are most likely to be under strong selective constraints (Fisher 1930). Here, we observed that the interindividual variance of transcription for parallel genes was almost half that measured for nondirectional genes (see Results). This suggests that genes with parallel transcription profiles experience stronger selective pressure than nondirectional ones. Thus, our results suggest both stabilizing selection within population and directional selection between populations are acting synergistically, therefore enhancing differential patterns of transcription across species pairs, while reducing transcription variance within each population for genes most likely to be under selective constraints (Cheung & Spielman 2002; Gilad *et al.* 2006).

That reduced interindividual transcription variance may truly reflect the consequences of selective constraints acting on directional parallel genes in nature is further supported, although indirectly, by the comparison with transcription variance observed in controlled conditions. Fish reared in controlled conditions for three generations have certainly been less subject to selective pressures, both during early life history stages (as evidenced by high survival rate; Lu & Bernatchez 1999), and throughout their life cycle vis-à-vis pressures associated with competition for resources (since always fed *ad libidum*), diseases (no mortality associated with pathogens has been recorded at LARSA; L.B. personal observation), and predation. Theory would predict that these relaxed conditions should allow for a higher range in phenotypic responses that could be measured by an increased variance in interindividual mean gene expression levels from controlled vs. natural environments (Fisher 1930). Our results supported this prediction; despite the fact that dwarf and normal whitefish lines maintained in the laboratory were initiated with a maximum of 40 breeders each (Lu & Bernatchez 1999), interindividual variance observed for parallel directional genes was more than twice higher in controlled relative to natural conditions (8.75 vs. 3.62, $P = 0.0012$).

Transcriptomic basis of trade-offs in life-history traits

Our second objective was to investigate how differential patterns of transcription between dwarf and normal whitefish could inform on the molecular mechanisms of life-history trade-offs potentially involved in the adaptive divergence of these species pairs. Based on their strikingly different life-history strategies, we predicted that differential trade-offs involving growth (and correlated fecundity) vs. survival between dwarf and normal whitefish should translate into predictable differences in patterns of gene transcription. Our results followed the general trend predicted under the hypothesis of trade-offs involving (i) survival via overall enhanced standard metabolism (for instance associated with increased feeding rate; Trudel *et al*. 2001) and swimming activity to occupy the limnetic niche in dwarf relative to normal whitefish; and (ii) increased growth rate (and associated increased fecundity) in normal relative to dwarf whitefish. Dwarf whitefish consistently showed significant over-expression of genes potentially associated with survival through increased activity (including genes involved in energy metabolism, iron homeostasis, lipid metabolism and detoxification; Table 5). Thus, when comparing normal and dwarf whitefish from natural and controlled environment, eight out of the nine energy metabolism genes were over-expressed in parallel in dwarf relative to normal whitefish. Genes involved in lipid metabolism and detoxification mechanisms were also generally over-expressed in dwarf whitefish, indicating enhanced metabolic activity. It is noteworthy that we also observed significant over-expression of genes involved in germ-line formation in dwarf relative to normal whitefish, despite the fact that fish of both species were of comparable age (data not shown). This corroborates the observation that dwarf whitefish become sexually mature at an earlier age. In contrary, genes more likely to be associated with growth, such as those involved in protein synthesis, cell growth and cell cycle promoting-genes were generally down-regulated in dwarf relative to normal whitefish. In the natural vs. controlled environment comparison, genes involved in various stages of protein synthesis pathways were over-expressed in parallel in normal whitefish (Table 5), thus corroborating previous observations on enhanced growth rate for this species (Rogers & Bernatchez 2005).

In the following sections, we discuss possible (hypothetical) functions of specific candidate genes that are the most likely to be involved in the differential life-history trade-offs distinguishing dwarf and normal whitefish species pairs. In doing so, one should bear in mind that a thorough appraisal of such functional links will require detailed mechanistic studies.

Dwarf whitefish are known to display more active swimming behaviours (Rogers *et al*. 2002) owing to their limnetic foraging habits and predator avoidance (Kahilainen & Lehtonen 2002). Accordingly, when comparing dwarf and normal whitefish populations from natural and control populations, we found over-expression of genes potentially associated with enhanced swimming activity in dwarf whitefish. Thus, relative to the normal, dwarf whitefish were prone to overexpression of genes playing key roles in energy producing mechanisms such as glycolysis (GDPH, aldolase A and B) and the TCA cycle (malate dehydrogenase), as well as genes facilitating transport of macromolecules such

as lipids towards cellular target compartments such as AFABP (Glatz *et al*. 2003). We also observed in dwarf whitefish parallel up-regulation for three genes related to other biological processes involved in the energy metabolism network (fructose-biphosphate aldolase B, malate dehydrogenase, GDP-L-fucose synthetase), as well as for three genes involved in lipid metabolism and transport (beta-2-glycoprotein I, gastrotropin, thyroid hormone-inducible hepatic protein). Conversely, three other lipid metabolism genes (apolipoprotein A-I-1, A-IV and CII) involved in lipid absorption, as well as one gene involved in the oxydative phosporylation of fatty acids (NADH-ubiquinone oxidoreductase chain 1) were down-regulated in dwarf whitefish, which would be consistent with an enhanced exportation of lipidic products towards energy demanding systems such as skeletal muscles. This hypothesis of enhanced lipid export activity in dwarf as opposed to normal whitefish liver tissue is supported by cluster analyses of transcription profiles for fish from natural populations (cluster 4, Fig. 2a). Thus, the aforementioned genes involved in lipid absorption and oxidative phosphorylation were grouped with genes potentially involved in vacuole formation and endocytosis (polyposis locus protein 1 homologue) as well as cytoskeleton-mediated vacuolar transport (Unknown CK990291, a beta actin homologue). Moreover, the clustering of these genes suggests that they are part of a common coregulation network (Quackenbush 2003).

Up-regulation of genes involved in detoxification pathways (glutathione-S-transferase and carboxylesterase 22, aldehyde dehydrogenase 9 family member A1-like 1, plasma glutathione peroxidase) observed in dwarf whitefish could also potentially be associated with higher metabolic activity and more active swimming behaviour. Thus, the higher energetic demands and metabolic activity would tend to generate a proportionally larger amount of metabolic by-products and wastes that would need to be detoxified in order to maintain proper muscle activity and avoid complications caused by oxydative stress (Claiborne 1998). For example, glutathione-S-transferase is known to play a role in protecting the plasma membrane against oxydating agents (Bioinformatic Harvester: http://harvester.embl.de/harvester/P464/P46439.htm). Another gene of interest also up-regulated in dwarf whitefish is ARF GTPase-activating protein GIT2, which is known for its possible involvement in DNA repair and apoptosis (Premont *et al*. 2000). Cells subjected to increased metabolic activity would tend to be faced with a greater production of metabolic by-products and free reactive oxygen species via an augmented respiratory activity in the mitochondria, which could potentially be DNA damaging (Mommsen 1998).

Dwarf whitefish are also characterized by a higher food consumption rate, perhaps to compensate for their less efficient food conversion efficiency, itself linked to higher energy demands for basal metabolism and/or swimming activity (Trudel *et al*. 2001). In turn, higher consumption rate should translate in differential transcription profiles between dwarf and normal whitefish for genes involved in catabolic functions. Accordingly, we observed parallel up-regulation in dwarf whitefish of three genes involved in the breakdown of proteins (trypsin II, homogentisate 1,2-oxygenase, elastase 2), a catabolic activity associated with lysosomes and digestive functions.

Other parallel up-regulated genes in dwarf whitefish could also be associated with adaptations to the limnetic niche. For instance, the heme oxygenase and two ferritin heavy chain gene clones, here classified in the iron homeostasis network (Bioinformatic Harvester: http://marvester.embl.de/marvester/O702/O70252.htm), could provide a more efficient way of ensuring oxygen transport by expressing higher levels of heme cleavage enzymes and keeping more iron ions readily available for oxygen transport (Rise *et al*. 2006). This would be consistent with the overall higher energetic demands of enhanced swimming and foraging activity in dwarf compared to normal whitefish. In addition to its potential role in respiratory functions, heme oxygenase is also associated with oxydative stress and inflammatory response (Stanford *et al*. 2003), consistent with enhanced activity and stress in the dwarf whitefish.

Two additional genes potentially associated with enhanced swimming activity were up-regulated in parallel in dwarf whitefish: troponin I, which is involved in the regulation of skeletal muscle contraction (UniProtKB/Swiss-Prot: http://c.expasy.org/uniprot/P13412), and the prostaglandin-H2 D-isomerase involved in the hormonal-dependent regulation of muscle contraction. This latter gene could play a role in a more efficient blood circulation of nutrients and energy molecules associated with increased metabolic demand, and is also known for its role as scavenger for harmful hydrophopic molecules (UniProtKB/Swiss-Prot: http://c.expasy.org/uniprot/O09114; http://c.expasy.org/uniprot/P22057).

Four genes involved in protein synthesis (*Onchorhynchus mykiss* calreticulin and 60S ribosomal protein L9, protein disulphide-isomerase A4 precursor and peptidyl-prolyl *cis*-trans isomerase B precursor) were up-regulated in normal whitefish, which would be consistent with their higher growth rate relative to dwarf whitefish (Rogers & Bernatchez 2005). The level of DNA synthesis may also be indicative of cell-cycle progression, and can therefore be associated with growth rate at the cellular and molecular level. Thus, two genes involved in nucleoside phosphate metabolism (nucleoside diphosphate kinases A and B) were up-regulated in normal whitefish, suggesting a higher DNA synthesis attributable to genome replication and cell division, consistent with an overall higher growth rate in normal whitefish. Up-regulation of three clones of the 'brain protein 44' gene in normal whitefish, a gene whose putative functions pertain to central nervous system development

and differentiation via apoptosis regulation (UniProtKB/Swiss-Prot: http://c.expasy.org/uniprot/BR44L_RAT), also corroborates higher growth rate and developmental activity in this species. Finally, up-regulation of the polyposis locus protein 1 gene (UniProtKB/Swiss-Prot: http://c.expasy.org/uniprot/Q60870) in normal whitefish, which is a putative membrane protein involved in cell signalling, membrane trafficking and indirectly in cellular division and cell cycle regulation, could hypothetically be associated with a higher responsiveness of cells to various growth signal. The role of the polyposis locus protein 1 gene, a human TB2 homologue, has been studied in yeast (YOP1) by Calero *et al*. (2001) who demonstrated that overexpression of YOP1 resulted in an accumulation of internal cell membrane and a block in membrane traffic. This accumulation of internal membrane accompanied by a reduction in cellular membrane surface and restriction in membrane traffic may be associated with early stages of mitosis (Boucrot & Kirchhausen 2007), and is consistent with an overall enhanced cell cycle activity and cellular division associated with higher growth rate observed in the normal whitefish. Finally, some other genes did not fit the pattern predicted by the trade-off hypothesis. For instance, expression profiles of two cell cycle regulation genes (ARF GTPase-activating protein, Table 2; XP8, Table 3) were not consistent with the hypothesis of accelerated cellular growth and replication expected in normal whitefish in association with their higher growth rate.

Conclusions

Parallelism in gene transcription, as well as patterns of interindividual variation across controlled and natural environments, provide strong indirect evidence for the role of selection in the evolution of differential regulation of genes that involve a vast array of potentially adaptive physiological processes between dwarf and normal whitefish, including energy metabolism, protein synthesis, cell cycle regulation, cell proliferation and differentiation, detoxification pathways, transport in the plasma, cellular intake of various nutrients and molecules, and immunity. Moreover, cluster analyses reveal significant grouping of genes with similar or related functions together, suggesting that clustered genes belong to common pathways that could share regulational effectors (Eisen *et al*. 1998; Ihmels *et al*. 2005). The fact that a relatively small number of clusters were observed also raises the hypothesis that the transcription regulation of these genes could be under the control of one or few genes with major pleiotropic effects on the genome. The nature of changes in DNA sequences underlying such differences between dwarf and normal whitefish remains elusive at this point. However, even if the actual coding sequence of functional genes was differentiated between dwarf and normal whitefish ecotypes, the overexpression we observed in dwarf for most of the parallel changes would suggest that their corresponding alleles would be functionally more similar together, compared to those found in the normal populations. This, in turn, would further support the evolution of adaptive divergence at the genetic level between dwarf and normal whitefish. Notwithstanding the apparently contradictory results observed for a few genes, our results also provide a first mechanistic genomic basis for the observed trade-off in life-history traits distinguishing dwarf and normal whitefish species pairs, wherein enhanced survival via a more active swimming, necessary for increased foraging and predator avoidance, engages energetic costs that translate into slower growth rate and reduced fecundity in dwarf relative to normal whitefish.

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This study is in continuation with our long-term research programme on the comprehension of genotype–environment interactions involved in the adaptive radiation of the lake whitefish. Jérôme St-Cyr is a graduate student working on the genomic bases of phenotypic divergence in the context of an adaptive radiation. Nicolas Darame is an assistant professor interested in the molecular response of genotype–environment interactions that lead to adaptive divergence. Louis Bernatchez's research focuses on understanding the patterns and processes of molecular and organismal evolution.

Supplementary material

The following supplementary material is available for this article:

Table S1 (a) Genes showing nondirectional transcription differences in natural populations. (b) Genes showing nondirectional transcription differences in natural populations and in controlled environment

This material is available as part of the online article from:
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