The Transcriptomics of Ecological Convergence between 2 Limnetic Coregonine Fishes (Salmonidae)

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Species living in comparable habitats often display strikingly similar patterns of specialization, suggesting that natural selection can lead to predictable evolutionary changes. Elucidating the genomic basis underlying such adaptive phenotypic changes is a major goal in evolutionary biology. Increasing evidence indicates that natural selection would first modulate gene regulation during the process of population divergence. Previously, we showed that parallel phenotypic adaptations of the dwarf whitefish (*Coregonus clupeaformis*) ecotype to the limnetic trophic niche involved parallel transcriptional changes at the same genes involved in muscle contraction and energetic metabolism relative to the sympatric normal ecotype. Here, we tested whether the same genes are also implicated in a limnetic specialist species, the cisco (*Coregonus artedi*), which is the most likely competitor of dwarf whitefish. Significant upregulation was detected in cisco at the same 6 candidate genes functionally involved in modulating swimming activity, namely 5 variants of a major protein of fast muscle and 1 putative catalytic crystallin enzyme. Moreover, 3 of 5 variants and the same putative catalytic crystallin enzyme were upregulated in cisco relative to the dwarf ecotype, indicating a greater physiological potential of the former for exploiting the limnetic trophic niche. This study provides the first empirical evidence that recent, parallel phenotypic evolution toward the use of the same ecological niche occupied by a specialist competitor involved similar adaptive changes in expression at the same genes. As such, this study provides strong support to the general hypothesis that directional selection acting on gene regulation may promote rapid phenotypic divergence and ultimately speciation.

Introduction

Species or populations occupying ecologically similar niches often display patterns of divergence that are strikingly similar, suggesting that parallel as well as convergent phenotypic evolution among independent, closely related lineages is a predictable consequence of natural selection (Harvey and Pagel 1991; Schluter 2000). Because selection ultimately acts on the genetic variation underlying trait variation, identifying the genes associated with parallel evolutionary changes among closely related lineages is essential toward identifying candidate genes implicated in convergent phenotypic adaptation. Haldane (1932) first proposed that parallel evolution of closely related lineages may be a result of both natural selection and constraints due to similar genetic architectures. The genetic basis of parallel phenotypic evolution is still debated around 2 competing hypotheses. On the one hand, negative pleiotropic constraints occurring within conserved protein-coding regions may force parallel phenotypic changes by involving the same gene-specific regulatory sequences (Stern 2000); conversely, in the absence of pleiotropy, different genes may be involved (Schluter et al. 2004). However, these alternate hypotheses have typically been tested using only one or a few candidate genes (Powers and Schulte 1998; Sucena and Stern 2000; Gompel and Carroll 2003; Hoekstra and Nachman 2003; Wittkopp et al. 2003; Colosimo et al. 2004; Cresko et al. 2004). Recently, studies of genome-wide expression profiles supported the view of King and Wilson (1975) expressed more than 30 years ago that changes in key regulatory genes may have a greater effect than those of structural genes on evolutionary changes. Genome-wide variation in gene expression between natural populations has been reported to underlie ecological adaptation (Oleksiak et al. 2002, 2005; Bochdanovits et al. 2003; Derome et al. 2006). These studies have cumulatively demonstrated that transcriptomics offers a powerful means for investigating the genomic basis of parallel, phenotypic divergence under natural environmental conditions.

Coregonine fishes (Salmonidae) are of particular interest for investigating the transcriptomics basis of parallel phenotypic evolution. The most taxonomically diverse genus among salmonids, *Coregonus*, has radiated during the Pleistocene to occupy a wide array of habitats throughout the Palearctic and Nearctic regions (Scott and Crossman 1973), presenting 2 basic morphological patterns: the "ciscoes" and the "true whitefishes" species (Lindsey 1981). Ciscoes and "true whitefishes" diverged 2–3 MYA (fig. 1) (Bernatchez 2004). Ciscoes typically occupy the lacustrine pelagic zones and are characterized by phenotypic adaptations for zooplankton feeding, such as a laterally compressed body and a large terminal mouth (Scott and Crossman 1973). Ciscoes are also characterized by higher swimming activity and metabolism than "true whitefishes" (Bernatchez and Dodson 1985). In contrast, the "true whitefishes" that most generally occupy the epibenthic habitat at the adult stage are less active swimmers and are characterized by a subterminal mouth and a more robust body shape. Such a dichotomy in forms has also occurred in North America at a lower phylogenetic level wherein 2 sympatric ecotypes, dwarf and normal, of lake whitefish (*C. clupeaformis*) diverged less than 15,000 years ago (fig. 1) (Bernatchez 2004). The dwarf ecotype occupies the functional niche of the cisco (*Coregonus artedi*) and is found in sympatry with the normal whitefish only where the cisco does not occur due to biogeographic reasons (fig. 2) (Fenderson 1964; Doyon et al. 1998). The fact that it is the most likely competitor of whitefish for zooplanktonic prey (Trudel et al. 2001) suggests that the absence of cisco created the ecological opportunity that promoted the recent evolution of dwarf whitefish populations derived from ancestral normal whitefish populations. Both cisco and the dwarf ecotype exhibit lower growth rates compared with the normal lake whitefish ecotype, due mainly to a 50–60%

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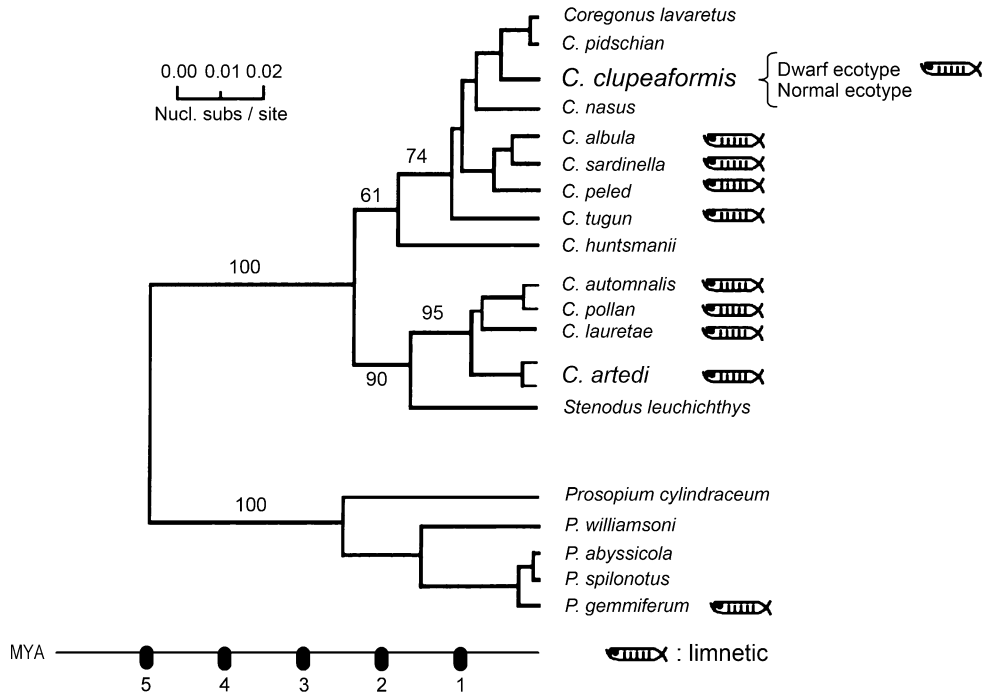

FIG. 1.—Kitsch phenogram based on a matrix of pairwise nucleotide substitutions per site estimated from mtDNA restriction fragment length polymorphism analysis among coregonine taxa. Values above the branches are bootstrap values >50 (in percent) obtained in a character-based parsimony analysis. Cisco species are identified by fish symbols. Modified from Bernatchez (2004).

higher energy allocation to increased swimming activity (Trudel et al. 2001).

Derome et al. (2006) recently tested the general hypothesis that the parallel phenotypic evolution of dwarf and normal whitefish ecotypes was accompanied by parallel changes in gene expression by investigating the white muscle, a tissue highly implicated in swimming activity and growth (Mommsen 2001; Martinez et al. 2004). As predicted (Trudel et al. 2001), genes showing the clearest evidence of differential expression belonged to functional groups associated with energetic metabolism and swimming activity and were upregulated in the dwarf whitefish. Two gene families were of particular interest. First, *parvalbumin* genes are intracellular calcium-binding proteins that play a major role in the regulation of muscle contraction (Rall 1996). Their upregulation is expected to favor faster swimming activity (Thys et al. 2001), as observed in the dwarf ecotype. Secondly, a *beta/gamma-crystallin* gene, potentially associated with catalytic activity (Jones et al. 1999; Piatigorsky 2003), most likely provides increased energetic output for swimming.

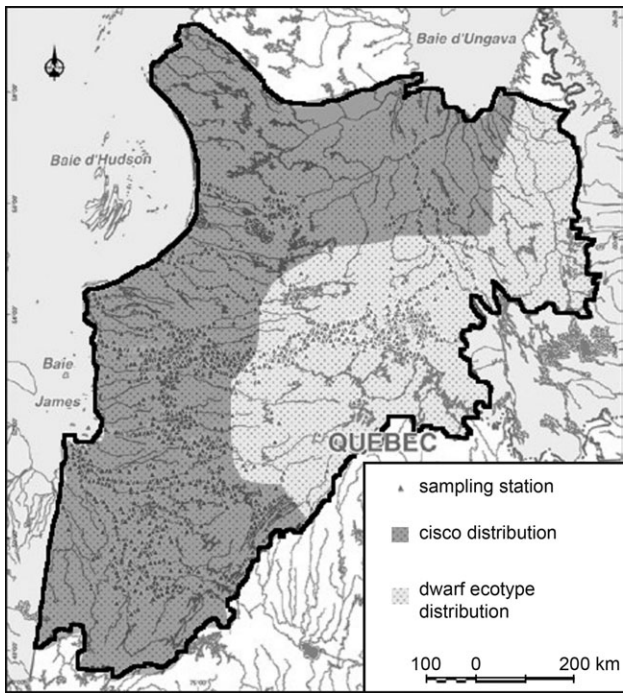

FIG. 2.—Geographic distribution of cisco (*Coregonus artedi*) and whitefish ecotypes (*Coregonus clupeaformis*) on the Quebec peninsula. The bold line delineates the distribution of the normal lake whitefish in northern Quebec. Modified from Verdon (2001).

The evolutionary convergence of the dwarf whitefish ecotype toward ciscolike phenotype and ecological characteristics suggests that both may have evolved under similar selective pressures associated with occupying the same habitat. Here, by comparing transcription profiles between cisco and lake whitefish, we found that the same changes underlying parallel divergence between dwarf and normal ecotypes are also involved in the differentiation between cisco and normal whitefish. The apparent ecological exclusion of dwarf whitefish by the cisco suggests that the latter outcompetes the former, partly because of more specialized physiological adaptations for occupying the pelagic niche. We therefore predicted and confirmed that genes involved in enhancing swimming activity, namely *parvalbumin* and *gamma-crystallin*, exhibited higher levels of expression in cisco relative to the dwarf whitefish ecotype.

Materials and Methods

Collection

Cisco from Lac des Trente et un Miles ($n = 10$) were sampled in August 2003 and cisco from Lac Florent ($n = 6$) were sampled in August 2004. Gillnets were inspected every 30 minutes to ensure fish mortality did not occur before tissue was sampled for RNA extractions. Dissected white muscle (250–350 mg) was immediately frozen in liquid nitrogen in the field and then stored at −80 °C in the laboratory. Only adult fish were sampled because metabolic rate differs between juvenile and adult fish (Rowan et al. 1997). White muscle was systematically sampled from the same body region than whitefish samples collected by Derome et al. (2006) (immediately below the dorsal fin) as gene expression in white muscle has been reported to vary along the body axis (Mommsen 2001). Dwarf and normal whitefish RNA samples used in this study are from the Indian Pond population studied in Derome et al. (2006).

RNA Isolation and Microarrays

RNA was extracted from white muscle tissue according to the Trizol Reagent protocol (Gibco BRL, Carlsbad, CA) and quantified with a GeneQuant spectrometer (Pharmacia, New York, NY). RNA integrity was verified with a 2100 Bioanalyzer (Agilent, Palo Alto, CA). Reverse transcriptase–polymerase chain reaction was performed using 15 μg of total RNA per sample following the SuperScript II Reverse Transcriptase protocol (Invitrogen life technologies, Carlsbad, CA). Indirect labeling was performed on individual cDNA following the Array 50 kit protocol (Genisphere, Hatfield, PA). Transcriptome profiles were obtained by using a 3,557 cDNA gene microarray developed for the Atlantic salmon (*Salmo salar*) by GRASP (Rise et al. 2004) (Genomic Research on Atlantic Salmon Project) and successfully tested for 2 other salmonid species (*Oncorhychus mykiss* and *C. clupeaformis*) and 1 osmeriform (*Osmerus mordax*). However, cross-hybridization can be a problem for spotted cDNA microarrays because of sequence polymorphisms between strains or paralogous genes that affect the signal for certain genes. Nevertheless, given that cisco (*C. artedi*) and whitefish ecotypes (*C. clupeaformis*) are equally divergent from the genus *Salmo*, the 2 species should be equally affected by cross-hybridization. Gene identification, with the corresponding expressed sequence tag (EST) sequence, is provided at: http://web.uvic.ca/cbr/grasp/. Functional array composition contains mostly ESTs associated to metabolism (65%) and only 0.48% of all ESTs are associated to regulation of muscle contraction (see Roberge et al. 2006 for details). The transcript levels inferred from fluorescent labels were quantified by scanning the chip using a ScanArray Express scanner (Packard Bioscience, Wellesley, MA). Spot location and quantification was done with the QuantArray (Perkin Elmer, Wellesley, MA) software, retaining the mean intensity value for each spot. Aberrant spot signals were removed before analysis, and their values were estimated using the "Row Average Imputer" function implemented in SAM software (Tusher et al. 2001). Genes with low-intensity data (mean intensity in both channels less than the mean of the empty spot controls plus 2.5 times their standard deviation [SD]) were considered as not expressed and, therefore, removed from the analysis. Finally, only genes expressed in both lakes were considered for the analysis. The data sets are available on the GEO Web site (http://www.ncbi.nlm.nih.gov/geo/).

Statistical Analyses

We used a paired design to minimize experimental variance (Churchill 2002; Yang and Speed 2002), whereby retrotranscribed RNA from cisco individuals of a given lake were randomly chosen to be hybridized along with both normal and dwarf lake whitefish ecotypes in separate experiments. Ten and 6 biological replicates were available per analysis for Lac des Trente et un Miles and Lac Florent populations, respectively. As each EST clone was duplicated on the array, fluorescence intensity values were averaged after correcting for local background (Draghici 2003). For genes spotted in quadruplicate, only twin replicates were averaged before statistical analysis. Potential biases in fluorophore intensity variation were minimized by dye swapping (Churchill 2002), wherein half of the cisco individuals were labeled with Cy3 dye and normal (or dwarf) ecotypes with Alexa 647 dye and vice versa for the other half. Raw intensity values were normalized using the regional LOWESS method implemented in the R/MANOVA software (Kerr et al. 2000), available at: http://www.jax.org/staff/churchill/labsite/software/Rmaanova, and by dividing each channel by the mean intensity. Initial analyses to detect differentially expressed genes occurring in both lake population pairs for both cisco/normal and cisco/dwarf comparisons were performed employing a permutation-based F-test ($F3$, with 1,000 sample permutations) using the R/MAANOVA software under a mixed-effects model (Cui and Churchill 2003) with the Array term as a random effect and the sample type (cisco, normal, or dwarf) and dye terms as fixed effects. As in Derome et al. (2006), significance for post hoc, multiple comparisons were adjusted using the Bonferroni correction (Bonferroni 1935). Genes with differential expression below a 1.05-fold change were considered as not differentially expressed.

Determination of Gene Functional Groups

Gene loci correspond to EST library annotations using databases from GenBank (see Rise et al. 2004 for details). Because several ESTs sometimes best matched the same GenBank locus, we are referring to gene clones as ESTs (see Results). Significant differentially expressed gene clones were classified into functional groups using the browser of Geneontology (http://www.geneontology.org/) and completed with references from the literature. Differentially expressed ESTs previously matched to unknown gene loci were resubmitted to "BlastN" and "BlastX" browsers from National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). Two main functional groups were defined: muscle contraction and energetic metabolism as they contain most of the differentially expressed genes and because they were both of particular interest given previous observation that both dwarf and cisco compared with normal whitefish ecotypes differ

Table 1
Expression Changes between Cisco and Normal Lake Whitefish Ecotype

Function[a]	EST[b]	Gene[c]	Fold Change[d] LT[e]	LF[f]
MC	nwh 6 27	= (AF538283) parvalbumin beta	2.86	3.30
—	nwh 18 44 [P]	= (AF538283) parvalbumin beta	1.70	2.68
—	nwh 10 49 [P]	= (AF538283) parvalbumin beta	1.39	2.23
—	nwh 11 21 [P]	= (AF538283) parvalbumin beta	2.05	1.95
—	nwh 20 43 [P]	= (AF538283) parvalbumin beta	1.69	1.45
EM	nwh 7 14 [P]	[JC2355] gamma-crystallin M1-2	2.15	2.17
—	nwh 7 14 [P]	[JC2355] gamma-crystallin M1-2	2.43	2.09
MC	nwh 8 70 [N]	[AF330142] actin	0.64	0.71
—	nwh 8 51 [P]	[O42161] beta-actin	0.69	0.82
—	rbha 1 72 [P]	[AAF80342] beta-actin	0.80	0.83
—	rblb 3 37 [P]	[AAK60615] myosin light chain 3	0.74	0.81
—	plnb 503 47 [P]	[P02593] calmodulin	0.79	0.75
EM	rbha 4 86 [P]	[S13164] creatine kinase	0.57	0.81
—	rblb 3 33 [N]	[BE518558] = triose phosphate isomerase	0.79	0.55
—	nwh 19 56 [P]	[NP_036627] aldolase	0.93	0.71
—	rgb 523 238 [N]	[AY024367] glycerol-3P dehydrogenase	0.75	0.77
OF	rgb 530 221 [P]	[O93484] collagen alpha 2(I) precursor	0.66	0.63
	rgb 523 11 [P]	[AAH19265] unknown	0.75	0.74
EM	plnb 506 341 [P]	[NP_612557] nucleoside diphosphate kinase	0.63	1.39
OB	nwh 1 23 [N]	[AF180482] beta-2 microglobulin	1.49	0.22
MC	nwh 20 13 [P]	= (SSPRVB2) parvalbumin beta	0.85	1.55
IR	lna 7 69 [P]	[P49946] ferritin H	0.75	1.29

[a] Functional classes are defined using AmiGO browser of Geneontology (http://www.geneontology.org/) and completed with references from the literature. EM: energetic metabolism; IB: iron binding; MC: muscle contraction; OB: oxygen binding; OF: other functions; PS: protein synthesis.

[b] EST clone number (56). Each number corresponds to a single EST sequence.

[c] Gene loci correspond to gene names assignation of each library's EST using databases from GenBank. See Rise et al. (2004) for details. '' = ''means unknown EST sequences resubmitted in this study to GenBank databases. Locus accession numbers are in brackets.

[d] Fold change correspond to a ratio of mean expression values of cisco to either normal or dwarf lake whitefish ecotype: >1 implies upregulated in cisco; <1 implies downregulated in cisco.

All *P* values are below a Bonferroni cutoff (*P* values < 2.97E-05, analysis of variance, *F*3-test).

[e] Lac des Trente et un Miles.

[f] Lac Florent.

mainly in terms of bioenergetics and swimming activity (Trudel et al. 2001).

Results

Cisco versus Normal Whitefish Ecotype

A total of 1,685 gene clones expressed in both cisco populations as well as in dwarf and normal whitefish ecotypes from Indian Pond hybridized on the 3,557 cDNA chip. Using a Bonferroni correction, 22 gene clones (1.3%) showed differential expression between the normal lake whitefish ecotype and both cisco populations, (table 1). Of these, 18 were differentially expressed in the same direction in both cisco populations (i.e., directional changes), 7 of which were upregulated in cisco, and 11 were upregulated in the normal whitefish ecotype. The 4 remaining genes were expressed in opposite directions in both populations (i.e., nondirectional changes). The mean level of expression between cisco and the normal whitefish ecotype differed between the genes that were upregulated in cisco and those that were upregulated in normal whitefish ecotype (fig. 3*A*). Thus, the mean fold change for the 7 gene clones that were upregulated in cisco was 2.15 (SD = 0.54) compared with 1.35 (SD = 0.088) for the 11 gene clones that were upregulated in the normal lake whitefish ecotype. Expression variances for these genes also differed between cisco and normal whitefish, whereby variance in expression for genes that were upregulated in both cisco populations was twice less (mean variance across genes = 3.9) than that observed for genes that were upregulated in the normal ecotype (mean = 6.29, $P = 0.09$, 1-tailed *t*-test). Moreover, expression variance of the 4 remaining nondirectional gene clones (mean = 16.88) was significantly higher than directional changes observed in both cisco and normal whitefish combined (mean = 5.35, $P = 0.0002$, 1-tailed *t*-test).

The functional groups involved in directional parallelism mainly concerned both muscle contraction and energetic metabolism (fig. 4*A*). For upregulated genes in cisco (table 1), the functional group representing muscle contraction was exclusively represented by 5 distinct clones of *parvalbumin* variants (mean upregulation of 2.13 in cisco relative to normal whitefish). The functional group associated with energetic metabolism was represented by a gamma-crystallin enzyme clone (mean upregulation of 2.21). Nine out of the 11 upregulated genes in normal whitefish belonged to either the muscle contraction or the energetic metabolism functional groups (table 1). The muscle contraction functional group was mainly represented by 3 distinct *actin* variants (mean upregulation of 1.33), a *myosin* (mean upregulation of 1.29), and a *calmodulin* (mean upregulation of 1.30). The energetic metabolism functional group was more diverse, being represented by: 1) 2 glycolytic

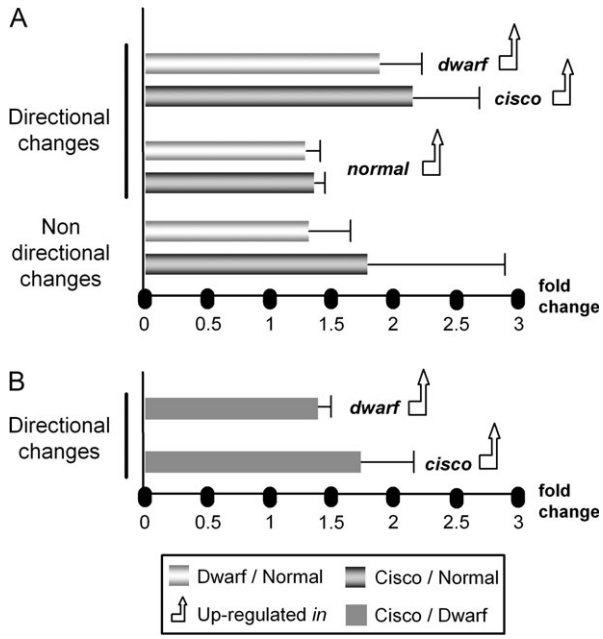

FIG. 3.—(*A*) Mean differential gene expression (fold change) between cisco and normal ecotype (this study) and between dwarf and normal ecotypes, modified from Derome et al. (2006), applying a Bonferroni correction. For each gene/group comparison, fold change is the ratio of the highest gene expression mean divided by the lowest. For example: fold change of upregulated genes in cisco relative to normal whitefish ecotype is gene expression mean in cisco divided by those in normal whitefish ecotype. (*B*) Mean differential gene expression (fold change) between cisco and dwarf ecotype (this study).

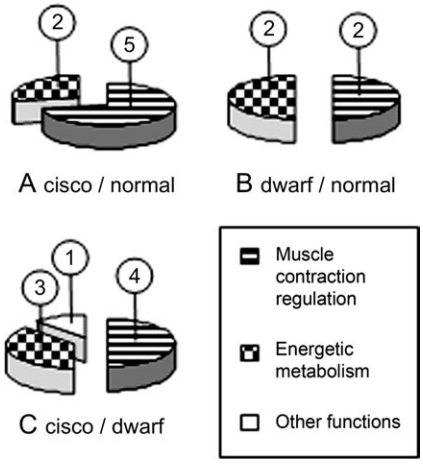

FIG. 4.—Breakdown of the main functional groups associated with upregulated genes in (*A*) cisco versus normal, (*B*) dwarf versus normal, modified from Derome et al. (2006), applying a Bonferroni correction, and (*C*) cisco versus dwarf. In circles: number of differentially expressed genes.

enzymes, namely, *aldolase* A and *triose phosphate isomerase* (mean upregulation of 1.82 and 1.22, respectively); 2) 1 oxidative phosphorylation gene: the *glycerol-3-phosphate dehydrogenase* (mean upregulation of 1.31); 3) a power exchange mediator, *creatine kinase* (mean upregulation of 1.45). Finally, 4 functional categories were also represented in the nondirectional genes, (table 1): 1 *parvalbumin* variant (muscle contraction), a *beta-2 microglobulin* (oxygen binding), *nucleoside diphospate kinase* (energetic metabolism), and *ferritin H* (iron binding).

Cisco versus Dwarf Whitefish Ecotype

Thirteen gene clones (0.8%) showed differential expression between the dwarf lake whitefish ecotype from Indian Pond and both cisco populations (table 2). All of these were differentially expressed in the same direction: 8 were upregulated in cisco with a mean fold change of 1.66 (SD = 0.44) and 5 were upregulated in dwarf lake whitefish with a mean fold change of 1.38 (SD = 0.09) (table 2, fig. 3*B*). Ten out of these 13 gene clones were shared with those that were differentially expressed between cisco and normal whitefish (tables 1 and 2), among which 9 showed directional changes between them. Five of these 9 gene clones were upregulated in cisco, relative to both normal and dwarf ecotypes, and concerned both energetic metabolism and muscle contraction (fig. 4*C*): 3 *parvalbumin* variants (mean upregulation in cisco versus dwarf of 1.73) and 2 clones of the *gamma-crystallin* gene (mean upregulation in cisco versus dwarf of 1.70) (table 2). Additional gene clones upregulated in normal whitefish, relative to cisco, were also upregulated in dwarf whitefish, relative to cisco; 2 *actin* variants (mean upregulation of 1.39) and *triose phosphate isomerase* (mean upregulation of 1.41) (table 2). Finally, a distinct *parvalbumin* variant that showed significant nondirectional changes between cisco and normal whitefish was upregulated in both cisco populations, relative to the dwarf ecotype.

Normal versus Dwarf Whitefish Ecotypes Revisited

For comparison with results of this study, data from Derome et al. (2006) were reanalyzed using the Bonferroni correction. This reanalysis reduced the number of parallel transcriptional changes from 55 to 11 gene clones; transcriptional changes between dwarf and normal whitefish ecotypes were significantly more numerous than between cisco and dwarf when taking respective divergence times into account (Fisher test, $P < 0.0001$). The mean fold change for the 4 gene clones that were upregulated in both dwarf populations was 1.89 (SD = 0.32) compared with 1.28 for the *ferritin* gene clone that was upregulated in both normal populations (fig. 3*A*). The 4 upregulated gene clones in dwarf concerned exclusively muscle contraction and energetic metabolism (fig. 4*B*), with 2 *parvalbumin* beta genes (mean upregulation of 1.96) and 2 clones of 1 *gamma-crystallin* gene (mean upregulation of 1.81), respectively. Finally, nondirectional changes involved 2 *parvalbumin genes*, both distinct from those that were upregulated in dwarf populations, 1 *enolase*, 1 *cytochrome c oxidase*, 1 *ubiquitin*, and 1 transcript with unknown function.

Discussion

Our main objective was to test the hypothesis that transcriptomic changes accompanying the parallel phenotypic evolution of dwarf lake whitefish ecotypes toward the exploitation of the limnetic trophic niche involved the same genes in the cisco, a highly specialized limnetic species. Confirming this hypothesis would further support the role of directional selection in driving the evolution of the dwarf ecotype toward their adaptation to the limnetic

Table 2
Expression Changes between Cisco and Dwarf Lake Whitefish Ecotype

Function[a]	EST[b]	Gene[c]	Fold Change[d]	
			LT	LF
MC	nwh 6 27	= (AF538283) parvalbumin beta	1.58	2.13
—	nwh 18 44 [P]	= (AF538283) parvalbumin beta	1.16	2.53
—	nwh 11 21 [P]	= (AF538283) parvalbumin beta	1.59	1.38
—	nwh 20 13 [P]	= (SSPRVB2) parvalbumin beta	1.30	1.34
EM	nwh 7 14 [P]	[JC2355] gamma-crystallin M1-2	1.57	1.87
—	nwh 7 14 [P]	[JC2355] gamma-crystallin M1-2	1.14	2.21
—	pitl 504 173 [P]	[NP_008448] cytochrome c oxidase subunit II	1.28	1.84
OF	rblb 1 60 [P]	[P87362] bleomycin hydrolase	1.32	2.30
MC	nwh 8 70 [N]	[AF330142] actin	0.71	0.67
—	nwh 8 51 [P]	[O42161] beta-actin	0.83	0.67
EM	rblb 3 33 [N]	[BE518558] = triose phosphate isomerase	0.84	0.58
PS	pha 501 321 [P]	[P27952] 40S ribosomal protein S2	0.81	0.68
	rgb 523 11 [P]	[AAH19265] unknown	0.80	0.67

[a] Functional classes are defined using AmiGO browser of Geneontology (http://www.geneontology.org/) and completed with references from the literature. EM: energetic metabolism; IB: iron binding; MC: muscle contraction; OB: oxygen binding; OF: other functions; PS: protein synthesis.

[b] EST clone number (56). Each number corresponds to a single EST sequence.

[c] Gene loci correspond to gene names assignation of each library's EST using databases from GenBank. See Rise et al. (2004) for details. '' = ''means unknown EST sequences resubmitted in this study to GenBank databases. Locus accession numbers are in brackets.

[d] Fold change correspond to a ratio of mean expression values of cisco to either normal or dwarf lake whitefish ecotype: >1 implies upregulated in cisco; <1 implies downregulated in cisco.

trophic niche. Given that cisco and dwarf lake whitefish are comparable vis-à-vis energetic metabolism (Trudel et al. 2001) and swimming activity (Rogers and Bernatchez 2005) and that genes that were upregulated in parallel in the dwarf ecotype involved genes belonging to both muscle contraction and energetic metabolism functional groups (Derome et al. 2006), we predicted that genes belonging to these functional groups should also be upregulated in the cisco when compared with the normal lake whitefish ecotype.

The results obtained strongly supported these predictions. Directional parallelism was observed for 7 gene clones that were upregulated in cisco (0.4% of the 1,685 expressed genes), of which 5 belong to muscle contraction and 2 to energetic metabolism functional groups (table 1, fig. 4*A*). Moreover, a 2.15 mean increase in expression of these genes was measured in cisco relative to the normal lake whitefish ecotype, which was higher than the mean 1.89 increase of expression observed for these same genes that were also upregulated in the dwarf ecotype when compared with the normal ecotype (Derome et al. 2006). In contrast, of the 11 parallel directional changes upregulated in the normal ecotype, differences in mean levels of expression were less dramatic with the normal ecotype exhibiting on average a 1.35 increase of gene expression, relative to cisco. This is very similar to the 1.28 increase in expression observed previously for the ferritin gene showing parallel directional changes in normal, relative to the dwarf ecotype.

Genes that showed parallel upregulation in both cisco (this study) and the dwarf ecotype (Derome et al. 2006) relative to the normal whitefish ecotype belonged exclusively to the muscular contraction and energetic metabolism functional groups (fig. 4). All of the 6 distinct EST clones of parvalbumin variants upregulated in the dwarf ecotype, relative to normal whitefish, were also upregulated in cisco. Five of these passed the Bonferroni cutoff, and all matched to 1 parvalbumin beta variant. High concentration of parvalbumin protein allows faster recovery between fast-twitch muscle contractions (Berchtold et al. 2000), which in turn may confer a faster swimming capacity. Therefore, parallelism in *parvalbumin* gene upregulation indicates that physiological adaptations recently (ca. 15,000 years before present) evolved in parallel in different dwarf whitefish ecotype for utilizing the limnetic niche, where more active swimming is required both for foraging zooplankton (Trudel et al. 2001) as well as avoiding predators (Kahilainen and Lehtonen 2002), involved the same transcriptomic changes as those evolved in the cisco approximately 2–3 Myr earlier.

As previously reported for the comparison between dwarf and normal ecotypes (Derome et al. 2006), the mean variance in expression was significantly reduced for genes that were upregulated in cisco relative to the normal whitefish ecotype, when including expression changes at permuted P values without Bonferroni correction. Reduced variance in expression for genes associated with increased swimming activity in both cisco and dwarf lake whitefish further supports the hypothesis that both *parvalbumin* and *gamma-crystallin* genes have been under strong directional selection in both species (Fisher 1930) that in turn, strongly suggest a genetic basis (Falconer 1989). Reduced variance in expression of these genes could also reflect similar genetic constraints being shared by closely related species and populations (Haldane 1932). The genetic constraint hypothesis would predict that genes under strong selection pressures should also be more likely to play a central role in their respective metabolic networks (Johnson and Porter 2000; Cork and Purugganan 2004). Our results are consistent with this prediction at least for the *parvalbumin beta* gene that plays a central role in muscle contraction networks (Rall 1996) and is essential in generating faster rates of contraction/relaxation and thus allowing in turn enhanced swimming speed in fish (Thys et al. 2001).

The putative catalytic gamma-crystallin enzyme is also likely to be highly implicated in the same network. Altogether, parallel changes in the regulation of these 2 genes between independently evolved dwarf whitefish ecotypes, as well as convergent evolution of the dwarf ecotype toward the cisco, provides strong evidence that their genetic architectures responded similarly to selective pressures imposed by the limnetic habitat and way of life, including diet, morphology, life history (Scott and Crossman 1973), as well as bioenergetics (Trudel et al. 2001). Overall, such a transcriptomic similarity further supports the role of natural selection in driving phenotypic divergence and, ultimately, speciation in coregonine species.

The twice as strong transcriptomic similarity observed in direct dwarf/cisco comparisons relative to the normal/cisco comparisons is all the more striking because variation among species has been reported to be affected primarily by neutral drift (Whitehead and Crawford 2006). Furthermore, it is noteworthy that the 3 *parvalbumin* clones and the *gamma-crystallin* gene that exhibited parallel changes between the 2 cisco populations relative to the dwarf whitefish ecotype were also upregulated in the cisco in all cases (table 2). Moreover, cisco showed reduced expression of both myosin and actin muscle proteins, compared with both normal and dwarf whitefish ecotypes, when including expression changes at permuted *P* values for cisco/dwarf comparisons. This differential pattern of expression may reflect a tradeoff between force and speed in muscle contraction as a reduced actomyosin filament concentration characterizes fastest twitch fibers, whereas high concentration of parvalbumin may be associated with increased rate of muscle contraction (Rome 2005). Correlatively, a gene directly implicated in muscle power supply of the actomyosin filament, such as *creatine kinase*, and 2 glycolytic enzymes were downregulated in cisco. Altogether, these patterns suggest that cisco may favor speed relative to strength. Furthermore, higher transcriptomic levels of both *parvalbumin* and *gamma-crystallin* candidate genes associated with fast swimming activity, combined with a lower amount of both actomyosin filament and glycolytic enzymes measured in cisco, when compared with dwarf whitefish ecotype, raises the hypothesis that cisco may be physiologically more efficient in the exploitation of the limnetic niche, partly by reducing energy allocation to muscle strength. This would be congruent with the higher energetic conversion efficiency observed in cisco relative to dwarf whitefish ecotype (Trudel et al. 2001). In addition, the large terminal mouth of the cisco, a morphological trait associated with efficient limnetic trophic niche exploitation, has not evolved as such in the dwarf whitefish ecotype, thus partially limiting its specialization to occupying the limnetic niche, perhaps due to constraints imposed by its genetic architecture. Overall, the dwarf whitefish ecotype has apparently converged toward a ciscolike way of life in many ways, including at the transcriptome level, yet not sufficiently so to compete effectively with a true cisco species. Indeed, several empirical observations support the prediction that cisco may outcompete the dwarf whitefish ecotype partly because they are physiologically better adapted toward the use of the pelagic niche. For instance, a drastic decline in dwarf whitefish abundance has been reported following the introduction of cisco (Bodaly et al. 1991). A similar observation was also reported in Europe following the introduction of the vendace (*Coregonus albula*), another limnetic specialist, which recently invaded several lakes in Norway and caused the decline of a limnetic form of the European whitefish (*Coregonus lavaretus*) (Amundsen et al. 1999).

Several reasons may account for the lower levels of expression observed in the dwarf whitefish ecotype, relative to cisco, for genes implicated in muscular contraction (e.g., *beta parvalbumin*) and energy metabolism (e.g., *gamma-crystallin* enzyme). Dwarf whitefish are always found in sympatry with the normal whitefish ecotype, and gene flow has been maintained between them in all cases documented to date (Pigeon et al. 1997; Lu and Bernatchez 1999; Campbell and Bernatchez 2004). Population differentiation often reflects a balance between the effects of divergent natural selection, whereby gene flow may impede adaptive divergence (Hendry et al. 2002; Nosil and Crespi 2004). Therefore, noticeable differences in transcription levels of both *parvalbumin* and *gamma-crystallin* genes observed between dwarf whitefish and cisco could partly result from different genetic constraints, potentially due to persistent gene flow between recently diverged sympatric whitefish ecotypes. Indeed, Lu and Bernatchez (1999) reported that phenotypic differentiation between dwarf and normal whitefish ecotypes was inversely correlated with the extant of gene flow between them. As a consequence, the lower ability of the dwarf whitefish ecotype, compared with cisco, in exploiting the limnetic trophic niche could partly result from limited transcriptomic changes at both *parvalbumin* and *gamma-crystallin* genes.

To conclude, this study provides further evidence for the determinant role of directional selection during the process of the recent and rapid adaptive divergence of the dwarf whitefish ecotype. Apparently, the process of adaptive divergence in whitefish involved a relatively small proportion of transcriptomic changes, which in turn suggests relatively few genetic changes at regulatory genes in muscle tissue. Muscle tissue is highly important for the 2 adaptive traits most differentiating limnetic and benthic ecotypes: swimming activity and growth rate. This is congruent with previous studies that showed that a small percentage of loci (less than 1.5%) exhibited both parallel patterns of divergence and significantly reduced gene flow between dwarf and normal whitefish ecotypes (Campbell and Bernatchez 2004; Rogers and Bernatchez 2005). Altogether, these studies are consistent with the general hypothesis that adaptive phenotypic divergence between closely related populations is accompanied by relatively few genetic changes, either in the form of structural or regulatory genes (Purugganan 1998). Such studies indicate that strong selective pressures may drive rapid adaptive divergence, and ultimately speciation, by involving similar transcriptional changes to the same genes belonging to fundamental gene network architectures, as was hypothesized by Haldane (1932).

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