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Pervasive Sex-Linked Effects on Transcription Regulation As Revealed by Expression Quantitative Trait Loci Mapping in Lake Whitefish Species Pairs (Coregonus sp., Salmonidae)

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ABSTRACT

Mapping of expression quantitative trait loci (eQTL) is a powerful means for elucidating the genetic architecture of gene regulation. Yet, eQTL mapping has not been applied toward investigating the regulation architecture of genes involved in the process of population divergence, ultimately leading to speciation events. Here, we conducted an eQTL mapping experiment to compare the genetic architecture of transcript regulation in adaptive traits, differentiating the recently evolved limnetic (*dwarf*) and benthic (normal) species pairs of lake whitefish. The eQTL were mapped in three data sets derived from an F_1 hybrid-*dwarf* backcrossed family: the entire set of 66 genotyped individuals and the two sexes treated separately. We identified strikingly more eQTL in the female data set (174), compared to both male (54) and combined (33) data sets. The majority of these genes were not differentially expressed between male and female progeny of the backcross family, thus providing evidence for a strong pleiotropic sex-linked effect in transcriptomic regulation. The subtelomeric region of a linkage group segregating in females encompassed >50% of all eQTL, which exhibited the most pronounced additive effects. We also conducted a direct comparison of transcriptomic profiles between pure *dwarf* and normal progeny reared in controlled conditions. We detected 34 differentially expressed transcripts associated with eQTL segregating only in sex-specific data sets and mostly belonging to functional groups that differentiate *dwarf* and normal whitefish in natural populations. Therefore, these eQTL are not related to interindividual variation, but instead to the adaptive and historical genetic divergence between *dwarf* and normal whitefish. This study exemplifies how the integration of genetic and transcriptomic data offers a strong means for dissecting the functional genomic response to selection by separating mapping family-specific effects from genetic factors under selection, potentially involved in the phenotypic divergence of natural populations.

INVESTIGATIONS of quantitative variation in gene transcription have begun to open up the "black box" connecting genotype to phenotype (Gibson and Weir 2005; Prud'homme *et al.* 2007). Expression quantitative trait loci (eQTL) mapping, which combines transcriptional profiling with linkage mapping methods, allows the investigation of the genetic architecture of gene regulation and quantifies the heritability of these regulation factors (Wayne and McIntyre 2002; Schadt *et al.* 2003; Petretto *et al.* 2006; Vuylsteke *et al.* 2006). This is also a crucial issue in evolutionary biology, as many authors argued that changes in key regulatory genes may have a greater and faster effect than changes in structural genes on early phenotypic diversification (Jacob and Monod 1961; King and Wilson 1975; Purugganan 1998), although this is still a contentious issue (Hoekstra and Coyne 2007). Transcription QTL mapping has almost exclusively been applied to model organisms, including yeast (Brem *et al.* 2002), human (Morley *et al.* 2004), mouse, maize (Schadt *et al.* 2003), and Drosophila (Jin *et al.* 2002); but see Kirst *et al.* (2005) for work on the eucalyptus tree. To date, eQTL mapping has not been specifically applied toward investigating the regulation architecture of genes involved in the process of recent population divergence in the wild. However, this should be a fundamental research goal since variation in gene expression within and between natural populations has been recently reported to underlie adaptation (Gibson 2002; Oleksiak *et al.* 2002, 2005; Bochdanovits *et al.* 2003; Whitehead and Crawford 2005; Derome and Bernatchez 2006; Derome *et al.* 2006; Roberge *et al.* 2007). Investigating the genetic architecture of

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transcriptome regulation in natural populations or closely related species exhibiting phenotypic divergence related to distinct environmental responses provides a powerful means to reach a more complete understanding of the molecular mechanisms related to the process of adaptive phenotypic divergence, which in turn are involved in the course of speciation events.

Clearly, adaptive trait variation is multifaceted (Reeves and Sherman 1993). In many species, differences between the sexes add another important level of phenotypic variation, as illustrated by substantial sex-linked effects in quantitative studies (Mckay 2001) and, more recently, studies of sex-biased gene transcription (Rantz *et al.* 2003; McIntyre *et al.* 2006; Yang *et al.* 2006; Itoh *et al.* 2007; Wayne *et al.* 2007). Indeed, speciation is intimately associated with the evolution of traits related to sex and reproduction, including those affecting hybrid incompatibilities (postzygotic isolation). Also, genes controlling such traits are particularly abundant on the sex chromosomes (Wang *et al.* 2001; Ellegren and Parsh 2007). However, to our knowledge, little attention has been paid to investigating the genomic basis of sex differences in the context of an adaptive radiation (Pröschel *et al.* 2006) or the evolutionary consequences of the sex linkage of genes involved in speciation (Servedio and Saetre 2006). However, sex-biased genes have been observed to be among the most rapidly evolving genes in various taxonomic groups such as nematodes, insects, fish, birds, and mammals (reviewed by Singh and Kulathinal 2000; Ellegren and Fridolfsson 2003; Zang *et al.* 2004; Richards *et al.* 2005; Ellegren and Parsh 2007). This phenomenon was observed as well for somatic tissues (Parisi *et al.* 2004; Marinotti *et al.* 2006; Mank *et al.* 2007), including muscle (Yang *et al.* 2006). Furthermore, these sex-biased genes have been reported to cause genetic incompatibilities by disrupting local adaptations or coadaptations within the genome (Parker and Partridge 1998; Fitzpatrick 2004). It thus appears crucial to investigate the genomic basis of sex-specific transcriptional regulation by providing new insights into the role of sex-linked regulation effects differentiating two recently diverged genomes.

Sympatric *dwarf* (limnetic) and normal (benthic) species pairs of the lake whitefish (Coregonus sp.) constitute a case of recent adaptive radiation that is particularly well suited for investigating the genomic architecture of gene transcription. Geographic isolation during the Pleistocene (500,000–18,000 YBP) led to genetic divergence between whitefish populations inhabiting distinct glacial refuges (Bernatchez and Dodson 1990; Lu *et al.* 2001). Secondary contact between these evolutionary lineages subsequently occurred ~15,000 YBP within at least six lakes of the Saint John River basin in Maine and southeastern Quebec. Ecological opportunity and character displacement have both contributed to the rapid evolution of a limnetic *dwarf* species, which has diverged in sympatry from the ancestral benthic normal species (Bernatchez 2004; Landry *et al.* 2007). Adaptive trait differences observed in the *dwarf* and normal whitefish dichotomy are supported by several genetically based phenotype–environment associations, including for life history (age of maturity, life span), morphological (size, weight), behavioral (swimming activity), physiological (metabolic rate and growth), and gene expression traits (Lu and Bernatchez 1999; Trudel *et al.* 2001; Rogers *et al.* 2002; Rogers and Bernatchez 2005; Derome *et al.* 2006). Indeed, *dwarf* exhibits a higher metabolic rate, partly associated with the cost of a more active swimming activity (higher position in water column, direction changes, burst swims), and lower bioenergetic conversion efficiency (growth rate/consumption rate ratio), associated with slower growth and younger age at sexual maturity in dwarf whitefish (Trudel *et al.* 2001) when compared to normal whitefish (Rogers *et al.* 2002; Rogers and Bernatchez 2007). Moreover, linkage mapping has been used to document the number and effects of quantitative trait loci (QTL) involved in controlling the expression of these adaptive traits (Rogers *et al.* 2007), and genome scans performed in natural populations provided evidence that directional selection is maintaining genetic divergence between sympatric dwarf and normal whitefish by restricting gene flow at more than half of these QTL (Rogers and Bernatchez 2007). Finally, functional genomic studies performed in these same natural populations on the white muscle tissue showed that *dwarf* whitefish overexpressed genes associated with muscle contraction speed, which is consistent with the higher swimming activity of limnetic foraging species (Derome *et al.* 2006). Thus, the accumulation of genetic differences during the allopatric phase of geographic isolation in conjunction with ecological divergence that subsequently occurred in sympatry led to reproductive isolation between *dwarf* and normal whitefish species pairs (Lu and Bernatchez 1998; Rogers and Bernatchez 2006).

Here, we investigate the genetic architecture of transcript regulation in the white muscle tissue differentiating the recently evolved *dwarf* and normal species pairs of lake whitefish by conducting an eQTL mapping study using a 16,006-gene cDNA salmonids-specific microarray (Von Schalburg *et al.* 2005). Transcript abundance was measured in the same progeny of an F_1 hybrid-*dwarf* backcrossed family for which a genetic map was recently built (Rogers *et al.* 2007). In this way, both genotypic and transcriptomic information were integrated to identify genes potentially underlying the divergence in adaptive traits between *dwarf* and normal whitefish, namely in terms of their energy metabolism and swimming activity (Derome *et al.* 2006). As there is a trade-off in energy allocated to growth *vs.* swimming activity (Trudel *et al.* 2001; Rogers and Bernatchez 2007), we paid particular attention to the relationship

between eQTL associated with muscle contraction, energetic metabolism, and protein synthesis. Namely, we predicted opposite additive effects between eQTL of transcripts related to growth and swimming activity.

Moreover, to test whether the architecture of transcriptomic profiles segregated between sexes, we compared sex-specific data sets to a combined data set termed "combined," including all individuals, but excluding the sex effect on transcript levels (see MATERIALS AND METHODS). Sexual dimorphism in gene expression may be substantial, as reported recently both for Drosophila (Jin *et al.* 2002) and for mouse (Yang *et al.* 2006). Therefore, to distinguish between sex-specific effects (independent from the segregating genetic background) and pleiotropic sex-linked effects potentially involved in the phenotypic divergence of *dwarf* and normal whitefish (dependent on the segregating genetic background), we contrasted transcripts that exhibited eQTL from expression profile mapping experiments to genes differentially expressed between sexes of the same backcross progeny. In this way, transcripts that exhibited eQTL in a male or a female data set, but were not differentially expressed between progeny of different sexes, were considered as pleiotropic sex-linked effects potentially involved in the adaptive divergence between whitefish ecotypes.

Finally, to further assess whether the eQTL detected were potentially relevant in the context of adaptive divergence, we conducted transcriptomic comparisons of pure *dwarf* and normal progeny issued from the grandparents of the backcross family and reared in controlled conditions. To identify potential candidate genes for which transcription levels may have evolved under directional selection between *dwarf* and normal whitefish, we paid particular attention to (i) transcripts associated with eQTL that were differentially expressed between pure *dwarf* and normal whitefish and (ii) eQTL belonging to functional groups that exhibited significant directional additive effects. The predominance of additive effects (*i.e.*, amount of a given transcript modulated by segregation of alleles between *dwarf* and normal genomes) in one direction for the loci underlying the quantitative variation of a given trait would provide further evidence for the role of selection in shaping genome architecture (Orr 1998).

MATERIALS AND METHODS

Linkage maps: The eQTL were mapped onto a hybrid (normal female × *dwarf* male) × *dwarf* backcross (BC1) linkage map built by Rogers *et al.* (2007). Briefly, sex-specific markers segregating in the backcross families were assigned to a linkage group under a LOD of 4 while orders and recombination distances of these groups were determined under the Kosambi mapping function independently for both families using MAPMAKER/EXP (Lander *et al.* 1987). Defining sex-specific linkage groups was necessary since salmonids exhibit a sex-specific recombination rate due to tetravalent formations in the males during meioses (Sakamoto *et al.* 2000; Gharbi *et al.* 2006). The hybrid × *dwarf* map was built using a total of 389 AFLPs and 23 microsatellites covering a total of 48 linkage groups, including 34 female linkage groups (average = 8.0 loci per linkage group, average distance between markers = 17.3 cM, and a total map length of 2800 cM) and 14 dwarf male linkage groups (average = 8.7 loci per linkage group, average distance between markers = 17.96 cM, and a total map length of 2127.5 cM). The homology of these mapped loci was verified in a second backcross family where 34 linkage groups of the $40n$ expected showed 83% colinearity among linked loci between both families (Rogers *et al.* 2007).

Tissue sampling, RNA preparation, labeling, and hybridization: Dissected white muscle tissue (250–350 mg) was sampled for 66 individuals from the hybrid × *dwarf* backcross mapping family and stored at −80° until RNA extraction. RNA was extracted according to the Trizol Reagent protocol (GIBCO BRL, Gaithersburg, MD) and quantified with a GeneQuant spectrometer (Pharmacia, Piscataway, NJ), and RNA integrity was verified with a 2100 Bioanalyzer (Agilent). Reverse transcriptase PCR was performed using 15 μg of total RNA per sample following the SuperScript II Reverse Transcriptase protocol (Invitrogen Life Technologies, San Diego). Indirect labeling was performed on individual cDNA following the Array 50 kit protocol (Genisphere). Transcriptome profiles were obtained by using the 16,006-cDNA gene microarray (version 2) developed for Atlantic salmon (*Salmo salar*) by cGRASP (Von Schalburg *et al.* 2005) (Consortium for Genomic Research on All Salmonids Project) and successfully tested and applied to other salmonid species, including *Coregonus clupeaformis* (Derome and Bernatchez 2006; Derome *et al.* 2006; Rise *et al.* 2007). Gene identification with the corresponding EST sequence can be found at http://web.uvic.ca/cbr/grasp/. Cross-hybridization is known to be potentially a problem for spotted cDNA microarrays because of sequence polymorphisms between strains or paralogous genes that affect the signal for certain genes. However, in this study, we did not compare Salmo to Coregonus transcripts, but a backcross family of whitefish species pairs that diverged <12,000 YBP. Therefore, differences in cross-hybridization between *dwarf* and normal whitefish alleles would be negligible and unlikely to affect the results obtained. The transcript levels inferred from fluorescent labels were quantified by scanning microarrays using a ScanArray Express scanner (Packard Bioscience). Spot location and quantification was done with the QuantArray (Perkin-Elmer, Norwalk, CT) software, retaining the mean intensity value for each spot. Aberrant spot signals were removed before analysis and their values were estimated using the "K-Nearest Neighbors Imputer" function implemented in SAM software (Tusher *et al.* 2001). Genes with intensity less than the mean of the empty spot controls plus 2.5 times their standard deviation were removed from the analysis. According to this criterion, 2255 among the 16,006 transcripts passed this threshold and were considered for subsequent analyses. Raw and processed data sets are available at the GEO website (http://www.ncbi.nlm.nih.gov/geo/).

Experimental design and statistical analyses applied to eQTL experiments: We used a loop design (Churchill 2002; Yang and Speed 2002) to maximize the number of sampled meioses. Each of 66 samples was technically replicated on two distinct slides, while performing dye swapping (Cy3 and Alexa 647) to estimate the dye intensity variation bias. The loop design allows for the evaluation of twice as many recombined genotypes for a given number of slides as does reference design (Kirst *et al.* 2005). After correcting for local background, raw intensity values were both $\log_2$ transformed and 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.

Then, the gene–genotype interaction effect (G) was estimated using the R/MAANOVA software under a mixed-effects model of variance analysis (Cui and Churchill 2003) with the array term kept as a random effect, while genotype, sex, and dye term effects were fixed. Note that the genotype term refers to the individual, as each offspring of the backcross family constitutes a unique event of recombination between the parental genotypes. Two ANOVA models were used, the first one being applied to the whole data set, hereafter termed a combined data set [$Y_{ijkgh} = \mu + A_i + D_j + T_k + G_g + S_h + (AD)_{ij} + (GA)_{gi} + (GD)_{gj} + (GT)_{gk} + (GS)_{gh} + \varepsilon_{ijkgh}$], and the second one used for separate sex data sets [$Y_{ijkg} = \mu + A_i + D_j + T_k + G_g + (AD)_{ij} + (GA)_{gi} + (GD)_{gj} + (GT)_{gk} + \varepsilon_{ijkg}$] ($A$, array; D, dye; G, gene; T, genotype; and S, sex; terms in parentheses are interaction terms).

Detections of eQTL: For each transcript, the estimates of the variation associated with gene–genotype interaction for the 66 individual progeny were used as the phenotypes along with the linkage map to perform genomewide eQTL detection from the combined and sex-specific data sets, using the UNIX version of QTL Cartographer (Batsen *et al.* 2002).

The combined data set comprised all of the 66 mapped individuals, and the two sex-specific data sets comprised 29 individuals for each male and female. Although 37 females were available, we chose to use the same sample size as for males. The analysis with 37 females did not improve significantly eQTL detection, compared to the 29-female data set (data not shown). For eQTL detection, we used an interval-mapping approach (model 3 of the ZmapQTL module). Likelihood-ratio (LR) profiles [$-2\ \ln(L_0/L_1)$], representing the ratio of the likelihood of the null hypothesis (L_0, no QTL in the marker interval) to the alternative hypothesis (L_1, presence of a QTL in the marker interval) were generated for each transcript at every 2-cM interval on the linkage map of Rogers *et al.* (2007), using a window size of 10 cM. Three empirical thresholds for experimentwise type I error rates (0.1, 0.05, and 0.01) were determined for all significant eQTL ($P < 0.05$) by approximating their null distribution (H_0). For each of them, we recorded the 10th-, 50th-, and 100th- ranked LR of 1000 random permutations. To achieve this, we developed a PYTHON language script for the UNIX version of QTL cartographer, which allowed the automatic running of iterations among traits. Users are first required to set up the analysis in a parameter file containing the list of traits to be analyzed, the number of iterations, the number of iterations for each trait (here 1000), and the percentile values to be sampled (*e.g.*, 99th, 95th, and 90th). The PYTHON script records the parameter values and calls the different QTL cartographer programs, including Rmap (reads the map), Rcross (reads the cross), and Zmapqtl (runs association analysis between traits and markers). For each trait (transcript), both during and following each iteration, a function reads the output from Zmapqtl and records the GlobalMax values with each percentile value fixed. The script is available from the authors upon request.

Transcripts corresponded to EST library annotations of Von Schalburg *et al.* (2005). Those annotated as "unknown" were submitted to BLAST nucleotide and translated protein searches to determine if new gene identifications were possible. The highest BLAST score with a known function was recorded, for $E \leq 1 \times 10^{-5}$, and successful transcript finds were annotated as "similar to."

Functional annotation was done according to cGRASP. Functional categories include various biological processes (functions as annotated by cGRASP are in parentheses) and are defined as follows for the three most represented: (i) energy metabolism (electron transport, glycolysis, ATP synthesis, tricarboxylic acid cycle intermediate metabolism, tricarboxylic acid cycle, malate metabolism, hydrogen transport), (ii) muscle proteins (regulation of muscle contraction, muscle development, cytoskeleton organization), and (iii) protein synthesis (protein biosynthesis, electron transport, transcription, translational elongation, ribosome biogenesis). According to these criteria, we observed among the 2255 significantly expressed genes that 158 of them (7%) coded muscle proteins, 310 (14%) belonged to energetic metabolism, 484 (21%) participate in protein synthesis, and 946 (42%) were unknown functionally, according to the cGRASP annotation.

To illustrate the genomic distribution of eQTL, we divided the genome into 17.6-cM bins, corresponding to the average distance between markers for male and female maps (Rogers *et al.* 2007), which resulted in 366 bins. A Poisson distribution was assumed for calculating the probability of observing a given number of eQTL within any bin along the map. The mean of the Poisson distribution for each data set was estimated as the number of eQTL linkages/366 bins detected at $\alpha = 0.05$. The proportion of positive *vs.* negative additive effects for eQTL (*i.e.*, effect of segregating alleles on the amount of a given transcript) belonging to a given linkage group or eQTL hotspots (numerous eQTL located at the same bin) was tested using a Mann–Whitney test.

Expression profile differences between males and females from the backcross family progeny: A classical paired analysis was conducted between 24 male and 24 female backcross progenies chosen among the 66 slides used for the eQTL mapping experiment This was the maximum number of slides available upon which a male was hybridized with a female. A dye swap between Cy3 and Alexa 647 fluorophores has been used because it minimizes bias inherent to the dyes' uneven fluorescence intensity (Churchill 2002). Transcription data were corrected for regional intensity-related bias, using an R-LOWESS algorithm, and analyzed by ANOVA, using the R/MAANOVA software package (Kerr *et al.* 2000) under a mixed-effect model [$Y_{ijgh} = \mu + A_i + D_j + G_g + S_h + (AD)_{ij} + (GA)_{gi} + (GD)_{gj} + (GS)_{gh} + \varepsilon_{ijgh}$], where sex ($S$, male or female) and dye (D) were fixed, array (A) was random, and (ε_{ijgh}) was residual error. Since our analysis model was across genes, and because gene expression levels may have different variances (Cui *et al.* 2005; Kristiansson *et al.* 2006; Mathur and Dolo 2007), we used the shrinkage F statistic (F_S) to detect significant differences (adjusted permuted P-values, $\alpha < 0.01$) in level of gene transcription between males and females (Cui *et al.* 2005). As recommended by the authors, the F_S null distribution was established by permutation analysis (1000 sample permutations), considering each array as a sampling unit.

Expression profile differences between pure *dwarf* and normal whitefish parental generations: *Dwarf* and normal whitefish reared in a control environment were originally sampled in 1998 in Témiscouata Lake and Aylmer Lake (Quebec), respectively, and held in captivity at the Laboratoire Régional des Sciences Aquatiques (LARSA) facilities (Université Laval, Quebec). Family crosses were made in 2001 to generate pure F_1 breeds. Both normal and *dwarf* lines were kept at all times under the same environmental conditions (water temperature, photoperiod, diet). In September 2004, 12 randomly selected individuals of comparable sizes for each ecotype were measured (mean fork length, *dwarf*, 22.9 cm, SD = 1.8 cm; normal, 28.9 cm, SD = 2.2 cm) and then killed with a 0.001% Eugenol solution just prior to tissue extraction. Muscle tissue samples were immediately frozen on dry ice and stored at −80°.

RNA extraction and microarray experiments were processed as described above. Two samples (one *dwarf* and one normal) were differentially colored by fluorescence (Cy3 and Alexa 647). The analysis focused on the same 2255 transcripts that were used for eQTL mapping. Before statistical analysis, data preparation was processed as described for the sex-paired

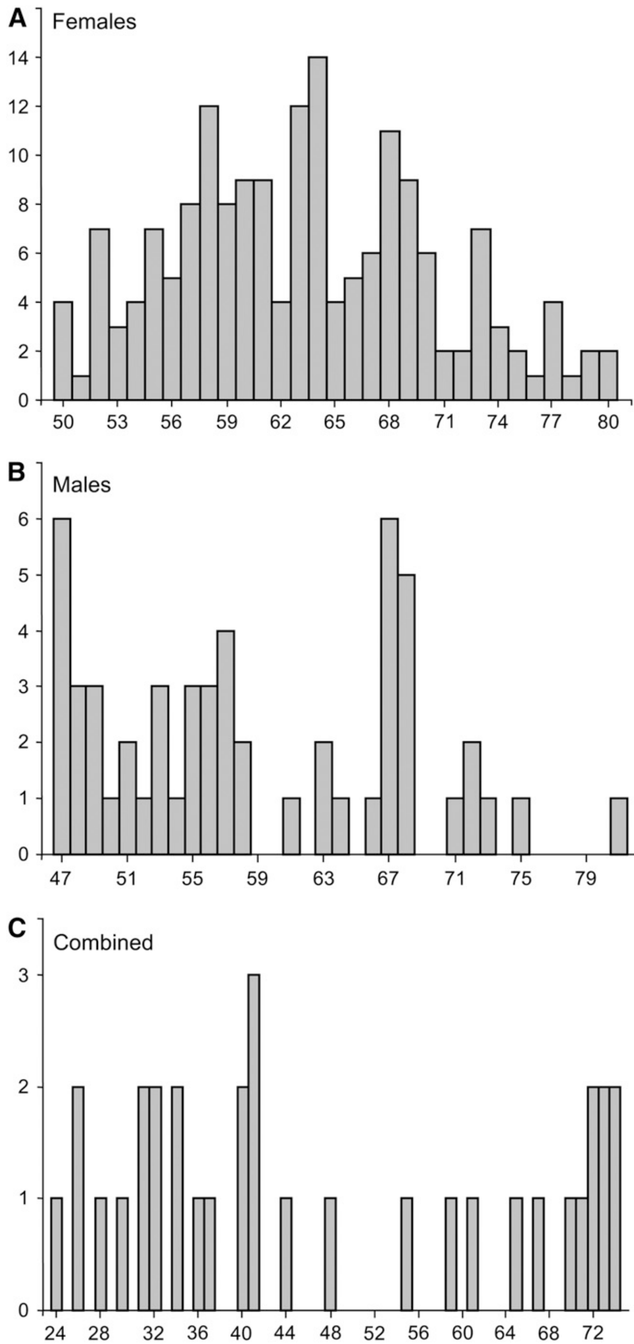

FIGURE 1.—Distribution of eQTL according to their proportion of variation explained (PVE) for all 2255 expressed genes. Female-specific (a), male-specific (b), and combined (c) data sets are shown.

analysis. The ANOVA was conducted under a mixed-effect model [$Y_{ijkg} = \mu + A_i + D_j + T_k + G_g + (AD)_{ij} + (GA)_{gi} + (GD)_{gj} + (GT)_{gk} + \varepsilon_{ijkg}$], where sample ($T$, *dwarf* or normal), gene (G), and dye (D) were fixed, array (A) was random, and (ε_{ijkg}) was residual error. A permutation-based F-test (F_S, 1000 sample permutations) was used to detect significant differences (permuted P-values, $\alpha < 0.05$) in level of gene transcription between *dwarf* and normal whitefish. Changes in transcription levels were calculated as a D/N ratio, where the mean transcription in *dwarf* individuals (D) was divided by mean transcription in normal individuals (N) for each gene.

TABLE 1

Number of eQTL detected for a given empirical threshold for experimentwise type I error rates of 0.10, 0.05, and 0.01 that were determined for each significantly mapped transcript ($P < 0.05$) by recording the 10th-, 50th-, and 100th-ranked LRs of 1000 random permutations

Data set	N	$\alpha < 0.10$	$\alpha < 0.05$	$\alpha < 0.01$
Combined	66	38 (35)	33 (30)	16 (14)
Males	29	75 (63)	54 (45)	20 (19)
Females	29	250 (91)	174 (117)	57 (51)

The numbers of transcripts (*i.e.*, associated with at least one significant eQTL) are in parentheses.

RESULTS

Detection of eQTL: Among the 66 backcross progeny examined, a total of 2255 genes were significantly transcribed (see MATERIALS AND METHODS). Striking differences in terms of numbers and effects of detected eQTL were observed when using the combined (a data set of 66 mapped individuals, analyzed with an ANOVA model discarding the sex effect) and the two sex-specific (29 individuals for each male and female) data sets. Thus, the proportion of variance explained (PVE) by individual eQTL was much higher for both males (median value of PVE = 66%; range, 47–81%) and females (PVE = 73%; range, 50–80%) than for the combined data set (PVE = 30%; range, 24–75%), which suggested a potential Beavis effect (LYNCH and WALSH 1998) and/or the conflict between sexes in the combined data set (supplemental Table S1, Figure 1). Moreover, for any given level of stringency, the number of eQTL detected in the female data set was significantly higher than that in both the male and the combined data sets (Table 1). Similarly, the number of eQTL detected in the male data set was always higher than that detected in the combined data set for any threshold value. This indicated that reduced sample size in the sex-specific data sets did not create a downward bias in eQTL detection and that contrasting patterns between sexes blurred eQTL detection when using the combined data set. Furthermore, the three data sets were also contrasted in terms of absolute values of additive effects (*i.e.*, amount of a given transcript modulated by segregation of alleles between *dwarf* and normal genomes). The female data set exhibited significantly more eQTL with absolute additive effects ≥1.00 (77/174) when compared to the male data set (3/55, P-value <0.001) and to the combined data set (1/30, P-value <0.0001). The mean absolute values of additive effects were also significantly higher in the female data set (mean = 0.99), when compared to the male (mean = 0.64, P-value = 0) and combined (mean = 0.58, P-value = 0) data sets.

Differences between the combined and sex-specific data sets were also found in terms of number of eQTL associated with different gene transcripts. Thus, at the

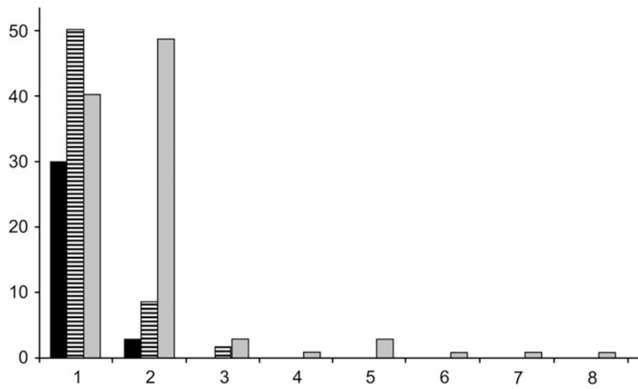

FIGURE 2.—Number of eQTL detected per transcript. Results are shown for combined (solid), male (striped), and female (shaded) data sets. The *x*-axis: classes of eQTL number per transcript; *y*-axis: class sizes.

permuted genomewide α of 0.10 for suggestive eQTL detection, the average number of eQTL associated with a given transcript was comparable for both the combined (1.085) and male (1.190) data sets but substantially higher for the female data set (2.747) (Fisher's test, $P < 0.0001$) (Figure 2). Thus, no more than two and three significant eQTL per transcript were found in the combined and male data sets, respectively, while up to eight significant eQTL per transcript were found in the female data set. The same differential pattern was still significant at a permuted genomewide α of 0.05, but not at $\alpha = 0.01$. This supports the observation that the *trans*-acting effects are generally of smaller effect than *cis*-acting effects and will not be detectable at overly conservative thresholds (GIBSON and WEIR 2005; PETRETTO *et al.* 2006). Finally, there were only three transcripts in common between male and female data sets, but with distinct eQTL locations: a nucleolar RNA helicase II (LG32f in male and LG19f in female), a 40S ribosomal protein S2 (LG25m in male and LG35m in female), and an ornithine decarboxylase antizyme (LG32f in male and LG14f in female). This suggests that transcript regulation was itself strongly influenced by sex.

Functional groups: At genomewide α of 0.05, 3 significant eQTL (8%) were detected, which belonged to energetic metabolism in the combined data set, 4 (11%) belonged to muscle proteins, and 13 (34%) belonged to protein synthesis functional groups. In the male-specific data set, 8 eQTL (15%) belonged to energetic metabolism, 7 (13%) belonged to muscle proteins, and 14 (26%) belonged to protein synthesis. In the female-specific data set, 23 eQTL (13%) belonged to energetic metabolism, 16 (9%) belonged to muscle proteins, and 30 (17%) belonged to protein synthesis and cell growth (Table 2, supplemental Table S1). These proportions of functional categories did not depart significantly from what was observed among the 2255 significantly expressed genes (see MATERIALS AND METHODS). Significant directionality of additive effect for two functional groups, energetic metabolism and/or muscle contraction regulation, was observed in the three data sets (Table 2). Energetic metabolism exhibited significant directionality in both the combined and the male data sets, but with opposite effects. Then, muscle contraction proteins exhibited significant directionality in both male and female data sets, but here again, with opposite effects: positive in males, negative in females.

TABLE 2

Summary of directionality of additive changes sorted by functional groups

Data set	Functional group	Transcript no.	Proportion in the same direction	Mean additive effect
Combined	EM	3	1.00**	−0.84
	MCR	4	0.50	−0.04
	PS	13	0.61	−0.20
Males	EM	8	1.00**	0.47
	MCR	7	0.86**	0.61
	PS	14	0.57	0.09
Females	EM	25	0.52	−0.08
	MCR	17	1.00***	−1.05
	PS	30	0.50	0.09

In every case, the direction of mean additive effects indicates the overall direction of transcripts belonging to a given functional group. The column titled proportion in the same direction indicates significance of the overall direction. A value of 1 in this column indicates all genes of the hotspot were in the same direction. A Mann–Whitney test was used to test for significant deviations from 1:1 proportional composition of positive and negative effects. Transcript sample sizes <4 were not tested. Functional group abbreviations are as follows: MCR, muscle contraction regulation; EM, energetic metabolism; PS, protein synthesis. $^{**}P < 0.01$; $^{***}P < 0.001$.

Nonrandom chromosomal distribution of eQTL: The eQTL linkages were not evenly distributed across the genome, particularly so for sex-specific data sets (Figure 3). Statistical significance of nonrandom distribution was assessed for a genomewide α of 0.05, for which totals of 33, 54, and 174 eQTL were detected for the combined, male, and female data sets, respectively (Table 1). For each of the data sets, we conservatively considered the occurrence of at least 4 eQTL within a given bin as an indication of eQTL hotspots. Indeed, the probability of observing ≥4 occurrences of eQTL in any given bin was <0.001 for the female data set when assuming a Poisson distribution. According to this criterion, both male and female data sets (but not the combined data set) revealed hotspots. In females, two major hotspots were located on the LG 25m subtelomeric region, with 53 eQTL located at position 229 and 34 eQTL at position193, both of which exhibited a significant mean directional additive effect (Figure 2, Table 3). Furthermore, absolute values of additive effects exceeded 1.00 for 77% of these 87 eQTL, whereas this proportion fell to 12% for the 79 remaining eQTL

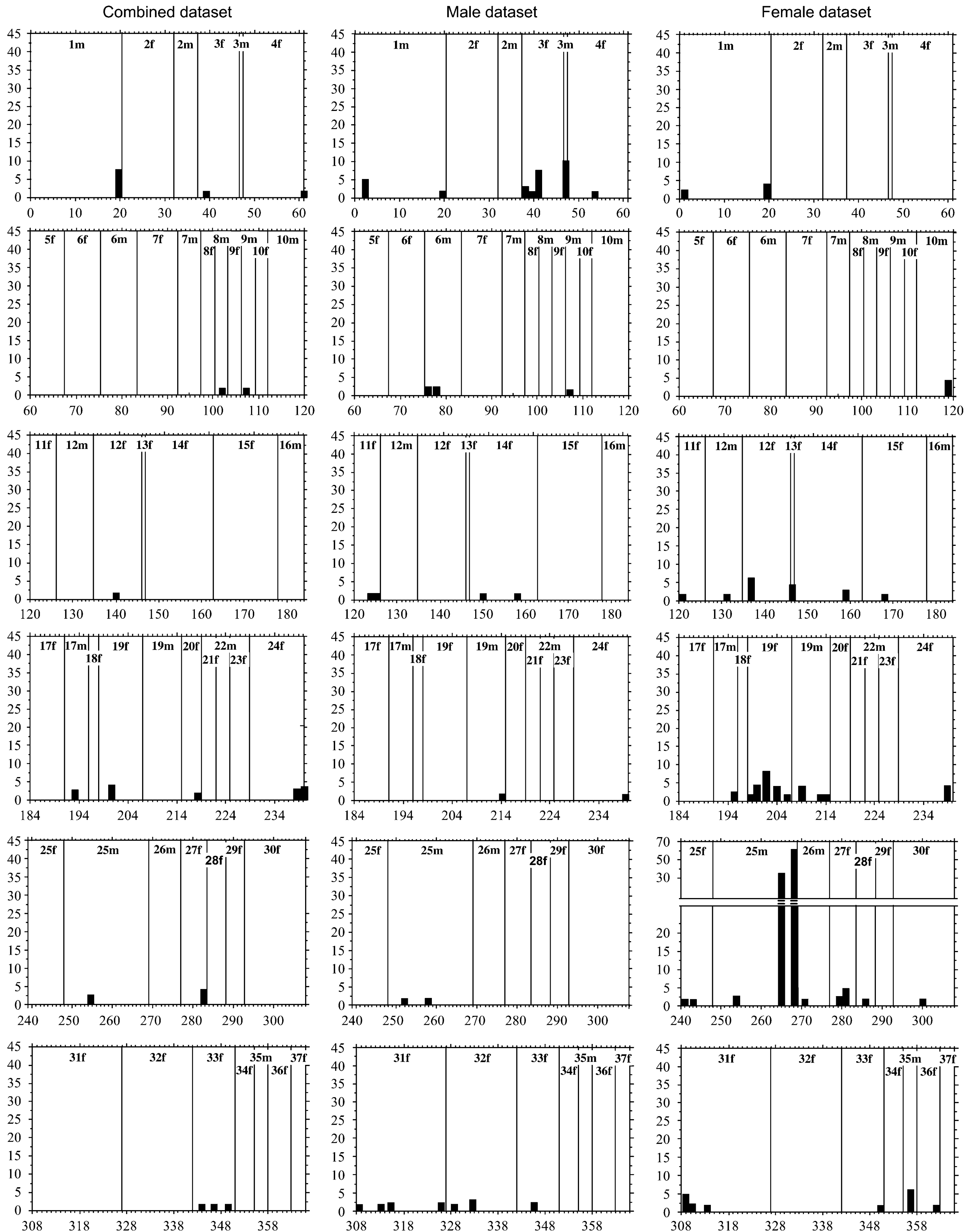

FIGURE 3.—Genome map showing eQTL ($\alpha < 0.05$) for the three data sets: combined, male, and female. The *x*-axes: numbers correspond to successively numbered markers on each linkage group where each interval corresponds to a 17.6-cM bin. Successive linkage groups are separated by a vertical line. The *y*-axes: number of eQTL per interval.

TABLE 3

Hotspots detected among the three data sets

Linkage group	Position (cM)	Transcript no.	Mean additive effect	Proportion in the same direction	Functions
			Females		
19f	56	5	−0.36	0.66	PS (1), MCR (1), EM (1)
25m	193	34	0.75	0.79***	PS (1), MCR (4), EM (2)
25m	226	4	0.58	0.75	PS (3), EM (1)
25m	229	53	0.62	0.74***	PS (3), MCR (7), EM (3)
35m	43	6	0.33	0.83	PS (4), EM (1)
			Males		
3f	63	5	0.23	0.80	EM (4), PS (1)

Number of transcripts, mean additive effect, proportion of transcripts either positive or negative, linkage group, chromosomal locations, and functional groups are shown. In every case, the direction of mean additive effects indicates the overall direction of the hotspot and is the direction referred to in the column labeled proportion in the same direction. A value of 1 in this column indicates all genes of the hotspot were in the same direction. A Mann–Whitney test was used to test for significant deviations from 1:1 proportional composition of positive and negative additive effects within hotspots with transcript number ranging from 5 to 25; >25, a normal approximation was used. Functional group abbreviations are as follows: MCR, muscle protein; EM, energetic metabolism; PS, protein synthesis. In parentheses: number of transcripts belonging to a given functional group. $^{***}P < 0.001$.

mapped outside the LG25m. This suggests that these particular chromosomal regions, both located on the same linkage group, have the most pervasive influence on transcript regulation and that this influence is strongly sex biased. Moreover, when considering functional groups, transcripts involved in protein synthesis, cell growth, and division exhibited systematically opposed additive effects to those associated with energy metabolism, proteolysis, muscle contraction, and repair. In this study, the additive effect of an eQTL represents the influence of a normal allele segregating in a *dwarf* genetic background on the expression regulation of a particular gene. For example, a positive additive effect means that the normal allele upregulated the transcription of a given gene. According to this, six transcripts involved in protein synthesis and cell growth, including an histone H1 homolog to protein, a ribosomal biogenesis NEP1 protein, a highly conserved transcript in eukaryotes and archaea identified as an essential growth factor regulator (Eschrich *et al.* 2005), a GDP-l-fucose synthetase, tightly involved in growth and development (Smith *et al.* 2002), and a RhoGTPase, which is involved in development of the striated muscles and in myofibrillogenesis in vertebrates (O'Brien *et al.* 2000), exhibited opposite additive effects to four enzymes involved in energetic metabolism (a triose phosphate isomerase, a sarcoplasmic/endoplasmic reticulum ATPase, a glycerol-3-phosphate dehydrogenase, and a kinase precursor) and to six muscle protein transcripts [two actins, two collagen α-precursors, and a myosin heavy chain (supplemental Table S1)]. In addition, there were three small hotspots encompassing respectively five, four, and six transcripts, which were located on LG19, LG25m, and LG35m, respectively. These harbored very few eQTL exceeding an additive effect of 1.00. Concerning the male data set, only one hotspot was detected on LG3f, located at position 62.89, featuring 4 eQTL: a ribosomal protein, an ATP synthase, a putative NADH, and an ubiquinol cytochrome c reductase, all of them exhibiting absolute values of additive effect <1.00.

Differentially expressed genes between progeny of different sexes: A total of 478 of the 2255 genes (21%) were differentially expressed between 24 male and 24 female progeny of the backcross family (adjusted permuted *P*-values, $\alpha < 0.01$) (supplemental Table S2). Genes belonging to several functional groups were clearly transcribed differentially between sexes. Namely oxidative phosphorylation genes were upregulated in males, whereas glycolytic enzymes, muscle proteins, and ribosomal proteins were upregulated in females. However, only 20 (4%) differentially expressed genes between males and females were associated with eQTL segregating in the female data set. This suggests that regulation settings for 96% of the transcripts differentially expressed between sexes were not sensitive to the genetic background of the backcross progenies, in which each individual represents a unique combination of *dwarf* and normal alleles.

Differentially expressed genes between *dwarf* and normal parental species: A total of 253 of the 2255 genes (11%) were differentially expressed between 12 pure *dwarf* and 12 normal whitefish (permuted *P*-values, $\alpha < 0.05$) (supplemental Table S3). Of these, 34 transcripts were associated with 53 eQTL (20% of the transcripts segregating among the three data sets), 22 in the female data set (Table 4A), 12 in the male data set (Table 4B), and one in the combined data set. The differentially expressed transcripts segregating in male and female data sets belonged mainly to three functional groups: muscle contraction, energy and repair (parvalbumin,

TABLE 4

Transcripts differentially expressed between pure *dwarf* and normal whitefish associated with eQTL

Description	Function	LG	Position(s)	Mean additive effect	PVE	D/N LARSA	*D/N* nat pop
A. In the female data set							
CB506671 [GO] [P30275] creatine kinase	MCR	12f	55	+	68	1, 11	IP 1, 24
CA057774 [GO] [P63260] actin	MCR	19f	57	–	64	1, 19	CL 1, 11
CB510655 [NR] [AAH65846] LIM domain binding 3	MCR	19m	31	–	76	0, 86	—
CB496426 [GO] [Q02566] myosin heavy chain	MCR	25m	229	–	52	1, 22	—
CA044548 [NT] [AJ250180] β-thymosin	MCR	25m	229	–	55	1, 91	—
CK991315 similar to NP_066270.1 kinase	EM	12f; 25m; 27f; 31f	55; 189; 229; 92; 33	–	66	1, 19	—
CA054312 [GO] [P52480] pyruvate kinase	EM	25m	143	–	68	1, 15	—
CB494468 [GO] [O55143] sarcoplasmic/endoplasmic reticulum calcium ATPase 2	EM	25m	193; 229	–	63	1, 32	CL 1, 14
CB494389 [GO] [P07310] creatine kinase, M chain	EM	25m	233	–	61	1, 32	IP 1, 24
CB499941 [GO] [P00417] cytochrome c oxidase subunit 3	EM	19f; 25m	109; 225	+	57	0, 69	IP 0, 78
CN442510 [GO] [P18929] NADH-ubiquinone oxidoreductase chain 1	EM	13f	18	+	63	0, 83	—
CB497579 [GO] [P48774] glutathione *S*-transferase	EM	28m; 33f	27; 127	–	66	0, 76	—
CK990291 similar to lysozyme	PM	25m	193; 229	–	56	1, 17	IP 1, 07
CA036950 [GO] [P15620] zinc finger protein 35	PS	19f; 25m	56; 225	+	70	0, 87	—
CA063904 [GO] [P62702] 40S ribosomal protein S4, X isoform	PS	10m; 27f	119; 86	–	58	1, 17	—
CA047190 [NR] [AAM34649] 60S ribosomal protein L35	PS	14f	191	–	54	1, 18	—
CB497613 [GO] [P97351] 40S ribosomal protein S3a.	PS	19f	41	–	56	1, 10	IP 1, 30
CB505812 [GO] [Q9CZX8] 40S ribosomal protein S19	PS	24f	138	–	61	1, 05	—
CA037733 [GO] [Q64438] angiogenin-2 precursor	OF	19f; 25m	129; 225	+	54	0, 82	—
CA057048 [NT] [AF504016] *Salmo salar* MHC class I	OF	19m	31	+	69	1, 13	—
CB491414 [GO] [Q9CQY1] Autophagy protein 12-like	OF	24f	138	–	60	1, 04	—
CA055233 unknown	—	27f	54	–	54	1, 18	—
B. In the male data set							
CB510281 [GO] [P32848] parvalbumin	MCR	3m	10	+	61	2, 22	CL 2, 13; IP 1, 82
CB497373 [GO] [P13412] troponin I	MCR	14f; 33f	176; 78; 82	+	47	0, 77	IP 0, 92
CB514461 [GO] [P63260] actin	MCR	3m	14	+	67	1, 19	—
CB498175 [GO] [P30275] creatine kinase	EM	3f	61; 64	+	54	1, 11	IP 1, 24
CA057881 [GO] [P62141] serine/threonine protein phosphatase	EM	3f	0	+	49	0, 85	IP 0, 89
CB493164 [GO] [P56384] ATP synthase lipid-binding protein	EM	32f	28	+	55	0, 79	IP 1, 16
CB493101 [NR] [XP_421325] similar to NADH dehydrogenase	EM	3f	63	+	47	0, 88	IP 0, 89
CA046558 [GO] [Q9CPQ8] ATP synthase g chain	EM	3f	63	+	47	0, 72	—
CA052161 [GO] [Q9VNE9] 60S ribosomal protein L13A	PS	6m; 37f	41; 52; 10	–	56	1, 19	—
CB515185 [GO] [P14206] 40S ribosomal protein SA	PS	24f	180	+	67	1, 11	—
CA050561 [NR] [CAB51372] transposase	OF	6m	0	–	50	0, 91	—
CB497026 [GO] [P06797] cathepsin L precursor	PM	3m	2	+	53	1, 09	—

Description, reference numbers of the transcript, name of the transcript; function, the functional group associated with the transcript: EM, energetic metabolism; MCR, muscle contraction regulation; OF, other function; PS, protein synthesis; LG, linkage group; position corresponds to a 17.5-cM interval; additive, additive effect of each eQTL; PVE, percentage of variance explained. *D/N* LARSA, the ratio of gene expression between *dwarf* and normal in controlled conditions; *D/N* nat pop, the same measure in natural populations obtained by Derome *et al.* (2006); IP, Indian Pond; CL, Cliff Lake.

myosin, actin, troponin, β-thymosin, sarcoplasmic/endoplasmic ATPase, and creatine kinase), energetic metabolism (pyruvate kinase, ATPase subunits G and C, NADH dehydrogenase, and cytochrome c oxidase polypeptide III), and protein synthesis (ribosomal proteins and zinc finger protein). Genes belonging to muscle contraction, energy, and repair were systematically upregulated in *dwarf*, except for troponin, whereas both energetic metabolism and protein synthesis functional groups were not (Table 4B). Also, 125 transcripts for which regulating regions segregated in male- and female-specific data sets were not differentially expressed between pure *dwarf* and normal whitefish, which suggests they may be associated with either interindividual differences or stabilizing selection.

DISCUSSION

Documenting the genetic architecture of gene expression provides a strong means for investigating the importance of the transcriptomic regulation during the course of an adaptive radiation in whitefish. Here, this approach revealed a complex genetic architecture of white muscle transcription, a tissue for which functional differences associated with swimming activity, energy metabolism, and growth have been well documented in the adaptive divergence of *dwarf* and normal whitefish species pairs (Derome and Bernatchez 2006; Derome *et al.* 2006). The first striking result of this study was the much higher number of chromosomal regions influencing transcript abundance in the female compared to the male data set of identical sample size. The second was the identification of two major nonrandom aggregations of highly significantly mapped transcript regulation loci (eQTL hotspots) in the female data set. Those were responsible for significant directional patterns of additive effects, therefore identifying potential candidate genes whose transcription levels may have coevolved under directional selection (Orr 1998) between *dwarf* and *normal* whitefish. Moreover, patterns of eQTL distribution were strongly contrasted between male and females data sets. Indeed, the number and distribution of eQTL per transcript provided strong indirect evidence for both putative *cis* and *trans* regulation, with *trans* regulation being apparently more prevalent in females than in males. The differential genetic architecture between sexes was also supported by a significantly higher number of transcripts showing segregation of their regulating regions in females relative to males. Furthermore, 20% of the transcripts associated with eQTL were differentially expressed between pure *dwarf* and normal progeny, and those mostly belonged to muscle energy and repair, energetic metabolism, and protein synthesis, therefore identifying potential candidate genes involved in the adaptive and/or historical genetic divergence of *dwarf* and normal whitefish.

Sex-specific genetic architecture of gene regulation: Sex-specific effect or adaptive sex-linked effects? As stated above, the most striking result of this study was the much higher number of chromosomal regions influencing transcript abundance in the female compared to the male data set of identical sample size. Although several studies recently reported sex-specific transcript regulation (Von Schalburg *et al.* 2005; McIntyre *et al.* 2006), including for muscle tissue (Yang *et al.* 2006), this possibility is challenged here by the alternative hypothesis that the sex-specific architecture we observed reflects adaptive sex-linked effects. In addition, other hypotheses concerning the genetic architecture of whitefish and the experimental cross itself have to be explored. Thus, it is well known that recombination is strongly repressed during male meiosis in salmonids (Sakamoto *et al.* 2000), with a female-to-male linkage map distance ratio of 6.4:1 reported in *S. trutta* (Gharbi *et al.* 2006). Such a discrepancy between male and female would significantly affect segregation of regulatory regions, which, in turn, could lead to differential eQTL detection between male- and female-specific data sets. Rogers *et al.* (2007) did not detect any significant sex differences between the recombination frequencies in the whitefish linkage map. They hypothesized that elevated recombination in female markers of hybrid origin was likely offset by suppressed recombination due to the heterogeneous genetic background of the female hybrids compared to the pure parent in the pedigree. This, in turn, would have resulted in comparable levels of recombination observed between sexes, in contrast with previous studies in salmonids. Despite this, we recorded significantly fewer eQTL in males but also in the whole data set compared to females. Moreover, despite numerous unique combinations between *dwarf* and normal alleles in the backcross family, >20% of expressed genes were differentially regulated between progeny of different sexes. Yet, 96% of these transcripts did not segregate in any of the three data sets, which suggests independence *vis-à-vis* their genetic background. Thus, most of the transcripts segregating in sex-specific data sets were not sex-biased genes. These transcripts were nonetheless under the control of the sex-determining region, which exhibited in that case a dependence *vis-à-vis* their genetic background. The fact that different transcripts segregated in either male or female data sets strongly suggests that the sex-determining region exerts pleiotropic effects. This in turn raises the hypothesis that pleiotropic effects of sex-determining regions may have played an important role in the adaptive divergence of *dwarf* and normal whitefish and may have contributed to their postzygotic reproductive isolation, as documented experimentally in previous studies comparing fitness of pure and hybrid crosses between *dwarf* and normal whitefish (Lu and Bernatchez 1998; Rogers and Bernatchez 2006).

Contrasting results among the combined and sex-specific data sets: Another salient result of this study was that there was no eQTL co-occurrence among the combined and the two sex-specific data sets. While the combined data set represents segregation effects on transcript regulation devoid of sex-linked effect, male and female data sets likely reflected contrasted eQTL occurrence due to, everything else been equal, differential patterns of recombination in the sex-determining region. Sex chromosomes in salmonids are at the early stages of differentiation (Phillip *et al.* 2001), and male heterogamety and female homogamety are accepted as a general rule in salmonids (reviewed in Philipps and Rab 2001). Moreover, X and Y chromosomes still share a similar repertoire of functional genes, conserved across a wide range of salmonid species (Woram *et al.* 2003, Artieri *et al.* 2006). Also, male-specific loci of the sex-determining autosomal region of two comparable young heterogametic sex chromosome systems (the medaka, *Oryzias latipes*, and the stickleback, *Gasterosteus aculeatus*), were observed to not recombine and to include the sex-determining gene (Matsuda *et al.* 2002; Peichel *et al.* 2004).

Aside from this, both size and weight at the same age differed significantly between males and females of the backcross family ($P_{\mathrm{size}} = 0.018$; $P_{\mathrm{weight}} < 1.10^{-6}$, one tailed *t*-test), associated with a variance four time lower in males, whereas these parameters did not vary between sexes either in *dwarf* or in normal whitefish from natural populations (data not shown). This suggests, first, that recombination between *dwarf* and normal genomes was responsible for both size and weight differences observed between sexes and, second, that these differences were mostly under the influence of a putative sex-determining region that did not recombine in males, as both weight and size variances were substantially reduced compared to females. This in turn suggests that *dwarf* Y-like-linked genes imposed hemizygous *trans*-acting effects among backcross males, despite autosomal *dwarf*/normal allele segregation. This hypothesis is reasonable because a majority of *trans*-regulation patterns occurred in the homogametic sex (see results) where segregation was theoretically possible along the whole sex-determining region. It is also consistent with the work of Wang *et al.* (2006), who observed a majority of sex-linked *trans*-regulation patterns in mouse. Taken together, it is likely that significantly lower eQTL occurrence in a male-specific sample would have resulted from a dominant effect of the *dwarf* Y-like nonrecombining autosomal region that would in turn impose strong pleiotropic effects in a 75% *dwarf* background genome of the backcross family. By contrast, free recombination between *dwarf* and normal X-like autosomal regions during meiosis in F_1 females would have caused higher eQTL occurrence in the backcross female-specific data set. In other words, eQTL detected in the males would correspond to sex-linked effects outside the nonrecombining region of the Y-like autosomal region, whereas those detected in females would correspond mostly to sex-linked effects encompassing the whole X-like chromosomal region.

The allopatric origin of the two evolutionary lineages used to build the backcross family may also partly underlie this striking difference of eQTL abundance between male and female data sets. The *dwarf* and normal whitefish populations belonged to different evolutionary lineages, Acadian and Atlantic–Mississippian, issued from distinct glacial origins (see materials and methods). Therefore, 18,000–500,000 years of allopatric genetic divergence between glacial races (Bernatchez and Dodson 1990) were enforced with ∼12,000 years of adaptive divergence that occurred between sympatric species and have potentially enhanced regional allelic incompatibilities between *dwarf* and normal genomes, perhaps also including the sex-determining region.

The major difference between males and females was further expressed in the nonrandom aggregation of eQTL at two tightly linked regulatory hotspots observed in females. Indeed, linkage group LG25m comprised >55% of all eQTL detected in the female data set, the majority (98%) of which were located at the distal tip of the linkage group. Furthermore, these two eQTL hotspots, which comprised 90% of all LG25m eQTL, exhibited a strikingly higher proportion of strong additive and directional effects, compared to eQTL found elsewhere in the genome (see results). Indeed, the mean absolute value of additive effects reached 1.22 (range = 0.52–1.87), which is the very upper range of additive effects observed in mouse (Petretto *et al.* 2006). Moreover, additive effects were significantly directional when considering eQTL belonging to separate functional groups, namely energy metabolism ($P = 0.016$), muscle proteins ($P = 0.004$), and protein synthesis ($P = 0.019$). Directional predominance of additive effects in one direction for the loci underlying the quantitative variation of a given trait provides strong indirect evidence for the role of directional selection in shaping genome architecture (Orr 1998). Altogether, when considering a functional group as a trait, this suggests that directional selection has been acting on patterns of gene regulation associated with these two tightly linked chromosomal regions. Strikingly, directionality of additive effects was systematically opposed between transcripts involved in different functional groups. Namely, normal whitefish alleles induced negative regulation effects on energetic metabolism and muscle proteins. On the contrary, normal whitefish alleles induced positive regulation effects on protein synthesis and cell growth functional groups.

This result is very consistent with both previous bioenergetic and population genomic studies on whitefish ecotype differences. First, Trudel *et al.* (2001) showed that *dwarf* ecotypes have a higher metabolic rate, partly associated with the cost of swimming, and a lower

bioenergetic conversion efficiency, both of them associated with slower growth and younger age at sexual maturity. In addition, parallelism in gene transcription of the same tissue in natural population pairs of whitefish ecotypes showed that adaptive transcriptional divergence involved mainly genes associated with swimming activity and energy metabolism (Derome and Bernatchez 2006; Derome *et al.* 2006). Such a concentration of eQTL belonging to diverse functional groups at these too subtelomeric positions (193 and 229) suggests that two single master regulators located at these positions are likely to control their expression. Moreover, all transcripts regulated at position 193 appeared also regulated on position 229. This raises the hypothesis that two duplicated regulating sequences are acting on the same suite of genes. This hypothesis is reasonable as positions 193 and 229 are separated by two markers that are associated with two regulating regions of, respectively, one and four different transcripts.

Among the transcripts that belong to both eQTL hotspots, there are four genes that are known to play fundamental roles in transcription regulation. First, the DPY-30-like protein gene encodes for a highly conserved protein throughout evolution, which is the upstream regulator of dosage compensation machinery of X chromosomes (Dong *et al.* 2005). In addition, this protein plays an essential role in sex determination (Hsu and Meyer 1993) and may direct various developmental processes via epigenetic control (Jenuwein and Allis 2001). Furthermore, it has been recently demonstrated in *Caenorhabditis elegans* that at least two separated DNA motifs, termed Rex1 and Rex2, act in combination or separately to regulate dosage compensation machinery via two DPY variants' activation. Also, Rex1 and Rex2 are, respectively, located close to one of the two distinct DPY sequences (Mcdonel *et al.* 2006). Such genetic architecture of DPY regulation could be consistent with the observation of both its tightly linked eQTL located on LG25m. Therefore, DPY-30-like protein appears to be a very relevant candidate as a master regulating locus for both LG25m hotspots. Another such candidate is the Histone H1 homolog protamine transcript, which is known to play a pervasive role in transcriptome regulation (Bustin *et al.* 2005). Interestingly, Nagy *et al.* (2001) have demonstrated an interaction between a DPY-30 homolog and a histone protein as a sex-specific epigenetic regulation in Drosophila, where histone methylation acts on chromatin dynamics. Therefore, as histone methylation is conserved from yeast to humans (Strahl *et al.* 1999), the coregulation of both DPY-30-like protein gene and histone H1 in whitefish could potentially account for regulating the suite of genes mapped on two tightly linked subtelomeric positions of a single chromosome. Another transcript potentially involved in the regulation of the two hotspots is the small ubiquitin-like modifier (SUMO). SUMO has emerged as an important regulator of diverse pathways and activities, including protein localization and transcriptional regulation (Hamard *et al.* 2007), and was observed to interact with RAD23 (Ortolan *et al.* 2000), an UV excision repair protein that is itself involved in chromatin-mediated repression of transcription, and is alleviated by both histone modifications and ATP-dependent chromatin-remodeling activities (Gong *et al.* 2006). Overall, these results exemplify how natural selection could act on a single master regulator controlling expression of a suite of numerous genes belonging to diverse functional groups in a tissue highly involved in the adaptive divergence of whitefish and that would be ultimately able to modulate notably the organism phenotype (Derome and Bernatchez 2006; Derome *et al.* 2006).

When extending the link between functional groups and directionality of additive effects to all linkage groups, only eQTL for transcripts belonging to muscle contraction proteins still have significant directionality, for which normal alleles induced a downregulation. However, the magnitude of their additive effects was weaker than that of those belonging to LG25m, suggesting a less profound effect in phenotypic differentiation between *dwarf* and normal whitefish. Significant directionality of additive effects on muscle contraction proteins and energy metabolism was observed in the male data set as well but with both opposite and smaller effects than those observed in females. Thus, sex differences of functional-specific directionality of transcript regulation add further support to the hypothesis that natural selection acted on a genetic architecture of transcript regulation mostly under the influence of the sex-determining region.

The eQTL hotspots on the LG25m are particularly striking because no such eQTL concentration was observed elsewhere on the map, and especially not on the LG25f, where alleles issued from the grandmother segregated. This suggests that this hotspot of transcriptomic segregation was specific to grandfather alleles, and, everything else been equal, these alleles would be linked to the X-like region homologous to the Y-like nonrecombining region. Indeed, loci associated with the sex-determining region in stickleback were reported to segregate only in male maps (Peichel *et al.* 2004). However, although many sex effects mapped to the LG25m, including a reproductive timing pQTL (Rogers and Bernatchez 2007), two eQTL of DPY-30-like protein, from which both regulating sequences and the gene itself were located on the X chromosome in *C. elegans* (Mcdonel *et al.* 2006), the genetic map was not dense enough to unequivocally determine a sex-determining locus.

Overall, these contrasting results between sexes revealed that two major tightly linked master regulating regions that influence expression of 34 common genes belonging to functional groups are likely involved in the adaptive divergence between *dwarf* and normal whitefish. Also, the additive effects of normal inherited alleles into the *dwarf* genetic background illustrate the trade-off

in energy allocation in these species pairs in accordance with previous bioenergetic and transcriptomic studies (Trudel *et al.* 2001; Derome and Bernatchez 2006; Derome *et al.* 2006; St-Cyr *et al.* 2008). As such, this work provides further insights into the genetic architecture of transcriptional regulation in the time course of an adaptive radiation, in which historical isolation during an allopatric phase followed as well by ecological interactions encountered in sympatry following secondary contact have been acting. Our results also add further support to the view that *trans*-acting factors play an important role in the evolution of gene expression (Wang *et al.* 2006), especially during the early stages of adaptive divergence.

Comparison between eQTL and transcripts differentially expressed between pure *dwarf* and normal progenies: The eQTL detection is dependent on alleles with significant regulatory effects segregating between two genomes, therefore encompassing both interindividual genetic differences and eventually regulatory changes differentiating distinct evolutionary lineages that may or may not be under selective effect. This study revealed that sex-linked pleiotropic effects have played pervasive effects in the transcript segregation. Here, there was no co-occurrence of eQTL between the three data sets, and only one transcript differentially expressed associated with one eQTL in the combined data set (*i.e.*, exhibiting transcriptomic segregation devoid of sex-linked effect). This supports further the importance of the sex-determining region in the genetic architecture differentiating *dwarf* and normal whitefish. Furthermore, 12 candidate genes were differentially expressed in the same direction between the parental *dwarf* and normal whitefish populations kept in controlled conditions at LARSA and that were used for genetic mapping, as well as one or two additional natural sympatric pairs studied by Derome *et al.* (2006): parvalbumin, actin, sarcoplasmic/endoplasmic reticulum calcium, creatine kinase, ATP synthase g, pyruvate kinase, lysozyme, and ribosomal protein were upregulated in *dwarf* whitefish whereas cytochrome oxidase subunit 3, putative NADH dehydrogenase, troponin, and serine/threonine protein phosphatase were upregulated in normal whitefish. This co-occurrence of directional changes for these 12 specific transcripts, along with segregation of their respective regulating regions, further suggests that the regulation of these genes evolved under natural selection during the adaptive radiation of the whitefish species complex.

Concluding remarks: This study exemplifies how the integration of genetic and transcriptomic data offers a strong means for dissecting the functional genomic response to selection by separating mapping family-specific effects from genetic factors under selection, potentially involved in the phenotypic divergence of natural populations. In particular, the genetic dissection of gene regulation architecture underlying phenotypic divergence between *dwarf* and normal whitefish provided new insights toward understanding the molecular mechanisms underlying their divergence and, ultimately, their reproductive isolation. First, the integration of a paired analysis comparing expression across sex and eQTL mapping of sex-specific data sets revealed that the sex-determining region would have imposed strong pleiotropic effects on adaptive traits, which could in turn at least partly account for their postzygotic reproductive isolation. Second, a higher number of eQTL were recorded in female progeny, for which two major hotspots of transcript regulation on two discrete positions on the LG25m encompassed more than half of all detected eQTL. This provides evidence for the existence of two master control loci modulating the same suite of genes, belonging to diverse functional groups involved in whitefish phenotypic divergence, including muscle contraction, energetic metabolism, and protein synthesis. The pronounced directionality and magnitude of additive effects of eQTL found in these chromosomal regions provided strong albeit indirect support for the role of historical selection in shaping patterns of transcription for this large suite of genes. Third, the transcripts associated with eQTL that were differentially expressed between pure *dwarf* and normal whitefish progeny added further insights into identifying candidate genes involved in the genetic divergence of these species. Overall, this study brings support to the hypothesis that natural selection may have acted on a few master regulating loci, which appeared to be under the influence of the sex-determining region. These master regulating loci, in turn, may have shaped the transcription profile at whole suites of genes through extensive pleiotropic effects and ultimately modulated phenotypic traits that underlay the adaptive divergence and speciation in *dwarf* and normal whitefish species pairs.

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