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Oxidative phosphorylation gene transcription in whitefish species pairs reveals patterns of parallel and nonparallel physiological divergence

M. L. EVANS* & L. BERNATCHEZ

Institut de Biologie Intégrative et des Systèmes, Pavillon Charles-Eugène-Marchand, Université Laval, Québec, QC, Canada

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Abstract

Across multiple lakes in North America, lake whitefish (*Coregonus clupeaformis*) have independently evolved 'dwarf' and 'normal' sympatric species pairs that exhibit pronounced phenotypic and genetic divergence. In particular, traits associated with metabolism have been shown to be highly differentiated between whitefish species. Here, we examine the transcription of genes associated with the five mitochondrial and nuclear genome-encoded oxidative phosphorylation (OXPHOS) complexes, the primary physiological mechanism responsible for the production of ATP, in whitefish species pairs from Cliff Lake and Webster Lake in Maine, USA. We observed OXPHOS gene transcription divergence between dwarf and normal whitefish in each of the two lakes, with the former exhibiting transcription upregulation for genes associated with each of the OXPHOS complexes. We also observed a significant influence of lake on transcription levels for some of the genes, indicating that inter-lake ecological or genetic differences are contributing to variation in OXPHOS gene transcription levels. Together, our results support the hypothesis that metabolic divergence is a critical adaptation involved in whitefish speciation and implicate OXPHOS gene upregulation as a factor involved in meeting the enhanced energetic demands of dwarf whitefish. Further studies are now needed to evaluate the contribution of genetically vs. plasticity driven variation in transcription associated with this critical physiological pathway.

Introduction

Physiological traits represent some of the most promising for studies of adaptation, given that their function is tightly linked to environmental conditions (Pörtner *et al.*, 2010; Seebacher *et al.*, 2010; Storz *et al.*, 2010). Indeed, the oxidative phosphorylation (OXPHOS) pathway is now considered a prime candidate for studies of eco-physiological adaptation (Gershoni *et al.*, 2009; Ballard & Melvin, 2010). The OXPHOS pathway is composed of a series of five protein complexes that are embedded within the inner mitochondrial membrane layer and which ultimately generate the majority of energy (ATP) available for use in cellular processes (Saraste, 1999; Kadenbach, 2003). During OXPHOS, electrons derived from the oxidation of nutrients such as glucose are passed along the first four complexes, which is also known as the electron transport chain: NADH or ubiquinone oxidoreductase (complex I), succinate/ubiquinone reductase (complex II), cytochrome bc_1 (complex III) and cytochrome oxidase (complex IV; Saraste, 1999). The passage of electrons between the complexes collectively generates a proton gradient across the inner mitochondrial membrane, and the kinetic energy associated with this proton gradient is harnessed by OXPHOS complex V, ATP synthase, to generate ATP from ADP and inorganic phosphate (Saraste, 1999). Importantly, recent growth in the

Correspondence: Melissa L. Evans, Institut de Biologie Intégrative et des Systèmes, Pavillon Charles-Eugène-Marchand, 1030 Avenue de la Médecine, Université Laval, Québec, QC, G1V 0A6, Canada.
Tel.: +1 709 864 3708; fax: +1 709 864 3220;
e-mail: melissa.lea.evans@gmail.com
*Present address: Ocean Sciences Centre, Memorial University of Newfoundland, St John's, NL A1C 5S7, Canada.

number of genomic tools available for nonmodel organisms has now made it possible to study molecular and transcription-level variation in the five OXPHOS complexes and the potential role of OXPHOS in eco-physiological interactions.

A number of studies have indicated that both coding and transcription-level variation in OXPHOS genes represent physiological responses to environmental variation. For instance, nonsynonymous nucleotide substitutions have been observed at OXPHOS genes in a variety of taxa, and it has been suggested that these genetic changes translate into adaptive differences in aerobic capacity or thermal tolerance (e.g. Mishmar *et al.*, 2003; Ruiz-Pesini *et al.*, 2004; Dalziel *et al.*, 2006; Scott *et al.*, 2010; Garvin *et al.*, 2011). At the transcription level, rufous-collared sparrows (*Zonotrichia capensis*) living at high altitudes show OXPHOS gene upregulation compared to populations found at low altitudes, a difference that may facilitate adequate energy production under extreme environmental conditions including hypoxia and cold (Cheviron *et al.*, 2008). Similarly, cytochrome c oxidase gene upregulation is an important mechanism used to cope with the increased energetic demands found in some populations of marine snails (*Littorina saxatilis*; Martinez-Fernandez *et al.*, 2010). Thus, inter-population variation at genes associated with the OXPHOS complexes may accurately reflect functional genomic responses to divergent environments.

Adaptation to diverging environments may also result in the formation of locally adapted gene complexes and reproductive barriers. It has been suggested that OXPHOS genes may play a particularly important role in the evolution of reproductive barriers among populations, and as a mechanism leading to speciation (Gershoni *et al.*, 2009). Although the OXPHOS complexes are embedded within the inner mitochondrial membrane layer, they are encoded by both the nuclear and mitochondrial genomes, which are expected to be tightly co-adapted (Blier *et al.*, 2001; Gershoni *et al.*, 2009). In the marine copepod *Tigriopus californicus*, inter-population hybridization leads to a reduction in cytochrome c oxidase enzyme activity in hybrids, suggesting that the disruption of co-adapted nuclear and mitochondrial OXPHOS genes may adversely affect fitness and promote reproductive barriers in this species (e.g. Rawson & Burton, 2002; Ellison & Burton, 2008, 2010). Studies in anthropoids have shown that nuclear and mitochondrial genes associated with OXPHOS complexes III and IV exhibit elevated rates of positive selection compared to other complexes, which may indicate that these regions of the genome are co-evolving (e.g. Rawson & Burton, 2002; Grossman *et al.*, 2004). Furthermore, genes associated with OXPHOS complex V have been implicated in Atlantic eel species (*Anguilla* spp.) reproductive isolation via cytonuclear incompatibility (Gagnaire *et al.*, 2012). Indeed, it has been hypothesized that mismatched mitochondrial and nuclear components may lead to the enhanced production of reactive oxygen species (ROS), which may affect DNA or functional molecules within cells, or even result in complete mitochondrial dysfunction (Grossman *et al.*, 2004; Bayona-Bafaluy *et al.*, 2005; Reinecke *et al.*, 2009). Taken together, these studies indicate that the co-adaptation of nuclear and mitochondrial OXPHOS components play critical roles in delineating boundaries to gene flow among populations.

Sympatric dwarf and normal species pairs of the lake whitefish (*Coregonus clupeaformis*) provide an ideal system for investigations of physiological traits, such as OXPHOS, and their roles in ecologically driven divergence. In at least six lakes in the St. John River basin in eastern North America, dwarf and normal whitefish have undergone repeated phenotypic divergence accompanied by variable degrees of reproductive isolation following the recession of the last glaciation event ~15 000 years BP (Pigeon *et al.*, 1997; Bernatchez *et al.*, 2010). Dwarf whitefish have evolved from the ancestral benthic form in response to competition deriving from the secondary contact of two previously allopatric benthic populations (Bernatchez *et al.*, 2010). Dwarfs allocate a greater proportion of their energy budget to metabolism, exhibiting a lower bioenergetic conversion efficiency compared to normal whitefish (growth rate/consumption rate ratio; Trudel *et al.*, 2001). Furthermore, microarray- and qPCR-based studies of gene transcription in whitefish have revealed marked differences between the dwarf and normal whitefish, with dwarfs typically exhibiting upregulation at genes associated with metabolism (Derome *et al.*, 2006; St-Cyr *et al.*, 2008; Jeukens *et al.*, 2009) and oxygen transport (Evans *et al.*, 2012). These differences may be genetically determined as other studies have shown divergence between dwarf and normal whitefish in genes associated with energy production (Renaut *et al.*, 2010, 2011). The allocation of energy towards metabolism by the dwarf whitefish is likely to have facilitated the alternative foraging and predator avoidance tactics needed to colonize the limnetic zone (Rogers *et al.*, 2002; Landry *et al.*, 2007; Landry & Bernatchez, 2010; also see Kahilainen & Lehtonen, 2003). Thus, studies of candidate genes associated with the OXPHOS pathway should help to further elucidate the key traits associated with whitefish expansion into divergent ecological niches and the factors contributing to the maintenance of reproductively isolated populations in sympatry.

Here, we test for OXPHOS complex I–V gene transcription divergence between dwarf and normal lake whitefish from Cliff and Webster lakes in Maine, USA. We predicted that dwarf whitefish would show higher levels of OXPHOS gene transcription compared to the normal whitefish, given the previously observed differences in metabolism between the two species. Whitefish from Webster Lake have not previously been

examined for transcription levels at any genes. However, morphological and population genetic differentiation is lower, and levels of gene flow are higher, between dwarf and normal whitefish from Webster Lake compared to Cliff Lake (Campbell & Bernatchez, 2004; Renaut *et al.*, 2011). Thus, we predicted that dwarf and normal whitefish from Cliff Lake would exhibit greater divergence in OXPHOS gene transcription levels compared to Webster Lake. We explored transcription correlations across all of the OXPHOS complexes using multivariate and correlation analyses, as these genes are expected to be tightly co-adapted. In particular, we highlight our results for the correlations between the nuclear and mitochondrial genes within complexes I, and III–V, given the potential role of genomic uncoupling in promoting species boundaries. Gene transcription coupling (correlation) was examined separately in each of the lakes, as the relatively high levels of gene flow in Webster compared to Cliff Lake could contribute to the breakdown gene transcription coordination within OXPHOS complexes (Ellison & Burton, 2008).

Methods

Sample collection

Dwarf and normal lake whitefish were sampled from Cliff (46°23′51″ N, 69°15′05″ W) and Webster lakes (46° 09′21″ N, 69°04′45″ W) in Maine, USA, in June 2010. Fish were caught live with gillnets, euthanized and immediately dissected to obtain fresh tissue samples and to determine sex. Approximately 30 mg of fresh muscle tissue was collected from each individual. Muscle was collected from the left side of each fish immediately above the lateral line and just anterior to the dorsal fin. Muscle samples were flash frozen in liquid nitrogen and later stored at −80 °C. We analysed gene transcription values in tissue samples from a total of 10 dwarf and 10 normal whitefish from Cliff Lake and 12 dwarf and 11 normal whitefish from Webster Lake.

Whitefish from Cliff and Webster lakes bear mitochondrial DNA descended from Atlantic and Acadian lineages (Pigeon *et al.*, 1997). Previous studies have shown that Cliff Lake dwarfs and normals are, respectively, fixed for the ancestral Acadian and Atlantic mitochondrial lineages (Bernatchez & Dodson, 1990; Pigeon *et al.*, 1997). However, both mitochondrial lineages are present in each of the dwarf and normal whitefish from Webster Lake (Pigeon *et al.*, 1997) due to introgressive hybridization in that lake (Lu *et al.*, 2001). To account for the potential influence of ancestral mitochondrial genomic background on gene transcription, we examined the mitochondrial lineage of six of the dwarf and eight of the normal whitefish from Webster Lake. Mitochondrial lineage was determined by sequencing a portion of the mitochondrial control (D-loop) region from each fish (Lu *et al.*, 2001). Briefly, DNA was isolated from fin clips using a salt extraction technique (Aljanabi & Martinez, 1997), and the primers TPro2 (5′-ACCCTTAACTCCCAAAGC-3′) and HN20 (5′-GTGTTATGCTTTAGTTAAGC-3′) were used to amplify the target gene (Gilbert *et al.*, 1988; Bernatchez & Danzmann, 1993).

RNA extraction and cDNA preparation

Total RNA was isolated from the muscle using the Ambion PureLink RNA Mini Kit according to the manufacturer's instructions (Ambion, Austin, TX, USA). All RNA extractions were treated with Superase-In RNase inhibitor and DNase I following the manufacturer's instructions (Ambion). RNA was quantified with the Nanodrop 2000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA), and RNA integrity was confirmed using the Experion Automated Electrophoresis system (Bio-Rad, Mississauga, ON, Canada). Complementary DNA (cDNA) synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Reverse transcription reactions were performed in 20-μL volumes containing 500 ng RNA and using random primers.

Oxidative phosphorylation gene transcription assays

Annotated whitefish transcriptome sequences (see Renaut *et al.*, 2010) were used as templates in the design of primers and TaqMan minor groove binder probes for use in quantitative PCR (qPCR) evaluation of OXPHOS gene transcription (Table 1, Ambion). Primers and probes were designed using Primer Express v.3.0 software (Applied Biosystems), and primer specificity was tested using conventional PCR and visualization of a single amplification product on agarose gel. In total, a set of 10 OXPHOS gene assays were developed. Specifically, assays were developed to evaluate transcription levels for one nuclear and one mitochondrial-encoded gene associated with complexes I, III and IV, one nuclear and two mitochondrial-encoded genes associated with complex V and one nuclear gene associated with complex II (Gershoni *et al.*, 2009; Table 1). However, gene transcription levels for the complex II assay were too low to be reliably evaluated and thus were excluded from our analyses.

qPCR analysis

We assessed the efficiency of each of the qPCR assays through a validation experiment (Applied Biosystems). We then utilized the comparative C_T method ($\Delta\Delta$ C_T method, Applied Biosystems) to quantify inter-individual variation in the relative amounts of target OXPHOS cDNAs in the muscle tissue. This method calculates the

Table 1 TaqMan qPCR assays used to evaluate oxidative phosphorylation (OXPHOS) transcription levels in the lake whitefish (*Coregonus clupeaformis*). TaqMan assays were designed in Primer Express v. 3.0, and the efficiency of each assay was tested in a validation experiment. Probe, forward (F) and reverse (R) primer sequences are indicated.

Target Gene*	Encoding Genome†	OXPHOS Complex	Primers (5′–3′)	Probe (5′–3′)	Efficiency (%)‡
NADH-ubiquinone oxidoreductase 75-kDa subunit (NADH75-N-I)	Nuclear	I	F-CAGGCCAACCAACTCTCACAR-GAGGAGAAACGAGAGGATTTGC	TGTGAATCAGACTCTTC	100.7
Succinate dehydrogenase (SUCC-N-II)	Nuclear	II	F-GCTACTGGTGGAATGGAGACAAG R-CCAACGGTAGGCCTGCATAA	ACCTGGGACCCGCTGT	98.2
Cytochrome b–c1 complex subunit 8 (CYTB-N-III)	Nuclear	III	F-GCCAAGATCAGGCATGTGATT R-GGAAAGCCCTCTGCTCGAA	CCTACAGTCTGTCCCC	100.9
Cytochrome c oxidase subunit 6A (CYTC-N-IV)	Nuclear	IV	F-CTTACCTGGTGTGGCCGTATG R-GGAGTGCTGCTGCATCTTCA	ATCGCCAACGCCTAC	100.1
ATP synthase subunit d (ATP-N-V)	Nuclear	V	F-AGCCGCGATCAACACACA R-GACGTAGGCAACAGCAGCTTT	AGGCCGAGGCGAA	119.4
NADH-ubiquinone oxidoreductase chain 5 (NADH5-M-I)	Mitochondrial	I	F-GGTCACCATCGGACTTAACCA R-GGCGTGGGTACAGATGTGAA	CCACAGCTGGCCTT	104.3
Cytochrome b (CYTB-M-III)	Mitochondrial	III	F-GCCATATTTGCCGAGATGTCA R-TGCTCCATTGGCGTGAATATT	CGGCTGACTTATCCG	100.8
Cytochrome c oxidase subunit 3 (CYTC-M-IV)	Mitochondrial	IV	F-GAGAGGGCACATTCCAAGGA R-TGCCGTAGCGTAGGCCTTT	ACCACACGCCCCC	98.4
ATPase subunit 6 (ATP6-M-V)	Mitochondrial	V	F-TCCGCCCCCTTGCTCTAG R-TAGAAGGTGGCCTGCCGTAA	CGTACGGCTTACAGCCA	86.1
ATPase subunit 8 (ATP8-M-V)	Mitochondrial	V	F-CTAACTGTTATTCCCCCCAAAGTC R-TGCTTTGTGAGGTAGGCTCATTT	TTGGCCACACCTTC	95.4

*The name of the putative target gene is based on sequence alignment with the annotated lake whitefish transcriptome. The gene name abbreviation is shown in brackets.

†The coding genome was determined based on the alignment of primer and probe sequences with the *Coregonus lavaretus* mitochondrial DNA sequence.

‡TaqMan efficiencies can occasionally exceed 100% when RT reactions are saturated at higher template concentration points in the calibration test.

relative quantity (RQ) of cDNA compared to a reference sample (calibrator), and RQ values represent fold changes in transcription relative to the reference sample, which has a value of one (Applied Biosystems). We amplified target OXPHOS genes from the cDNA in triplicate using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems) and TaqMan Universal PCR Master Mix (Applied Biosystems). All quantitative PCRs (validation and comparative C_T) were conducted in 10-μL reactions, and we used the default thermocycler setting for Fast qPCR TaqMan assays, consisting of an initial denaturation step at 95 °C for 20 s, and then 40 cycles of 95 °C for 3 s and 60 °C for 30 s of annealing/extension. The quantity of amplification product was assessed following each PCR cycle. We used ABI's Human Euk 18S rRNA gene assay (Applied Biosystems) as an endogenous control to normalize the total quantity of cDNA loaded into the PCR wells. The Human Euk 18S gene has previously been shown to be appropriate for the normalization of transcription assays in whitefish (Jeukens *et al.*, 2009) and in other salmonids (Olsvik *et al.*, 2005; Jørgensen *et al.*, 2006). We ran an RT reaction without reverse transcriptase and tested for amplification in the qPCRs to ensure the efficacy of our DNase treatment (Bustin *et al.*, 2009). We also included negative controls (no template) in each qPCR.

Statistical analyses

We examined the influence of mitochondrial lineage (Atlantic, Acadian) on gene transcription levels (RQ) in Webster Lake using a multivariate analysis of variance (MANOVA) that included all nine OXPHOS genes as dependent variables, and species (dwarf or normal) and mitochondrial lineage as fixed factors. The identity function was used as the response specification. MANOVA was also used to investigate differences in transcription of the nine OXPHOS genes between the two lakes and species. In this model, lake and species (main effects), and the interaction between lake and species, were included as fixed factors. Sex may affect the transcription of genes in the lake whitefish (Jeukens *et al.*, 2009), and thus, we also included sex as a fixed factor in the model. As the multivariate gene transcription response variable was not normally distributed, we examined the significance of the MANOVA model using Pillai's Trace statistic, as this statistic is robust to deviations from normality (Zar, 1999). To

visually examine standardized multivariate gene transcription differences among individual whitefish, we calculated canonical scores using the eigenvectors associated with the first two MANOVA roots, which were significant for both Cliff and Webster lake whitefish. To ensure that we had sufficient power to detect any differences in transcription levels among the groups, we ran a *post hoc* power analysis on our data set in G*Power v.3.0 (Faul *et al.*, 2007). Using a MANOVA test with repeated measures and within–between interaction specifications at $\alpha = 0.05$, our power $(1 - \beta)$ to detect differences among the groups was 0.96.

To confirm any observed relationships between the lake, species and lake × species factors and OXPHOS gene transcription, we conducted a permutation test in SAS (SAS Institute Inc., Cary, NC, USA). Permutation tests are distribution-free tests, and thus, the results of this analysis are robust to violations of normality. In the permutation test, the *F*-statistic associated with each factor, derived from the original MANOVA test, was compared to a distribution of *F*-statistics calculated from a randomization of group assignments (lake, species and lake × species) across the observed gene transcription values. The randomization was repeated for 1000 iterations to generate the distribution of *F*-statistics. When the observed (original) *F*-statistic fell significantly ($P < 0.05$) outside of the permutation distribution, this was taken as evidence of a relationship between that factor and gene transcription levels (see Good, 2000).

Univariate two-way ANOVAs were run following the MANOVA to examine the influence of lake, species, the lake × species interaction and sex on the transcription of individual genes. We used Spearman's rank correlations to examine gene transcription-level similarities among the complexes and between the mitochondrial and nuclear genes within each of the OXPHOS complexes in Cliff and Webster lakes. All statistical tests, excluding the permutation test, were run in JMP v.9.0 using an alpha = 0.05 as the threshold for statistical significance. Means are reported ±1 SD.

Results

Transcription levels between species and lakes

We did not observe an effect of mitochondrial lineage on OXPHOS gene RQ in whitefish from Webster Lake (MANOVA model: Pillai's Trace = 1.52, $F_{8,18} = 1.41$, $P = 0.321$; species: $F_{3,9} = 1.68$, $P = 0.366$; mtDNA lineage: $F_{3,9} = 0.75$, $P = 0.678$). However, across the two lakes, we observed significant lake, species and lake × species effects on RQ for the complement of OXPHOS genes (Table 2). The significant interaction between lake and species indicates that while the levels of OXPHOS gene transcription found in dwarf and normal whitefish differ, the effect of species on transcription levels of each of the genes also varies by lake.

In the permutation analysis, the observed F-statistics associated with the lake, species and lake × species factors were significantly higher than expected when compared to the distribution of F-statistics generated when lake, species and lake × species identities were randomized across the observed gene RQ values (lake: $P < 0.001$; species: $P = 0.020$; lake × species: $P = 0.031$). These results confirmed the MANOVA results, indicating that significant differences in OXPHOS gene transcription occur both between dwarf and normal whitefish and between Webster and Cliff lakes, independent of species.

Given the significant interaction effect in the MANOVA model, we present the results of this analysis separately for Webster and Cliff lakes. For Cliff Lake, canonical axes one and two explained 31% and 28% of the variation in multivariate OXPHOS gene RQ, respectively. For Webster Lake, axes one and two explained 38% and 33% of the variation in OXPHOS gene RQ, respectively. Projections of the canonical scores for the dwarf and normal whitefish show clear gene transcription differentiation between species in each of the lakes (Fig. 1a,b). For Cliff Lake, dwarf and normal whitefish exhibited group differentiation primarily along axis one, with dwarfs exhibiting higher canonical scores than normals (Fig. 1a). Higher canonical scores along axis one in Cliff Lake are associated with higher RQ for all nine of the OXPHOS genes included in the model, all of which showed positive loadings along this axis (Fig. 1c). In Webster Lake, the canonical scores for dwarf and normal whitefish gene RQ exhibited more overlap than in Cliff Lake (Fig. 1b). Here, gene transcription differentiation is driven along both axes, with Webster dwarfs tending to exhibit lower and higher canonical scores, respectively, along axes one and two compared to the normal whitefish (Fig. 1b). In particular, lower scores along axis one are associated with higher RQ at the NADH5-M-I,

Table 2 Results of MANOVA examining the influence of lake and species on oxidative phosphorylation (OXPHOS) gene transcription in the lake whitefish (*Coregonus clupeaformis*). Sex was also included as a fixed factor in the model. The overall model was tested using Pillai's Trace statistic and each factor using approximate *F*-statistics. Numerator and denominator degrees of freedom (Num d.f., Denom d.f.) and the significance of the relationship between gene transcription and each factor (*P*-value) are indicated. Significant *P*-values (<0.05) are indicated in bold.

	Pillai's Trace	*F*	Num d.f., Denom d.f.	*P*
Model	1.42	2.01	36,132	**0.002**
Intercept		33.87	9,30	< **0.001**
Lake		3.26	9,30	< **0.001**
Species		2.41	9,30	**0.034**
Lake × Species		2.25	9,30	**0.047**
Sex		0.57	9,30	0.812

CYTB-N-III, ATP8-M-V and NADH75-N-I genes, whereas higher scores along axis two are associated with higher RQ at the CYTC-M-IV and ATP8-M-V genes (Fig. 1d).

The univariate ANOVAs revealed a lake (main) effect on RQ of both of the complex I genes (NADH5 and NADH75) and the ATP6-M-V gene, with both dwarfs and normals in Cliff Lake exhibiting significantly higher transcription levels than observed in Webster Lake whitefish (Table 3; *post hoc* Student's *t*-test: $P < 0.05$). We observed a significant species (main) effect on RQ levels of OXPHOS genes NADH5-M-I, CYTB-M-III and ATP8-M-V (Table 3) with dwarfs showing gene upregulation in both Cliff and Webster lakes (*post hoc* Student's *t*-test: $P < 0.05$, Fig. 2). We also observed a near-significant trend for dwarf whitefish to exhibit higher CYTC-M-IV RQ than normals (Table 3, Fig. 2). Higher-order lake × species effects were observed at the CYTB-N-III and CYTC-N-IV genes. Specifically, Cliff dwarfs exhibited CYTB-N-III gene upregulation compared to Webster dwarfs and normal whitefish from both lakes (Tukey's *post hoc* test: $P < 0.05$). Cliff dwarfs also exhibited significant gene upregulation compared to Cliff normals at the CYTC-N-IV gene (Tukey's *post hoc* test: $P < 0.05$; Table 3, Fig. 2), whereas no difference was observed between dwarfs and normals from Webster Lake. Sex was not a significant predictor of RQ for any of the genes (Table 3).

Nuclear and mitochondrial gene transcription correlation

Relative transcription quantities (RQ) for the nuclear and mitochondrial genes associated with complex I were not significantly correlated in either Cliff or Webster Lake (Table 4). However, the RQ of nuclear and mitochondrial genes within complexes III and IV were significantly correlated (Table 4). For complex V, the nuclear and mitochondrial genes exhibited uncorrelated RQ, whereas the two mitochondrial complex V genes,

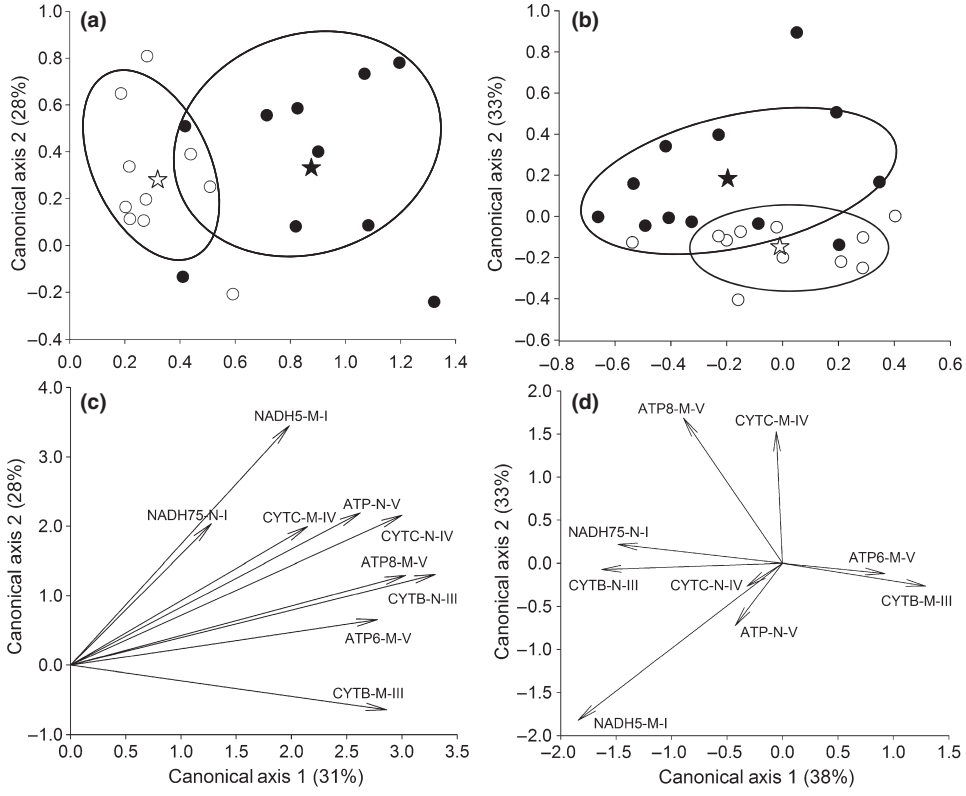

Fig. 1 MANOVA results examining the influence of species (dwarf, normal) on oxidative phosphorylation (OXPHOS) gene transcription levels in lake whitefish (*Coregonus clupeaformis*) from Cliff and Webster lakes in Maine, USA. (a, b) show OXPHOS gene transcription canonical scores projected onto canonical axes one and two, for dwarf (black circles) and normal (open circles) whitefish examined in Cliff and Webster lakes, respectively. The canonical scores for each individual were calculated from the eigenvectors of the first two MANOVA roots. The stars within each figure represent the mean canonical scores, and the ellipses encompass 75% of the data points for each species. (c, d) show the mean centroid values for each of the nine OXPHOS genes plotted across the first two canonical axes for Cliff and Webster lakes, respectively.

Table 3 Results of univariate ANOVA tests examining the contribution of lake and species to the transcription of oxidative phosphorylation (OXPHOS) genes in lake whitefish (*Coregonus clupeaformis*). Definitions for the abbreviated gene names can be found in Table 1. For each factor, the *F*-statistic and *P*-value (in brackets) are indicated. Model and error degrees of freedom (d.f.) are indicated for each model, and d.f. for each factor are indicated. Significant *P*-values (<0.05) are indicated in bold text.

	DF	NADH75-N-I	NADH5-M-I	CYTB-N-III	CYTB-M-III	CYTC-N-IV	CYTC-M-IV	ATP-N-V	ATP6-M-V	ATP8-M-V
Model	4,38	1.89 (0.131)	3.65 (**0.013**)	11.74 (<**0.001**)	2.14 (0.094)	4.17 (**0.006**)	1.31 (0.285)	1.63 (0.186)	10.44 (<**0.001**)	2.91 (**0.034**)
Lake	1	5.64 (**0.023**)	4.41 (**0.043**)	23.97 (<**0.001**)	0.60 (0.443)	0.68 (0.414)	0.01 (0.987)	1.99 (0.166)	28.76 (<**0.001**)	2.72 (0.107)
Species	1	1.07 (0.307)	4.30 (**0.045**)	17.30 (<**0.001**)	7.43 (**0.001**)	7.16 (**0.011**)	3.89 (0.056)	0.01 (0.982)	3.42 (0.072)	7.08 (**0.011**)
Lake × Species	1	1.07 (0.307)	2.63 (0.113)	4.22 (**0.047**)	0.01 (0.998)	7.59 (**0.009**)	0.18 (0.672)	0.09 (0.756)	2.69 (0.109)	0.04 (0.838)
Sex	1	0.04 (0.828)	1.46 (0.235)	0.89 (0.895)	1.21 (0.279)	1.26 (0.269)	1.26 (0.267)	2.37 (0.131)	0.84 (0.364)	0.86 (0.361)

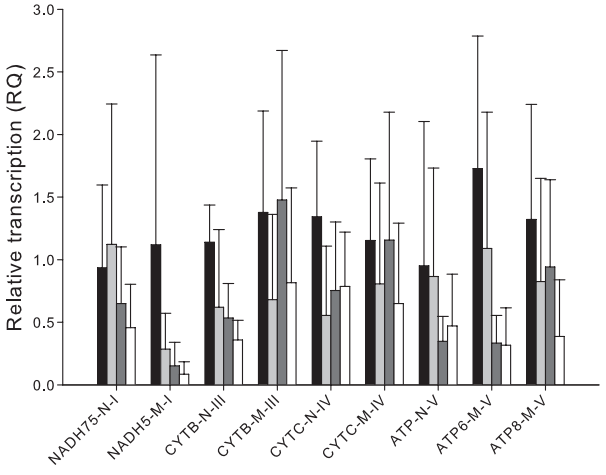

Fig. 2 Variation in oxidative phosphorylation (OXPHOS) gene transcription levels in dwarf and normal lake whitefish (*Coregonus clupeaformis*) from Cliff and Webster lakes in Maine, USA. Relative gene transcription quantities (relative quantity, RQ) ± 1 SD are shown for each of the nine OXPHOS genes examined in Cliff Lake dwarfs (black bars), Cliff Lake normals (light grey bars), Webster Lake dwarfs (dark grey bars) and Webster Lake normals (white bars). The gene name abbreviations shown on the *x*-axis correspond to those outlined in Table 1. See Table 3 for the results of the univariate ANOVA models examining the contribution of species (dwarf, normal) and lake to variation in OXPHOS gene transcription. Note that RQ cannot be compared among genes as different calibration samples were used across some of the gene transcription assays.

Table 4 Correlation of gene transcription quantities (relative quantity) within each of the nuclear and mitochondrial-encoded oxidative phosphorylation (OXPHOS) complexes (I, III–V) in the lake whitefish (*Coregonus clupeaformis*) from Cliff and Webster lakes in Maine, USA. Spearman's rank correlation coefficients (ρ) and *P*-values for each test are shown. Significant *P*-values (<0.05) are indicated in bold text.

		Cliff Lake		Webster Lake	
Complex	Genes	ρ	*P*	ρ	*P*
I	NADH5-M and NADH75-N	−0.014	0.955	0.372	0.081
III	CYTB-M and CYTB-N	0.596	**0.006**	0.723	<**0.001**
IV	CYTC-M and CYTC-N	0.553	**0.011**	0.668	<**0.001**
V	ATP6-M and ATP-N	−0.018	0.939	0.078	0.723
V	ATP8-M and ATP-N	−0.057	0.811	0.228	0.295
V	ATP6-M and ATP8M	0.532	**0.016**	0.610	**0.002**

ATP6 and ATP8, showed significantly correlated RQ in both lakes (Table 4). Examining all genes, we observed 18 significant and three nearly significant correlations in transcription levels between genes in Cliff Lake and 17 significant and two nearly significant correlations in transcription levels between genes in Webster Lake (Table S1).

Eight and 10 of these significant correlations were observed between the five mitochondrially encoded genes in Cliff Lake and Webster Lake, respectively.

Discussion

Parallelism in OXPHOS gene transcription

The pronounced parallel divergence of metabolic traits between multiple pairs of dwarf and normal whitefish has led to their recognition as key attributes involved in ecological speciation (Bernatchez *et al.*, 2010). Here, we found strong evidence of differential gene transcription in the muscle tissues of dwarf and normal whitefish from Cliff and Webster lakes through both multivariate and univariate analyses of gene transcription patterns. Indeed, at least one gene from each of OXPHOS complexes I, III, IV and V exhibited significant or near-significant upregulation in dwarf compared to normal whitefish in both lakes. These results support previous research showing higher levels of transcription in dwarf compared to normal whitefish for genes associated with metabolism (Derome *et al.*, 2006; St-Cyr *et al.*, 2008). For example, Derome *et al.* (2006) used the salmonid 3557 cDNA microarray to examine differential gene transcription in the muscle tissues of lake whitefish from Cliff Lake and Indian Pond in Maine, USA, and found evidence of upregulation in Cliff dwarfs for two ESTs associated with cytochrome c oxidase (complex IV), albeit the same pattern was not observed in Indian Pond dwarfs. However, the authors did observe gene transcription upregulation in dwarfs from both populations at an EST associated with ATP synthase (Derome *et al.*, 2006). Therefore, our results confirm previously observed patterns of OXPHOS gene transcription upregulation in dwarf whitefish from microarray studies. Moreover, the observed parallelism in OXPHOS gene transcription divergence between dwarf and normal whitefish from Cliff and Webster lakes lends further support to the hypothesis that metabolic traits play a key role in the diversification of the lake whitefish.

Diverging ecological factors, both biotic and abiotic, could contribute to the general pattern of OXPHOS gene upregulation observed in dwarf compared to normal whitefish (see Guderley, 2004). For instance, the upregulation of genes associated with OXPHOS has been observed in other systems, in response to both cold temperatures and hypoxia-induced stress (e.g. Gracey *et al.*, 2001, 2004; Storey, 2007; Cheviron *et al.*, 2008). While hypoxia and temperature differences do occur between lake zones in Webster and Cliff lakes, these factors are unlikely to explain the observed upregulation of gene transcription in the dwarf whitefish, as, at least in the summer months, dwarf and normal whitefish experience similar temperatures and oxygen saturation levels (Landry *et al.*, 2007). However, there could be divergent energetic demands placed on dwarf and normal whitefish due to differences in prey utilization or predation pressures. Studies have shown that dwarf whitefish typically forage in the limnetic environment on zooplankton, whereas normal whitefish forage on benthic macroinvertebrates (Trudel *et al.*, 2001; Landry *et al.*, 2007; Landry & Bernatchez, 2010; also see Robinson & Wilson, 1994; Kahilainen *et al.*, 2004). While we are not currently aware of any studies examining the influence of diet of OXPHOS gene transcription in wild populations, a recent study in cattle showed an influence of feed type on the transcription of genes associated with OXPHOS (Kelly *et al.*, 2011). Little is currently known about predation pressures faced by the dwarf and normal whitefish in Cliff and Webster lakes or the potential influence of predation on lake whitefish metabolism. However, studies of European whitefish (*C. lavaretus*) have shown that dwarfs face greater predation pressures (Kahilainen & Lehtonen, 2003), which may contribute to the evolution of their smaller, more streamlined size and associated differences in behaviour and metabolism compared to normal whitefish (Trudel *et al.*, 2001). Furthermore, increased predation risk is associated with the upregulation of genes associated with metabolism in the stickleback (*Gasterosteus aculeatus*), albeit this has only been examined in brain tissues (Sanogo *et al.*, 2011). Taken together, the results from this and other studies suggest that ecological factors, including differences in diet and predation risk, are important drivers of OXPHOS gene transcription divergence between dwarf and normal whitefish.

Nonparallel patterns of OXPHOS gene transcription between lakes

Natural replicates of ecological speciation events provide an exceptional opportunity to examine not only the factors driving trait parallelism but also those contributing to nonparallel variation (Landry & Bernatchez, 2010; Rosenblum & Harmon, 2011; Fan *et al.*, 2012; Kaeuffer *et al.*, 2012). While we observed parallel patterns of transcription divergence between dwarf and normal whitefish in Cliff and Webster lakes for some of the OXPHOS genes, we also observed significant differences between the lakes, independent of species (Landry & Bernatchez, 2010; also see Kaeuffer *et al.*, 2012). First, whitefish from Cliff Lake generally exhibited higher levels of gene transcription than in Webster Lake for the nuclear and mitochondrial complex I genes and the ATP subunit 6 gene. We also observed significant upregulation in dwarfs at the nuclear cytochrome b and cytochrome c genes in Cliff Lake but not in Webster Lake. Ecological variation between the two lakes examined in this study could have profound effects on the patterns of observed OXPHOS gene transcription. For example, Webster and Cliff lakes are significantly

diverged in prey availability and size, with Webster Lake exhibiting larger prey at lower densities than is found in Cliff Lake (Landry & Bernatchez, 2010). Furthermore, gill raker morphology shows more overlap between dwarf and normal whitefish in Webster than in Cliff Lake, suggesting that that two species exploit relatively similar foraging opportunities in Webster Lake (Landry *et al.*, 2007). Our results suggest that natural replicates of whitefish species pairs exhibit as many differences as they do similarities in patterns of OXPHOS gene transcription, which could be related to the nonparallel ecological conditions experienced by whitefish among lakes.

In addition to ecological factors, nonparallel patterns of OXPHOS gene transcription could be related to differences in the genetic background of the whitefish populations. Dwarf and normal whitefish exhibit lower levels of genetic differentiation and more gene flow in Webster than in Cliff Lake (Bernatchez & Dodson, 1994; Pigeon *et al.*, 1997; Lu & Bernatchez, 1999; Campbell & Bernatchez, 2004; Renaut *et al.*, 2011), which could contribute to the less-pronounced divergence we observed in OXPHOS gene transcription between the species in Webster Lake. We found no evidence that ancestral mitochondrial lineage influences gene transcription levels, albeit our ability to relate mitochondrial haplotype to transcription was somewhat constrained by sample size and thus requires further study. However, it is also possible that more recently derived genetic differences between the populations could play a role in mediating OXPHOS gene transcription. For instance, a recent study of functional genetic differentiation between dwarf and normal whitefish showed that single nucleotide polymorphism (SNP) markers associated with NADH-ubiquinone oxidoreductase chain 5 (complex I) and cytochrome c oxidase subunit 3 (complex IV) are highly diverged between dwarf and normal whitefish in Cliff Lake, but exhibit no differentiation in Webster Lake (Renaut *et al.*, 2011). Thus, there is at least some evidence that coding regions associated with OXPHOS genes exhibit differing genetic backgrounds, which could lead to variation in gene transcription levels between the populations.

Gene translation, whereby the products of gene transcription (messenger RNA) are decoded into amino acids, has also been implicated as a potentially important mechanism contributing to metabolic capacities in fish species. Indeed, differences in mitochondrial content among individuals may relate as much to translational differences as gene transcription (Hardewig *et al.*, 1999; Moyes, 2003). In a study of billfish (Xiphiidae and Istiophoridae) and tuna (*Thunnus* spp.), Dalziel *et al.* (2005) observed no difference between the two groups in transcription levels of citrate synthase, an indicator of mitochondrial content in cells. However, the groups exhibited significant differences in citrate synthase enzyme content in their muscle, suggesting that gene translation is the major factor regulating levels of this enzyme (Dalziel *et al.*, 2005). Here, we observed differences among populations in the transcription profiles of OXPHOS genes, but it is possible that dwarf whitefish from Cliff and Webster lakes take different functional pathways to colonize the limnetic zones in each lake (see Derome *et al.*, 2006). Thus, the functional significance of our results must be interpreted with some caution until studies of enzymatic function in dwarf and normal whitefish from each of the lakes can be conducted.

Nuclear and mitochondrial OXPHOS gene co-transcription

It has been suggested that the co-adaptation of nuclear and mitochondrial genes of the OXPHOS pathway is an important mechanism contributing to the formation of reproductive barriers between locally adapted populations (Blier *et al.*, 2001; Gershoni *et al.*, 2009). In whitefish, we found evidence of correlated nuclear and mitochondrial gene transcription within OXPHOS complexes III and IV, suggesting that the nuclear and mitochondrial genomic regions encoding this portion of the OXPHOS pathway are co-adapted. In contrast, the transcription of the nuclear and mitochondrial genes examined from complexes I and V appears to be uncorrelated. Complex IV is thought the be the rate-limiting complex in OXPHOS, and complex III is one of the primary producers of ROS; therefore, the consequences of nuclear and mitochondrial transcription decoupling for these complexes may be of particular importance to individual fitness (see Lenaz, 2001; Grossman *et al.*, 2004). Similar to our results, studies in humans have shown that OXPHOS complex I genes exhibit uncorrelated transcription, and it has been suggested that the widespread genomic distribution of the genes encoding for the ~43 complex I subunits may complicate coordinated transcription (Garbian *et al.*, 2010; also see Van Waveren & Moraes, 2008). Thus, it is possible that our results for complex I and complex V, which is composed of the largest number of subunits save for complex I (Ballard & Melvin, 2010), reflect similarly complicated regulation of nuclear and mitochondrial gene transcription. Overall, our results show that correlated transcription of nuclear and mitochondrial-encoded genes occurs in the lake whitefish and suggest that transcription coordination is of particular importance in the central complexes of the OXPHOS pathway.

In contrast to our prediction of lower nuclear and mitochondrial gene transcription correlation in Webster compared to Cliff Lake, we observed similar patterns in the two lakes. This result suggests that gene flow between dwarf and normal whitefish may not be an important factor influencing the coordination of OXPHOS gene transcription. However, it is possible that

the natural rates of gene flow between dwarf and normal whitefish are too low in either of the lakes examined to cause a breakdown in OXPHOS gene transcription. Indeed, estimates of migration rate (per generation) in Cliff and Webster lakes are only 0.0004 and 0.0006, respectively (Campbell & Bernatchez, 2004). Experimental studies examining OXPHOS gene transcription in dwarf–normal hybrids are needed to investigate how mitochondrial and nuclear genomic mismatch may contribute to the breakdown of co-adapted OXPHOS genes, particularly in complexes III and IV, and to test whether the decoupling of genes contributes to reduced fitness and reproductive isolation.

Conclusions

This study points to the importance of eco-physiological interactions in the repeated ecological speciation of the lake whitefish. Dwarf whitefish showed OXPHOS gene upregulation in both Webster and Cliff lakes, suggesting that parallel ecological conditions faced in the two lakes lead to the requirement for enhanced ATP production via OXPHOS in dwarfs. Further studies are needed to examine the extent to which these differences are regulated via plastic responses to environmental variation and evolutionary genetic changes in each population. Nevertheless, our results, in combination with previous studies that have shown a heritable basis for transcription variation in other populations, point to OXPHOS gene transcription divergence as a factor potentially involved in delineating reproductive barriers between dwarf and normal whitefish (St-Cyr *et al.*, 2008; Whiteley *et al.*, 2008; see Gershoni *et al.*, 2009). The patterns of OXPHOS gene transcription were also highly variable between the two lakes potentially reflecting divergence in response to nonparallel ecological conditions, differing evolutionary (genetic) backgrounds, or variation in the functional pathways used to achieve similar phenotypes in each lake. Contemporary ecological and historical genetic differences across lakes could lead to an evolutionary trade-off that influences the trajectory of gene transcription divergence in each species pair. Our results thus highlight the importance of investigating parallel and nonparallel aspects of trait variation to fully understand how ecologically driven selection produces optimal phenotypes in nature.

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Data accessibility

Gene transcription and mtDNA haplotype data are available at DRYAD doi:10.5061/dryad.s4kc8.

References

Aljanabi, S.M. & Martinez, I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res.* **25**: 4692–4693.

Ballard, J.W. & Melvin, R.G. 2010. Linking the mitochondrial genotype to the organismal phenotype. *Mol. Ecol.* **19**: 1523–1539.

Bayona-Bafaluy, M.P., Muller, S. & Moraes, C.T. 2005. Fast adaptive coevolution of nuclear and mitochondrial subunits of ATP synthetase in orangutan. *Mol. Biol. Evol.* **22**: 716–724.

Bernatchez, L. & Dodson, J.J. 1990. Allopatric origin of sympatric populations of lake whitefish (*Coregonus clupeaformis*) as revealed by mitochondrial-DNA restriction analysis. *Evolution* **44**: 1263–1271.

Bernatchez, L. & Danzmann, R.G. 1993. Congruence in control-region sequence and restriction-site variation in mitochondrial-DNA of brook charr (*Salvelinus fontinalis* Mitchill). *Mol. Biol. Evol.* **10**: 1002–1014.

Bernatchez, L. & Dodson, J.J. 1994. Phylogenetic relationships among palearctic and nearctic whitefish (*Coregonus* sp.) populations as revealed by mitochondrial DNA variation. *Can. J. Fish. Aquat. Sci.* **51**: 240–251.

Bernatchez, L., Renaut, S., Whiteley, A.R., Derome, N., Jeukens, J., Landry, L. *et al.* 2010. On the origin of species: insights from the ecological genomics of lake whitefish. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **365**: 1783–1800.

Blier, P.U., Dufresne, F. & Burton, R.S. 2001. Natural selection and the evolution of mtDNA encoded peptides: evidence for intergenomic co-adaptation. *Trends Genet.* **17**: 400–406.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Hugget, J., Kubista, M. *et al.* 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* **55**: 611–622.

Campbell, D. & Bernatchez, L. 2004. Generic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. *Mol. Biol. Evol.* **21**: 945–956.

Cheviron, Z.A., Whitehead, A. & Brumfield, R.T. 2008. Transcriptomic variation and plasticity in rufous-collared sparrows (*Zonotrichia capensis*) along an altitudinal gradient. *Mol. Ecol.* **17**: 4556–4569.

Dalziel, A.C., Moore, S.E. & Moyes, C.D. 2005. Mitochondrial enzyme content in the muscles of high-performance fish: evolution and variation among fiber types. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**: R163–R172.

Dalziel, A.C., Moyes, C.D., Fredriksson, E. & Lougheed, S.C. 2006. Molecular evolution of cytochrome c oxidase in high-performance fish (Teleostei: Scombroidei). *J. Mol. Evol.* **62**: 319–331.

Derome, N., Duchesne, P. & Bernatchez, L. 2006. Parallelism in gene transcription among sympatric lake whitefish (*Coregonus clupeaformis* Mitchill) ecotypes. *Mol. Ecol.* **15**: 1239–1249.

Ellison, C.K. & Burton, R.S. 2008. Interpopulation hybrid breakdown maps to the mitochondrial genome. *Evolution* **62**: 631–638.

Ellison, C.K. & Burton, R.S. 2010. Cytonuclear conflict in interpopulation hybrids: the role of RNA polymerase in mtDNA transcription and replication. *J. Evol. Biol.* **23**: 528–538.

Evans, M.L., Præbel, K., Peruzzi, S. & Bernatchez, L. 2012. Parallelism in the oxygen transport system of the lake whitefish: the role of physiological divergence in ecological speciation. *Mol. Ecol.* (in press), doi:10.1111/j.1365-294X.2012.05682.x.

Fan, S., Elmer, K.R. & Meyer, A. 2012. Genomics of adaptation and speciation in cichlid fishes: recent advances and analyses in African and Neotropical lineages. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **367**: 385–394.

Faul, F., Erdfelder, E., Lang, A.G. & Buchner, A. 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* **39**: 175–191.

Gagnaire, P.-A., Normandeau, E. & Bernatchez, L. 2012. Comparative genomics reveals adaptive protein evolution and a possible cytonuclear incompatibility between European and American eels. *Mol. Biol. Evol.* (in press), doi:10.1093/molbev/mss076.

Garbian, Y., Ovadia, O., Dadon, S. & Mishmar, D. 2010. Gene expression patterns of oxidative phosphorylation complex I subunits are organized in clusters. *PLoS ONE* **5**: e9985.

Garvin, M.R., Bielawski, J.P. & Gharrett, A.J. 2011. Positive Darwinian selection in the piston that powers proton pumps in complex I of the mitochondria of Pacific salmon. *PLoS ONE* **6**: e24127.

Gershoni, M., Templeton, A.R. & Mishmar, D. 2009. Mitochondrial bioenergetics as a major motive force of speciation. *Bioessays* **31**: 642–650.

Gilbert, T.L., Brown, J.R., Ohara, P.J., Buroker, N.E., Beckenbach, A.T. & Smith, M.J. 1988. Sequence of transfer Rna-thr and transfer Rna-pro from white sturgeon (*Acipenser transmontanus*) mitochondria. *Nucleic Acids Res.* **16**: 11825.

Good, P. 2000. *Permutation Tests: A Practical Guide to Resampling Methods for Testing Hypotheses*. Springer, New York.

Gracey, A.Y., Troll, J.V. & Somero, G.N. 2001. Hypoxia-induced gene expression profiling in the euryoxic fish *Gillichthys mirabilis*. *Proc. Natl Acad. Sci. USA* **98**: 1993–1998.

Gracey, A.Y., Fraser, E.J., Li, W., Fang, Y., Taylor, R.R., Rogers, J. *et al.* 2004. Coping with cold: an integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. *Proc. Natl Acad. Sci. USA* **101**: 16970–16975.

Grossman, L.I., Wildman, D.E., Schmidt, T.R. & Goodman, M. 2004. Accelerated evolution of the electron transport chain in anthropoid primates. *Trends Genet.* **20**: 578–585.

Guderley, H. 2004. Locomotor performance and muscle metabolic capacities: impact of temperature and energetic status. *Comp. Biochem. Physiol. B, Biochem. Mol. Biol.* **139**: 371–382.

Hardewig, I., van Dijk, P.L., Moyes, C.D. & Pörtner, H.O. 1999. Temperature-dependent expression of cytochrome-c oxidase in Antarctic and temperate fish. *Am. J. Physiol.* **277**: R508–R516.

Jeukens, J., Bittner, D., Knudsen, R. & Bernatchez, L. 2009. Candidate genes and adaptive radiation: insights from transcriptional adaptation to the limnetic niche among Coregonine fishes (*Coregonus* spp., Salmonidae). *Mol. Biol. Evol.* **26**: 155–166.

Jørgensen, S.M., Kleveland, E.J., Grimholt, U. & Gjoen, T. 2006. Validation of reference genes for real-time polymerase chain reaction studies in Atlantic salmon. *Mar. Biotechnol.* **8**: 398–408.

Kadenbach, B. 2003. Intrinsic and extrinsic uncoupling of oxidative phosphorylation. *Biochim. Biophys. Acta* **1604**: 77–94.

Kaeuffer, R., Peichel, C.L., Bolnick, D.I. & Hendry, A.P. 2012. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. *Evolution* **66**: 402–418.

Kahilainen, K. & Lehtonen, H. 2003. Piscivory and prey selection of four predator species in a whitefish dominated subarctic lake. *J. Fish Biol.* **63**: 659–672.

Kahilainen, K., Malinen, T., Tuomaala, A. & Lehtonen, H. 2004. Diel and seasonal habitat and food segregation of three sympatric *Coregonus lavaretus* forms in a subarctic lake. *J. Fish Biol.* **64**: 418–434.

Kelly, A.K., Waters, S.M., McGee, M., Fonseca, R.G., Carberry, C. & Kenny, D.A. 2011. mRNA expression of genes regulating oxidative phosphorylation in the muscle of beef cattle divergently ranked on residual feed intake. *Physiol. Genomics* **43**: 12–23.

Landry, L., Vincent, W.F. & Bernatchez, L. 2007. Parallel evolution of lake whitefish dwarf ecotypes in association with limnological features of their adaptive landscape. *J. Evol. Biol.* **20**: 971–984.

Landry, L. & Bernatchez, L. 2010. Role of epibenthic resource opportunities in the parallel evolution of lake whitefish species pairs (*Coregonus* sp.). *J. Evol. Biol.* **23**: 2602–2613.

Lenaz, G. 2001. The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. *IUBMB Life* **52**: 159–164.

Lu, G.Q. & Bernatchez, L. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): Support for the ecological speciation hypothesis. *Evolution* **53**: 1491–1505.

Lu, G., Basley, D.J. & Bernatchez, L. 2001. Contrasting patterns of mitochondrial DNA and microsatellite introgressive hybridization between lineages of lake whitefish (*Coregonus clupeaformis*); relevance for speciation. *Mol. Ecol.* **10**: 965–985.

Martinez-Fernandez, M., Bernatchez, L., Rolan-Alvarez, E. & Quesada, H. 2010. Insights into the role of differential gene expression on the ecological adaptation of the snail *Littorina saxatilis*. *BMC Evol. Biol.* **10**: 356.

Mishmar, D., Ruiz-Pesini, E., Golik, P., Macaulay, V., Clark, A. G., Hosseini, S. *et al.* 2003. Natural selection shaped regional mtDNA variation in humans. *Proc. Natl Acad. Sci. USA* **100**: 171–176.

Moyes, C.D. 2003. Controlling muscle mitochondrial content. *J. Exp. Biol.* **206**: 4385–4391.

Olsvik, P.A., Lie, K.K., Jordal, A.E., Nilsen, T.O. & Hordvik, I. 2005. Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon. *BMC Mol. Biol.* **6**: 21.

Pigeon, D., Chouinard, A. & Bernatchez, L. 1997. Multiple modes of speciation involved in the parallel evolution of

sympatric morphotypes of lake whitefish (*Coregonus clupeaformis*, Salmonidae). *Evolution* **51**: 196–205.

Pörtner, H.O., Schulte, P.M., Wood, C.M. & Schiemer, F. 2010. Niche dimensions in fishes: an integrative view. *Physiol. Biochem. Zool.* **83**: 808–826.

Rawson, P.D. & Burton, R.S. 2002. Functional coadaptation between cytochrome c and cytochrome c oxidase within allopatric populations of a marine copepod. *Proc. Natl Acad. Sci. USA* **99**: 12955–12958.

Reinecke, F., Smeitink, J.A.M. & van der Westhuizen, F.H. 2009. OXPHOS gene expression and control in mitochondrial disorders. *Biochim. Biophys. Acta* **1792**: 1113–1121.

Renaut, S., Nolte, A.W. & Bernatchez, L. 2010. Mining transcriptome sequences towards identifying adaptive single nucleotide polymorphisms in lake whitefish species pairs (*Coregonus* spp. Salmonidae). *Mol. Ecol.* **19**: 115–131.

Renaut, S., Nolte, A.W., Rogers, S.M., Derome, N. & Bernatchez, L. 2011. SNP signatures of selection on standing genetic variation and their association with adaptive phenotypes along gradients of ecological speciation in lake whitefish species pairs (*Coregonus* spp.). *Mol. Ecol.* **20**: 545–559.

Robinson, B.W. & Wilson, D.S. 1994. Character release and displacement in fishes – a neglected literature. *Am. Nat.* **144**: 596–627.

Rogers, S.M., Gagnon, V. & Bernatchez, L. 2002. Genetically based phenotype-environment association for swimming behavior in lake whitefish ecotypes (*Coregonus clupeaformis* Mitchill). *Evolution* **56**: 2322–2329.

Rosenblum, E.B. & Harmon, L.J. 2011. "Same same but different": replicated ecological speciation at White Sands. *Evolution* **65**: 946–960.

Ruiz-Pesini, E., Mishmar, D., Brandon, M., Procaccio, V. & Wallace, D.C. 2004. Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science* **303**: 223–226.

Sanogo, Y.O., Hankison, S., Band, M., Obregon, A. & Bell, A. M. 2011. Brain transcriptomic response of threespine sticklebacks to cues of a predator. *Brain Behav. Evol.* **77**: 270–285.

Saraste, M. 1999. Oxidative phosphorylation at the fin de siecle. *Science* **283**: 1488–1493.

Scott, G.R., Schulte, P.M., Egginton, S., Scott, A.L.M., Richards, J.G. & Milsom, W.K. 2010. Molecular evolution of cytochrome c oxidase underlies high-altitude adaptation in the bar-headed goose. *Mol. Biol. Evol.* **28**: 351–363.

Seebacher, F., Brand, M.D., Else, P.L., Guderley, H., Hulbert, A.J. & Moyes, C.D. 2010. Plasticity of oxidative metabolism in variable climates: molecular mechanisms. *Physiol. Biochem. Zool.* **83**: 721–732.

St-Cyr, J., Derome, N. & Bernatchez, L. 2008. The transcriptomics of life-history trade-offs in whitefish species pairs (*Coregonus* sp.). *Mol. Ecol.* **17**: 1850–1870.

Storey, K.B. 2007. Anoxia tolerance in turtles: metabolic regulation and gene expression. *Comp. Biochem. Physiol., Part A Mol. Integr. Physiol.* **147**: 263–276.

Storz, J.F., Scott, G.R. & Cheviron, Z.A. 2010. Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. *J. Exp. Biol.* **213**: 4125–4136.

Trudel, M., Tremblay, A., Schetagne, R. & Rasmussen, J.B. 2001. Why are dwarf fish so small? An energetic analysis of polymorphism in lake whitefish (*Coregonus clupeaformis*). *Can. J. Fish. Aquat. Sci.* **58**: 394–405.

van Waveren, C. & Moraes, C.T. 2008. Transcriptional co-expression and co-regulation of genes coding for components of the oxidative phosphorylation system. *BMC Genomics* **9**: 18.

Whiteley, A.R., Derome, N., Rogers, S.M., St-Cyr, J., Laroche, J., Labbe, A. *et al.* 2008. The phenomics and expression quantitative trait locus mapping of brain transcriptomics regulating adaptive divergence in lake whitefish species pairs (*Coregonus* sp.). *Genetics* **180**: 147–164.

Zar, J.H. 1999. *Biostatistical Analysis*. Prentice Hall, New Jersey.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Pairwise correlations of transcription levels for the nine oxidative phosphorylation genes examined in lake whitefish (*Coregonus clupeaformis*) from Cliff and Webster lakes in Maine, USA.

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