Linkage maps of the dwarf and Normal lake whitefish (*Coregonus clupeaformis*) species and their hybrids reveal the genetic architecture of population divergence

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ABSTRACT

Knowledge about the genetic architecture of interspecific hybrids enables the detection of genomic regions implicated in population divergence, hence providing insight into their role as potential barriers to gene flow. We assembled genetic linkage maps for the dwarf and Normal lake whitefish species complex and their hybrids. A total of 877 AFLP loci and 30 microsatellites were positioned. The homology of mapped loci between families supported the existence of 34 linkage groups (of 40n expected) exhibiting 83% colinearity among linked loci between these two families. Classes of AFLP markers were not randomly distributed among linkage groups. Both AFLP and microsatellites exhibited deviations from Mendelian expectations, with 30.4% exhibiting significant segregation distortion across 28 linkage groups of the four linkage maps in both families (P< 0.00001). Eight loci distributed over seven homologous linkage groups were significantly distorted in both families and the level of distortion, when comparing homologous loci of the same phase between families, was correlated (Spearman R = 0.378, \( p = 0.0021 \)). These results suggest that substantial divergence incurred during allopatric glacial separation and subsequent sympatric ecological specialization has resulted in several genomic regions that are no longer complementary between dwarf and Normal populations issued from different evolutionary glacial lineages.
INTRODUCTION

Understanding the genetic consequences of population divergence is central to evolutionary biology (COYNE and ORR 2004, Edmands 2002, DE QUEIROZ 2005). Namely, the ability to detect genetic regions implicated in this evolutionary process may provide insight into the genomic regions involved and the evolution of their role as potential barriers to gene flow. This remains challenging without knowledge of the genetic architecture, i.e., the number, location, and effect of genomic locations contributing to differentiation within and among populations or species (RIESEBERG 1998, ORR and TURELLI 2001). As genetic architecture may either promote or constrain divergence (HAWTHORNE and VIA 2001), such genome-wide perspectives are integral towards a complete understanding of the functional genomic response to the evolutionary processes incurred by populations as they diverge (TING et al. 2001, EMELIANOV et al. 2004, WU and TING 2004).

Genetic linkage mapping approaches have several advantages towards addressing these issues (RIESEBERG 1998). Such an approach has led to the detection of genomic regions resistant to introgression (e.g. RIESEBERG et al. 1999, ROGERS et al. 2001, LEXER et al. 2003), the identification of adaptive QTL and the dissection of complex traits (e.g. PEICHEL et al. 2001, SAINTAGNE et al. 2004), and has proven valuable towards the mapping of gene expression profiles (eQTL, e.g., KIRST et al. 2005). Comparative linkage mapping among species has also allowed inference about the types of genomic changes that may accompany divergence (e.g., KUITTINEN et al. 2004, LEXER et al. 2005, Gharbi et al. 2006). Altogether, genetic mapping has provided an efficient means for improving our
understanding of the consequences of natural selection on the genetic architecture of complex trait variation (e.g. SAINTAGNE et al. 2004, ROGERS and BERNATCHEZ 2005).

Studies on the genetic architecture of adaptive population divergence have primarily focused on invertebrates (e.g., LYNCH et al. 1999, HAWTHORNE and VIA 2001) and plants (e.g., BRADSHAW et al. 1995, RIESEBERG et al. 1999, SCOTTI-SAINTAGNE et al. 2004), while fishes represent the exception in studies of vertebrate population divergence (e.g., PEICHEL et al. 2001, ALBERTSON et al. 2003). Northern temperate fish populations offer several characteristics advantageous for studying the genetic consequences of population divergence as several fish species that colonized postglacial lakes following the retreat of glacial ice are currently undergoing rapid evolution within these environments (BERNATCHEZ et al. 1999, ROBINSON and SCHLUTER 2000). Accordingly, the use of controlled crosses originating from these young diverging populations is more likely to reveal genomic regions implicated in the first steps of the process of divergence itself, rather than regions which may have become progressively resistant to introgression (due to genetic drift and natural selection) following speciation events.

Within several northern temperate lakes, the lake whitefish (Coregonus clupeaformis) species complex embodies this example of rapid evolutionary change. Five lineages diverged in allopatry within refugia during the Pleistocene glaciation (18 000 to 500 000 ya, BERNATCHEZ et al. 1999), whereby recession of glacial ice (15 0000 ya) resulted in secondary contact between populations followed by introgressive hybridization (LU et al. 2001, ROGERS et al. 2001). Geographical isolation in these glacial refugia may have been sufficient for the development of genetic incompatibilities between populations prior to secondary contact in postglacial times (LU and BERNATCHEZ 1998, ROGERS and BERNATCHEZ 2006). However, postglacial parallel phenotypic evolution of sympatric pairs
(BERNATCHEZ et al. 1999) exhibit bimodal adult size distributions whereby divergent selection for differential growth has maintained and promoted sympatric divergence between dwarf and Normal populations associated with the use of distinct limnetic and benthic trophic niches (CHOUINARD et al. 1996, CAMPBELL and BERNATCHEZ 2004, ROGERS and BERNATCHEZ 2005). Altogether, these populations display adaptive trait differentiation with respect to life-history (BERNATCHEZ et al. 1999), behavioural (ROGERS et al. 2002), physiological (ROGERS and BERNATCHEZ 2005), transcriptional (DEROME et al. 2006), and morphological traits (LU and BERNATCHEZ 1999, ROGERS et al. 2002, BERNATCHEZ 2004). A linkage mapping approach in young diverging populations of the lake whitefish could: 1) substantially improve our understanding of the genetic architecture of historically contingent and adaptive trait differences contributing to the population divergence between species and, 2) help identifying genomic regions permeable or resistant to gene flow.

Altogether, we predicted that the genetic architecture of hybrids could reveal genomic regions implicated in population divergence. The objective of this study was therefore to first assemble genetic linkage maps of dwarf and Normal lake whitefish, and their hybrids. Linkage mapping was performed based on a hybrid backcross-like design for each of both dwarf and Normal whitefish, using primarily dominant AFLP markers with microsatellites, in an attempt to achieve full genome coverage on the 40(n) chromosomes hypothesized to comprise the karyotype of this species (PHILLIPS and RAB 2001). Because increasing evidence suggests that sex-specific chromosomal differences results in suppressed recombination in male chromosomes (SAKAMOTO et al. 2000), four sex-specific genetic linkage maps were generated from distinct dwarf and Normal genetic backgrounds.
Under the hypothesis that recombination of genomic regions that have incurred substantial divergence may contribute to elevated hybrid inviability and therefore have a substantial influence on the level of recombination observed (OTTO and NUISMER 2004, BUTLIN 2005), the impact of hybridization on recombination frequencies among linked markers between the dwarf and Normal backcrosses was investigated. In addition, because heterospecific interactions between hybrid crosses are known to influence Mendelian segregation of loci (VOGL and XU 2000, FISHPMAN et al. 2001, MYBURG et al. 2004), the impact of the intensity and direction of segregation distortion on genetic architecture was also compared between the dwarf and Normal backcrosses. Finally, as AFLP are increasingly being used to generate linkage maps of unexplored genomes (PARSONS and SHAW 2002, BENSCH and AKESSON 2005), the null hypothesis that AFLP primer categories are randomly distributed across the genome was tested.

The results we obtained support the hypothesis that genetic divergence incurred during allopatric glacial separation and subsequent sympatric ecological specialization has resulted in several non complementary genomic regions between dwarf and normal populations. They also provide insight into the evolutionary implications of hybridization in diverging genomes and illustrate how linkage mapping may assist in the elucidation of these processes, particularly the identification of genetic regions associated with reproductive barriers.
METHODS

Experimental hybrid crosses: Hybrids were produced between parents representing two allopatric whitefish populations belonging to different glacial races. The parental generation of the Acadian glacial origin (dwarf) and Atlantic-Mississippian glacial origin (Normal) were sampled from Témiscouata Lake (47°36′N, 68°45′W) and Aylmer Lake (45°50′N, 71°26′W), respectively. Previous studies based on AFLP (Campbell and Bernatchez 2004) and microsatellites (Lu and Bernatchez 1999) revealed high levels of heterozygosity for both dwarf and Normal populations. The $F_1$ consisted of we considered as pure dwarf, pure Normal, and their hybrids (detailed in Lu and Bernatchez, 1998). Two distinct backcross-like crosses were used for mapping: 1) between a ♀ hybrid (♀ Normal x ♂ dwarf) and a ♂ dwarf, denoted as hybrid x dwarf, and; 2) between a ♀ hybrid (♀ dwarf x ♂ Normal) and a ♂ Normal, denoted herein as hybrid x Normal (Figure 1). As these crosses were not derived from inbred lines of pure dwarf and Normal fish, but were instead heterogeneous, these crosses should be considered as backcross-like.

In 2001, 250 individuals from each family were tagged with passively integrated transponder (PIT, Biomark Inc.) tags providing a barcode by which individuals could be followed throughout their life history. A biopsy (50mg) of adipose fin tissue was sampled from each individual at the age of 1+ and total genomic DNA was extracted using a standard phenol-chloroform procedure.

AFLP genotyping: In total, 336 backcross progeny (n = 198 in the hybrid x dwarf cross and n = 138 in the hybrid x Normal cross) plus the two parents from each family were
genotyped with AFLP markers. The AFLP plant mapping kit (Applied Biosystems, Inc.) was used according to the protocol of Vos et al. (1995). Following the pre-selective amplification step, 15 selective primer combinations were used to amplify AFLP loci (Table 2). AFLP locus notation consisted of the di-nucleotide extensions representative of their selective primer combination (EcoRI Axx: MseI Cxx) followed by the average locus size in base pairs (to 1 decimal point) calculated over all individuals genotyped in this study (see Rogers et al. 2001 for more details on AFLP amplification and scoring).

**Microsatellite genotyping:** For additional anchoring of the AFLP map, 30 polymorphic microsatellites were used with 18 species-specific and the remainder cross-amplified from other salmonid sources (Appendix 1). A total of 75 individuals were genotyped from each of the hybrid x dwarf and hybrid x Normal backcrosses. Microsatellites loci were amplified via PCR and alleles scored from 8% polyacrylamide gels using an FMBIO II scanner (Hitachi) with the Genescan-500 size standard (Applied Biosystems, Inc.) following protocols specific for each locus (Appendix 1).

**Linkage analysis and genetic map construction:** AFLP genotypes were scored in each family according to two patterns of segregation: 1) 1:1 ratio resulting from the presence of a fragment only in either the female (i.e., ♀Aa : ♂aa) or the male (i.e., ♀aa : ♂Aa); and 2) 3:1 ratio resulting from the presence of a segregating fragment in both parents (i.e., ♀Aa : ♂Aa). Data points were scored as missing in cases where scoring was questionable. Sex-specific marker assignments were maintained throughout the linkage analysis to build two sets of linkage maps within each family. Only AFLP and microsatellite markers
segregating under a pseudo-testcross model of segregation (1:1 ratio) were used for the four linkage maps generated in this study.

Mendelian segregation ratios of both AFLP and microsatellite markers were assessed using a chi-square test implemented in MAPDISTO v1.5 (Lorieux, M., http://mapdisto.free.fr/). The significance of distorted segregation ratios was corrected for multiple comparisons \( [\alpha = 0.05/ k, \text{where } k \text{ was the number of tests performed (RICE 1989)}] \), while all distorted markers with table-wide significant deviations were monitored throughout linkage analysis in efforts to guard against the formation of pseudolinkages (CLOUTIER et al. 1997).

For each family, all segregating markers were assigned to linkage groups using the GROUP algorithm performed in MAPMAKER/EXP (LANDER et al. 1987) employing a LOD of 4.0 and a minimum recombination fraction of 0.35 under the backcross model using the notation H and A to represent heterozygotes and homozygote recessive genotypes, respectively. Pairwise recombination frequencies between all loci were also calculated using a linkage analysis package for outcrossed families with male or female exchange of the mapping parent (LINKMFEX, Danzmann, www.uoguelph.ca/~rdanzmann/software/). Based on a LOD significance threshold of 4.0, linkage groups were designated using the LINKGRP command also enabling comparisons between both estimates as an additional verification of the data. Although there is currently no consensus in the literature about what initial LOD should be used when generating an initial estimate of the number of linkage groups, an LOD of 3 is typically employed (LIU 1998, WANG and PORTER 2004; HUBERT and HEDGECOCK, 2004). However, LANDER et al. (1987) acknowledged that a conservative approach is needed when the haploid number is not known or there is no pre-existing data for a given system. Given the lack of pre-existing
data in the whitefish system, grouping according to an LOD threshold of 4 was deemed a more conservative approach.

MAPMAKER/EXP was used for the remainder of the map construction. Based on the pairwise recombination frequencies, anchors within each of the identified groups were first designated to test locus assignments in efforts to reduce spurious linkage and to reveal cases of conflicting data where markers may show linkage to more than one group (LANDER et al. 1987, ALBINI et al. 2003). Following this step, loci were individually assigned to the linkage groups via the ASSIGN command with a minimum threshold set to a LOD of 4.0. This was particularly important to determine if distorted markers may have been contributing to pseudolinkages cojoining independent chromosomal regions (LORIEUX et al. 1995, CLOUTIER et al. 1997, LIVINGSTONE et al. 1999). In cases of a marker showing a conflict between linkage groups, LOD scores were compared. If the difference in LOD scores for the two linkage groups was 3.0 or greater, the marker was assigned to the group with the higher LOD (SLATE et al. 2002). Loci that did not meet these criteria or that showed irresolvable conflicts between linkage groups remained unassigned.

For linkage groups with less than eight loci, ordering of loci within each linkage group was estimated using the COMPARE command which estimates the likelihood of all possible orders. For linkage groups with more than eight loci, the ORDER command established a framework order for a subset of five loci (with parameter values set to thresholds of LOD > 3.0 and θ less than 20 cM). Remaining loci were ordered between the framework marker intervals using the PLACE command under the threshold criterion of 2.0<LOD<3.0. In some cases, placement of loci was below this threshold whereby the TRY command established relative likelihoods along intervals allowing for ordering of these accessory loci. This frequently consisted of groups of loci separated by less than 5
cM where placement on either side of the framework was equally as likely or in other cases, some markers were positioned beyond the framework order on the ends of the linkage groups. Markers that could not be ordered via the PLACE command with a likelihood of LOD 2.0 were considered ‘accessory’ loci. The presence of double recombinants and candidate genotyping errors was checked with the GENOTYPE command and an error detection threshold set to 1% (Lincoln and Lander 1992). Resulting orders were also checked with the LOD tables and the RIPPLE command. Final map distances between linked loci were calculated from the recombination frequency using the MAP command employing the Kosambi mapping function (Kosambi 1944) as salmonid fish are known to exhibit crossover interference (Thorgaard et al. 1983). Because MAPMAKER does not recognize linkage in the repulsion phase, the final dataset was duplicated with reversed matrix genotypic coding for all loci (H for A and A for H), enabling an identification of sex-specific markers linked in repulsion. Linkage homologies within families (between male and female linkage groups) were implied upon detecting significant linkage in the repulsion phase between sex-specific markers. Genome length was calculated as $G_0 = G_F + X_0(L - R)$, where $G_F$ is the total length of the map in cM, $X_0$ is the observed maximum distance in cM between two linked markers at a minimum LOD (4.0), $L$ is the observed number of linkage groups, pairs of loci, and unlinked loci, and $R$ is the haploid number of chromosomes (Hulbert et al. 1988).
**Comparison of linkage maps between sexes and families**

**Establishing homology for AFLP between families:** AFLP loci of the same molecular weight (within 0.3 bp) from the same selective primer combinations between families were considered homologous based on similar studies that detected an error rate of 0.008% with sequencing observations under this assumption (Parsons and Shaw 2002). The sharing of a homologous locus among linkage groups between the two backcrosses established homology while the degree of colinearity among families was assessed by comparing the location and frequency that homologous loci exhibited the same order between families.

**Comparison of recombination frequencies between sexes and families:** To test the null hypothesis that recombination is reduced in male linkage groups compared to females, we compared significant pairwise recombination estimates (above a LOD threshold of 4.0) between the sex-specific maps of each family. We performed the same comparison using pairwise distances between marker intervals.

To explore the hypothesis that a genetic basis for hybrid inviability may result in differential levels of recombination, we compared significant pairwise recombination estimates (above a LOD threshold of 4.0) among linkage maps generated from both crosses. For this test we also performed an additional comparison of inter-marker distances using co-linear marker intervals between families.

**Comparison of segregation distortion between families:** Patterns of segregation distortion for all homologous loci between families were also compared to explore the hypothesis that loci exhibiting segregation distortion underlie genomic regions resistant to introgression caused by differential viability of segregating loci (Fishman and Willis
2001, MYBURG et al. 2004). Therefore, for these homologous loci segregating in the same phase in both families, we calculated the percentage of genotype frequency distortion using the frequency of the segregating heterozygote genotype under a 1:1 expectation (frequency of $Aa$ - 0.5) x 100%. A correlation in the direction of segregation distortion for loci between families would support the hypothesis that heterospecific interactions between genomes may have contributed to differential viability in segregating loci and that these loci would represent the genomic regions most likely implicated in a genetic basis for resistance to introgression between populations.

In addition, the potential impact of loci deviating from Mendelian segregation on both map order and distance between loci was investigated by comparing distorted loci under classic recombination frequencies and under Bailey’s mapping function using MAPDISTO. Bailey’s mapping function incorporates a maximum likelihood estimation of recombination frequency under a hypothesis of segregation distortion of the informative allele relative to the recessive, thereby potentially more appropriate for mapping distorted markers (BAILEY 1949, LORIEUX et al. 1995). Homologous loci between families that showed significant segregation distortion were chosen for the comparisons. Recombination frequencies were calculated for two pairwise loci surrounding each of these distorted loci between families. The most likely map orders of three loci under each estimate were then compared using the RIPPLE command in MAPDISTO.

**Test of random AFLP distribution:** A $\chi^2$ test was used to compare the observed and expected frequencies of loci per selective primer combination segregating onto linkage group to test the hypothesis that AFLP loci were randomly distributed across the linkage maps in each family. As linkage groups within families were sex-specific, we used only
linkage groups where locus information was available for both parents in the calculations of the observed and expected locus frequency. The null hypothesis was that AFLP fragments amplified from a primer pair combination should segregate randomly over all linkage groups. Therefore, the expected frequency of AFLP loci was calculated as the frequency of loci per selective primer combination segregating over the entire map (i.e., expected frequency = total number of loci per selective primer combination / all loci).

The observed number of AFLP originating from each of the 15 primer combinations was then counted for each linkage group. To discern the absolute expected number of AFLP from a primer combination that one should find on each linkage group, we multiplied the total number of loci per linkage group by the expected frequency. This analysis was performed in both families and the significance of these $\chi^2$ tests was corrected for multiple comparisons (RICE 1989).
RESULTS

Marker polymorphism

**AFLP:** A total of 931 and 1280 AFLP loci were scored from the 15 selective primer combinations in the hybrid x *dwarf* and hybrid x Normal families, respectively (Table 1). Of these, 59.2% were polymorphic (551/931) in the hybrid x *dwarf* and 59.4% (760/1280) in the hybrid x Normal backcrosses (Table 1). A high percentage of the polymorphic loci were informative for mapping with 401 (72.7%) loci in the hybrid x *dwarf* and 476 (62.6%) in the hybrid x Normal families revealing a 1:1 segregation pattern, while the remaining segregated 3:1 (Table 1). The number of markers in male and female parents with 1:1 segregation patterns was comparable between families, with 54.2% (216/401) and 43.9% (209/476) of loci shown to be maternally informative within hybrid x *dwarf* and hybrid x Normal families, respectively (Table 1). Overall, 122 polymorphic loci were considered homologous and informative exhibiting 1:1 segregation patterns across both families (Table 1).

**Microsatellites:** Of the 30 microsatellites genotyped for mapping, 21 were polymorphic in the hybrid x *dwarf* family while 20 were polymorphic in the hybrid x Normal family. Overall, 10 polymorphic loci were common between families (Appendix 1).
Segregation distortion of loci: Both AFLP and microsatellite loci across both families exhibited highly significant levels of segregation distortion. In the hybrid x dwarf family, 133 loci (32.9%) deviated from Mendelian expectations at a 0.05 tablewide significance threshold (P < 0.00001) with 32.7% of AFLP and 17% of microsatellites exhibiting this pattern. Loci deviated in both directions, with 54 loci underrepresented and 79 loci overrepresented by heterozygous genotypes (Table 2). At a significance threshold of $\alpha < 0.001$, the number of loci exhibiting significant segregation distortion rose to 178 (44%), with 171 AFLP (43.4%) and 7 microsatellites (35%) exhibiting deviations from Mendelian expectations. In the hybrid x Normal family, 130 loci (27.8%) deviated from Mendelian expectations at the 0.05 tablewide significance threshold (P< 0.00001) with 28.5% of AFLP and 15% of microsatellites exhibiting this pattern (Table 2). At the P< 0.001 level of significance, 201 loci (43%) exhibited segregation distortion. Loci were again distorted in both directions, with 92 loci underrepresented and 38 loci overrepresented by heterozygote genotypes in the segregating progeny.

Linkage maps: The majority of AFLP and microsatellite loci were successfully mapped among dwarf and Normal backcrosses (Figure 2). In the hybrid x dwarf family, a total of 389 AFLP and 20 microsatellites were mapped. A total of 11 AFLP remained unassigned, with six showing no evidence of linkage to any other markers and five with irresolvable linkage conflicts. A total of 34 female and 14 male linkage groups were found and mapped with an average number of 8 loci per female linkage group and 8.7 loci per male linkage group. Including all loci in the hybrid female map, there was an average distance of 17.3 cM between loci and an overall map length of 2800 cM. The map length of the dwarf male was 2127.5 cM with an average distance of 18.0 cM between loci. The total number of
identified linkage groups was 37, below the haploid number of 40(n) chromosomes expected (Figure 2).

In the hybrid x Normal backcross, a total 452 AFLP and 14 microsatellites were mapped. A total of 17 loci remained unassigned, nine exhibiting no evidence of linkage and eight conflicting loci. A total of 23 female and 29 male linkage groups were mapped with an average number of 8.2 loci per female loci and 8.1 loci per male linkage group. The hybrid female map was 2383 cM with an average distance of 16.9 cM between loci. For the male parent, the map length was 3198.9 cM with an average distance of 16.9 cM between loci. The total number of identified linkage groups was 41, above the haploid number of 40 expected. Several markers that could not be assigned within the current linkage groups within this family grouped as small marker clusters (Figure 2).

**Linkage map comparisons between families:** A total of 122 homologous AFLP and microsatellites were mapped across families, resulting in over 34 linkage groups supported by homologous linkages between families. Overall, 91.4 % of loci were linked to the same groups between families, when including homologous loci that were informative in the opposite sex between families (e.g., Lg2, Lg5 and Lg6, (Figure 2). When considering linkage groups that consisted of multiple linked homologous loci (88 out of the 122 total), over 83% were co-linear and exhibited the same order of loci in each family while inversions or differential localization between linkage groups were observed for the remaining (Figure 2). On linkage group Lg8, an additional homologous locus (GGTG081.7) showed weak (LOD = 2.4) but ultimately insignificant linkage to the Lg8f in the hybrid x dwarf family and thus it was not included in the map. However, this observation as well as unpublished data from QTL in both families supports homology
between families at Lg8 and for this reason these groups are tentatively placed together in the map (Figure 2). It should be noted that because this locus was not included, it had no impact on the overall results on the degree of homology detected between families. For the remaining loci, either a single locus was homologous across linkage groups between families (25 out of 122 loci), or a conflict was observed meaning there was no synteny and consequently the locus were assigned to different linkage groups between families (10 out of 122 loci).

**Comparison of recombination frequencies between sexes and families:** Pairwise recombination frequencies among significantly linked loci greater than a LOD of 4.0 (for hybrid x *dwarf* cross n = 3034 female pairwise values, 2227 significant male pairwise values while for hybrid x Normal cross n = 1759 female pairwise values and n = 5816 significant male pairwise values) revealed similar recombination levels between sexes and differential recombination levels between families. A LSD post hoc comparison among all groups (between sexes and families) indicated that there was a marginal difference between the male and female recombination frequencies in the hybrid x *dwarf* (0.260 vs. 0.264, \( p = 0.041 \)) family and no observable difference in the hybrid x Normal (0.231 vs. 0.227, \( p = 0.295 \)). In contrast, these pairwise values were significantly reduced in the hybrid x Normal family (\( p = 0.000008 \)) compared to the hybrid x *dwarf* (Figure 4). However, when the same comparison was performed between 12 colinear marker intervals between families, there was no significant difference (t-test, \( F = 1.69, p = 0.39 \), Table 3). Overall, these results also suggested a more elevated heterozygosity in the pure cross males compared to the female-
derived maps, given that the density of both maps (similar numbers of markers) and the average distance between markers was similar in both backcrosses.

Comparison of segregation distortion between families: Loci exhibiting segregation distortion were generally distributed genome-wide with 28 linkage groups implicated overall in both families (Figure 2). Within a given linkage group, the segregation distortion was almost exclusively unidirectional (e.g. Lg3) suggesting that linked loci elicited the same genotypic response and that genotypic ratios among individuals were not random. Comparisons of distorted homologous loci between families revealed that they were distributed over seven common linkage groups, indicating that many parallel genomic regions in these hybrid backcrosses were potentially implicated in unfavourable interactions between genomes (Table 4).

There were overall 64 homologous loci of the same phase (i.e., same sex) among both families. These 64 loci were distributed over 26 linkage groups and consisted of AFLP originating from 12 different selective primer combinations. A comparison of the level of allelic frequency distortion for all of these loci revealed that degree of segregation distortion between families was correlated (Spearman R = 0.378, \( p = 0.0021 \), Figure 5) to a certain extent.

This level of segregation distortion had only a marginal influence on the observed map distances. An average change in recombination frequency of 5.7 % in the hybrid x dwarf and 1.4 % in the hybrid x Normal was observed over all loci when comparing the standard and Bailey’s recombination fraction for backcrosses (Table 5). However, in a few cases, such as the 70% reduction in distance for loci linked to GGTG120.8 on Lg3, the
recombination change was considerable (Table 5). Estimation of the most likely order of these loci indicated that in only one linkage group (Lg25, locus CAAT060.4) did the change in recombination fraction under Bailey’s recombination fraction result in another order that was 10 times more likely than that of the classic estimate (LOD = 1.04, Table 5). Thus, loci exhibiting significant segregation distortion in these families had a slight impact overall on mapping distance between loci and a limited impact on the most likely order.

**Distribution of AFLP loci:** The distribution of AFLP loci amplified from different selective primer combinations over these linkage groups was not random in either family. Comparisons of the observed and expected relationships for 11 linkage groups for which information was available for both sexes (15 selective primer combinations x 11 linkage groups = 165 tests for each family where a 5% tablewide significance threshold was inferred when \( P < 0.0003 \)) indicated that 10 out of 15 primer combinations exhibited non-random distribution to at least one linkage group in the hybrid x dwarf map (\( p < 0.0001 \)) while 14 out of 15 selective primer combos exhibited non-random distributions in the hybrid x Normal (\( p < 0.0001 \), data not shown). Of the 11 linkage groups in each family that had information for both parents, six were homologous allowing comparisons between families. Of these homologous linkage groups, four of six illustrated parallel clustering of the same primer combination in both families (CATA on Lg7; CCTC on Lg9; AGAC on Lg12; and CAAT on Lg25, Figure 3).
DISCUSSION

Linkage maps provide a powerful genome-wide framework towards elucidating the genetic footprints of changes that have occurred as a result of population divergence (Whitkus 1998). Comparative genetic maps of reciprocal hybrids are particularly advantageous because their architecture is based on the genomic composition and viability of hybrid genotypes upon recombination in the F₁ hybrids (e.g. Chu and Howard 1998, Rieseberg et al. 2000, Myburg et al. 2004).

In this study, our main objective of elucidating the genetic architecture of population divergence in the lake whitefish was advanced through the mapping of 877 AFLP and 30 microsatellite loci in the construction of two sex-specific linkage maps in each dwarf and Normal backcross. Comparisons between backcrosses found significant evidence for 34 of the 40 linkage groups presumably corresponding to the haploid number of 40 chromosomes in the lake whitefish species complex. However, we cannot exclude the possibility that some chromosomes are represented by more than one linkage group. These four linkage maps provided insight into facets of genetic architecture because of the high degree of colinearity across both genetic backgrounds, comparable patterns of resistance to introgression in both AFLP and microsatellite loci, and non-random clusters of AFLP distribution. Overall, these results will contribute towards understanding the genetic architecture of this species complex and offer some insight into the potential genomic consequences of introgressive hybridization in cases of recent population divergence.

The extensive use of primarily dominant markers coupled with a pseudo-testcross strategy for map construction (Grattapaglia and Sederoff 1994) attests to their continued utility (e.g. Parsons and Shaw 2002, Nichols et al. 2003, Liu et al. 2003,
Wang and Porter 2004), particularly in unexplored genomes where these marker systems provide an ability to proceed without a priori genetic knowledge (Whitkus 1998, Parsons and Shaw 2002). Here, over 91% of homologous loci (segregating 1:1) were linked to the same linkage group across families suggesting that AFLP linkage relationships were highly concordant and likely represented homologous genomic regions between the dwarf and Normal parents (Figure 2). Moreover, there was a high degree of synteny with previous linkage relationships from a smaller subset of hybrid × dwarf individuals at 100 linked loci and 12 primer combinations (Rogers et al. 2001, Figure 2). Small inversions were also observed between some linkage groups (e.g., Lg4, Figure 2), and these were often associated with homologous loci informative in the opposite sexes between families. These inconsistencies in marker order were likely the result of doubtful positioning, particularly from loci considered as accessory markers within the current linkage map (Pelgas et al. 2005, 2006). An additional explanation for non-colinearity may also be that despite the stringent criteria used for defining homology, homoplasy is still a likely source of mis-scoring.

**Nonrandom distribution of loci:** AFLP loci amplified from selective primer combinations clustered non-randomly across particular linkage groups. Parallel distributions observed across both families revealed a pattern unlikely to have arisen by chance alone. Although not statistically tested before, observations of AFLP clustering in other species have previously prompted the suggestion that the distribution of AFLP loci across the genome is not random (Peng et al. 2000, Strommer et al. 2002). In linkage maps this pattern has been observed at three levels. The first includes AFLP that map to non-random clusters regardless of the restriction enzymes and selective primer combinations employed,
exhibiting a non-random distribution relative to other marker intervals typically presumed to be result of reduced recombination around the centromere (Tanksley 1993, Qi et al. 1998, Young et al. 1998, Wang and Porter 2004). The second is AFLP loci amplified from different restriction enzymes that exhibit non-random distribution across linkage groups (Young et al. 1999, Peng et al. 2000, Nichols et al. 2003, this study). Finally, non-random distribution of AFLP amplified from selective primer combinations has also been observed (Lashermes et al. 2001, Schwarz-Sommer et al. 2003, Campbell and Bernatchez 2004).

The implications of these observations for linkage mapping suggests that many selective primer combinations and restriction enzymes combined with other marker systems may be required in order to ensure complete coverage of the genome. This may also contribute to potential gaps in the maps, which in some cases may cause the number of linkage groups to exceed the actual number of chromosomes (Wang and Porter 2004). However, they also beg for an explanation as to how genomic dynamics may explain these types of patterns. It has been proposed that AFLP markers generated from EcoRI sites cluster outside or between gene rich regions resulting from the ability of EcoRI to digest methylated DNA associated with noncoding regions (Young et al. 1999, Schwarz-Sommer et al. 2003, Takata et al. 2005). An additional possibility is the presence of repetitive DNA potentially influencing the probability of repetitive AFLP sequences in the whitefish genome. It has been estimated that as much as 70% of the salmonid genome contains repetitive DNA (Britten and Kohne 1968). In some species the process by which repetitive sequences are generated has been suggested to lead to genome expansion (Livingstone et al. 1999) coupled with observations that there is an increase in retro-elements of large genome species when compared to smaller-genome species (SanMiguel
et al. 1996). Thus, it is possible that transposable elements interspersed across gene-rich and neutral regions may partially account for these observations, whereby AFLP markers may indeed be associated with repetitive elements (Hamada et al. 1997, Young et al. 1999, Park et al. 2003, Wang et al. 2005). This is supported by recent studies of comparative genomics between species that have revealed a number of highly conserved sequence motifs confirming that sequences of genomes may not be random (Dermitzakis et al. 2005). Overall, these explanations may also partially explain why complete coverage of the genome was not observed with 15 selective primer combinations.

Finally, many microsatellites mapped to the terminal regions of the genome. Given the non-random segregation of markers with the AFLP, this may have influenced estimates of linkage in microsatellites. For example, many studies have suggested that AFLP loci are more centromeric than microsatellites (Haanstra et al. 1999, Young et al. 1999), which could have partially explained these patterns. The non-random distribution of microsatellites may also be associated with a stabilizing function in the architecture of chromatin possibly contributing to the degree of segregation bias at the terminal ends (Blackburn and Greider 1995, Takayanagi et al. 1997, de Caceres II et al. 2004). Unfortunately, we did not position enough microsatellite loci to detect any significant tendency within the current data set compared to the AFLP.

Comparing the genetic architecture of both families: In salmonids, few intrinsic barriers to hybridization among closely related species are hypothesized to exist (Taylor 2004). However, sex-specific differences in meiotic recombination rate within species appear to be common in vertebrates (Devincent and Tanksley 1993, Otto and Lenormand 2002, Lenormand and Dutheil 2005) with the highest differences among all species being
reported in salmonids (6.4:1 female to male linkage map distance ratio in brown trout, *Salmo trutta*, Gharbi et al. 2006). This is hypothesized to result from the formation of multivalents during meiosis in males hindering recombination in the distal region of chromosomes (potentially due to structural constraints) during meiosis I (Wright et al. 1983, Sakamoto et al. 2000).

In stark contrast, no gender differences were found between the recombination frequencies or marker interval distances in either family. This is unusual when considering that some degree of sex-specific differences have been observed in all other salmonid species studies to date, including rainbow trout (*Onchorhynchus mykiss*, Sakamoto et al. 2000, Nichols et al. 2003, O’Malley et al. 2003), arctic charr (*Salvelinus alpinus*, Worman et al. 2004), and Atlantic salmon (*Salmo salar*, Moen et al. 2004). However, because F₁ hybrid females were utilized in generating both backcross families in this map, hybrids might also have suppressed recombination compared to pure species because of differentiation between the homologous chromosomes of the parental species (Tenhopen et al. 1996, Chetelat et al. 2000, Yin et al. 2004, Worman et al. 2004). Thus, it is possible that the elevated recombination in our female markers of hybrid origin may have been offset by suppressed recombination due to the heterogenous genetic background of the female hybrids compared to the pure parent in this pedigree (Pломон and Oмаллее 1996, Yin et al. 2004). This, in turn, would have resulted in comparable levels of recombination observed between sexes, in contrast with previous studies in salmonids. This pattern is consistent with observations in a study on Arctic charr where females of hybrid origin were used in mapping families (Worman et al. 2004).

In contrast, when comparing the recombination frequencies of all linked loci, we observed a significant difference in recombination between hybrid backcrosses, with
reduced recombination in the hybrid x Normal family when compared to the hybrid x dwarf family (Figure 2). Yet, when this comparison was made using strictly colinear marker intervals between families, there was no significant difference (Table 3). Differences in recombination may result from differences in the inviability between hybrid families of variable genetic backgrounds. If so, hybrid crosses are expected to exhibit differences in recombination because of the potential fitness consequences incurred in two distinct sources; recombinant gametes or upon the formation of zygotes (Butlin 2005). While the first prediction is indirectly supported by results between sexes, the second prediction is partially supported by the results between families, given that the degree of hybrid inviability was much higher in the hybrid x Normal family (21% survival upon hatching) compared to the hybrid x dwarf family (45% survival upon hatching, Rogers and Bernatchez 2006). If differential levels of inviability between hybrid crosses are the result of selection for or against hybrid genotypes during development, this may be observed as differential levels of recombination across genetic backgrounds in linkage maps. Overall, outbreeding depression may result from various genetic mechanisms (Burke and Arnold 2001) and we are still not sure of the mechanism in many cases (Pélabon et al. 2005). For example, Ferreira and Amos (in press) found evidence for some heterozygote fitness advantages in inbred and outbred lines of Drosophila, while the linkage disequilibrium effects of these extended up to 10Mb, thereby influencing the frequency of recombination as well as the preferential segregation of certain genotypes in these regions of the genome. However, Ferreira and Amos (in press) also found that increased fitness was sometimes associated with homozygous regions as well, possibly due to the effects of selection, demonstrating that the consequence of crossing divergent lines may be variable depending on the region of the genome. Presumably, the effect may be
genome-wide or may manifest along specific linkage groups, but could overall result in a change in the recombination frequencies compared to what would have been observed in crosses of their pure counterparts.

Heterozygosity also appeared elevated in the pure cross male derived maps compared to the female-derived maps, given that the density of both maps (similar numbers of markers) and the average distance between markers was similar in both families. Consequently, there were approximately equal numbers of informative markers for each of the male and female maps (Table 1, Figure 2). There are several possibilities to explain this observation: 1) increased heterozygosity in pure forms compared to inbred lines (e.g., Lynch and Walsh 1998, Jiggesen et al. 2005) and/or 2) decreased heterozygosity in female gametes due to outbreeding depression (e.g., Ferreira and Amos, in press). Both hypotheses are entirely plausible given the allopatric separation of these glacial races (> 200 000 years of divergence, Bernatchez and Dodson 1990) and the subsequent ecological divergence of dwarf and Normal populations (>10000 years, Lu et al. 2001) used to generate these mapping families.

First, with respect to increased heterozygosity in pure forms, our outbred crossing design, based on dominant markers and the pseudo-testcross strategy (Grattapaglia and Sederoff 1994), likely had considerable impacts on mapping with the main effect being an increase in the number of segregating markers (Myburg et al. 2003, Yin et al. 2004, Pelgas et al. 2006). Consequently, the number of markers segregating 3:1 was also likely inflated, particularly when considering the parents originated from divergent sources (Jiggesen et al. 2005, Pelgas et al. 2006). Overall, over one third of the markers showed a 3:1 segregation ratio and this was much higher than the than the proportion expected by chance alone when using dominant markers.
There is also the possibility of the second prediction that heterozygosity was reduced in females as a function of outbreeding depression. For example, emerging empirical studies on the fitness consequences of genotypes in inbred and outbred crosses are also finding that the fitness of early generation hybrids may reflect both the action of dominance effects (hybrid vigor) and recombination (hybrid breakdown) within the same family (e.g., Ferreira and Amos, in press, Pélabon et al. 2005, Johansen-Morris and Latta 2006). These experiments demonstrate how a single hybridization event within one family can result in a number of outcomes including hybrid vigor, hybrid breakdown, and transgressive segregation, which ultimately interact to determine long-term hybrid fitness. Notably, mechanisms such as transgressive segregation have been observed in hybrid whitefish (Rogers and Bernatchez 2006), but their impact on linkage mapping remains largely unknown. For example, recent observations of developing hybrid embryos have found that hybrid genotypes on linkage group 1 may suffer a fitness consequence upon hybridization (Rogers and Bernatchez 2006). We observed that loci segregating in Lg1 exhibited the same heterozygous pattern in both families and that in this particular linkage group it was the male-based pure markers that were heterozygous (Figure 2). Either explanation will require further studies to confirm the mechanisms and demonstrate that the observed heterozygosity levels reflect the possible consequence of crosses between divergent taxa. For now, these hypotheses remain speculative.

The notion that hybridization can have vast evolutionary consequences is without question (Burke and Arnold 2001, Taylor 2004). Yet, without an understanding of the genetic response to hybridization these results demonstrate just how challenging it may be to elucidate the evolutionary consequences of population divergence, particularly when the
genetic architecture of divergence seems to involve numerous chromosomal regions, such as in lake whitefish.

**Segregation distortion:** A large percentage of AFLP and microsatellite loci across families exhibited segregation distortion. Deviations from Mendelian expectations implicated several homologous loci and linkage groups across families (Figure 2, Table 2), supporting the hypothesis that segregation distortion resulted from heterospecific interactions between genomes due to divergence at over 30% of the loci included in our map. Both Whitkus (1998) and Fishman et al. (2001) observed up to 50% distortion in interspecific hybrid crosses of plants where segregation distortion was presumed to be a consequence of hybridization between divergent genomes. Such postzygotic barriers typically manifest themselves in linkage maps of reciprocal hybrid crosses resulting from selection on the F₁ gametes or by selection on recombinant BC zygotes (Kim and Rieseberg 1999, Li et al. 1997), where the degree of segregation distortion appears to be correlated with increasing genetic divergence between parental lines (Zamir and Tadmor 1986, Quillet et al. 1995, Grandillo and Tanksley 1996).

Several causes may explain these observations. Rieseberg et al. (2000) proposed that ‘donor’ alleles favoured in hybrid genetic backgrounds might represent ‘selfish’ genes (genes that enhance the success of gametes they inhabit even if they pose a substantial fitness cost for diploids, reviewed in Lyttle 1991). Other factors that may explain this pattern include the genetic basis of inbreeding and, in the case of hybrids, outbreeding depression (Remington and O’Malley 2000). These predictions require knowledge of the fitness cost of distorted alleles or aberrant gene expression in hybrids during development. Fixed loci that have an impact on fitness should be heterozygous in the F₁ hybrid, segregate
in both hybrid backcrosses, and exhibit a deficiency of the respective donor alleles in at least one of the two backcrosses (MYBURG et al. 2004). This is consistent with the Dobzhansky-Muller model of negative heterospecific interactions resulting in intrinsic incompatibilities caused by the inviability of zygotes that inherited specific diploid hybrid genotypes (ORR and TURELLI et al. 2001, COYNE and ORR 2004). Our results may fit this expectation when considering that several genomic regions elicited parallel genotype frequency distortions between families, although it is important to note that we did not establish a role for epistasis (Figure 4). Furthermore, a recent experiment in the same family employed 100 of the mapped loci to follow Mendelian segregation ratios during embryonic development (ROGERS and BERNATCHEZ 2006). This experiment confirmed that elevated mortality in these hybrid backcrosses was associated with a significant genotypic response among linked loci in at least seven linkage groups during development. More loci than expected on two linkage groups (Lg3 and Lg18) exhibited a significant shift in Mendelian segregation ratios during development, supporting a role for these incompatibilities contributing to be associated with intrinsic hybrid inviability (ROGERS and BERNATCHEZ 2006). Thus, chromosomal regions implicated in differential mortality are also associated with elevated segregation distortion, indicating that incompatible developmental gene expression may be contributing to the elevated mortality occurring during these embryonic developmental phases in the hybrid genotypes.

Overall, these results indicate that negative interactions among alleles at different loci contribute to the reproductive barriers between diverging whitefish populations. Because this mechanism would select against unmatched genotypes at pairs of interacting loci rather than favouring alleles from one parent, it should also generate distortion in both parental directions (VOGL and XU 2000, FISHMAN et al. 2001, MYBURG et al. 2004), such
as observed here for several genomic regions that showed parallel positive and negative allele frequency distortion correlations across both families (Figure 4). It will be important that future mapping studies determine the change in the level of linkage disequilibrium between loci as the physical distance from the distorted regions increases to determine the distribution of the size of the isolation unit.

**Linkage mapping considerations in divergent hybrid backcrosses:** A limitation to this study was imposed by the use of primarily dominant markers and a backcross design hindering distinctions between sources of error. For example, segregation distortion appeared to result from heterospecific interactions between genomes as opposed to inbreeding depression. For inbreeding depression to substantially distort genotypic frequencies in BC, a lethal or semi-lethal recessive allele heterozygous in one parent must be transmitted to the F1, and upon segregation in higher inbred crosses cause differential zygote mortality and a deficit of carrier parental heterozygotes (LYNCH and WALSH 1998). Inbreeding depression can be a major source of segregation distortion in linkage mapping populations (REMINGTON and O'MALLEY 2000). However, loci analyzed in these hybrid backcrosses were derived from two outbred populations with considerable genetic variation present in the parental individuals. This was supported by the high level of heterozygosity observed in the F1 parental lines given that approximately 30% of AFLP loci segregated 3:1 within both families indicating both parents were heterozygous (Aa) at these loci (LYNCH and WALSH 1998, JIGGINS et al. 2005). The history of these lineages also suggests that sufficient divergence has occurred for populations to have acquired separate, co-evolved gene complexes, to the extent that reduced zygote viability are likely the result of outbreeding depression (LU and BERNATCHEZ 1998, LYNCH and WALSH 1998, ROGERS and
BERNATCHEZ 2006). Future experimental designs will need to take these considerations into account in order to increase the likelihood of correctly inferring which genetic mechanisms are responsible for incompatible interactions between genotypes.

It is equally important to consider possible non-genetic sources of segregation distortion; sampling biases, co-migration, and scoring errors (Remington et al. 1999, Myburg et al. 2004). For instance, genotyping errors can be difficult to identify, particularly in dominant markers (Remington et al. 1999), and consequently may impact marker placements or recombination fractions (Buetow 1991, Ehm et al. 1996, Collins et al. 1996, Li et al. 2005). Systematic segregation distortion may also artificially inflate intermarker distances or result in tight clustering of markers, depending on the direction of the distortion (Bailey 1949, Lorieux et al. 1995, Liu 1998, Fishman et al. 2001). A pair of linked markers distorted in the same direction will have an apparent excess of non-recombinant homozygotes or heterozygotes relative to an otherwise similar pair of undistorted markers (Fishman et al. 2001, Hackett and Broadfoot 2003). We tested the influence that significant segregation distortion among homologous loci had on linkage map distance and order by comparing classic recombination fractions with those estimated under Bailey’s recombination fraction which has been purported to be better suited for distorted loci (Lorieux et al. 1995). We found that in some cases distances were significantly different between estimates but in other cases the most likely order remained unchanged. This is consistent with simulations that have suggested that the presence of segregation distortion renders linkage mapping efforts more difficult, but overall may not necessarily cause strong biases in linkage associations (Cloutier et al. 1997, Hackett and Broadfoot 2003, Fishman et al. 2001).
Overall, because loci deviating from Mendelian expectations appear to underlie integral aspects of population divergence, studies of segregation distortion will eventually need to consider the genomic region implicated, the direction, and the relative influence of the distortion over different genetic backgrounds. Namely, it is unclear to what extent the effect of distortion on interval lengths will diminish the utility of the map for QTL analysis. For example, permutations commonly employed to establish QTL significance may need to be calculated separately for the contiguous distorted region (Doerge and Churchill 1996, Fishman et al. 2001). More simulations need to be performed in order to compensate for observed levels of segregation distortion and their impact on recombination fraction.

**Summary:** How many genomic regions can we expect to be implicated during population divergence and how many actually contribute to reproductive barriers? There is no single answer to this question but the current consensus is that they correspond to ‘ordinary loci’, that is, loci not associated with speciation *per se* (Coyne and Orr 2004, Orr 2005). Our data suggest that population divergence in the lake whitefish may implicate several genomic regions and therefore knowledge of the genetic architecture of phenotypic traits differentiating dwarf and Normal whitefish will be necessary to elucidate the potential evolutionary forces that have contributed to their divergence. Several aspects of their evolutionary history and their recent sympatric divergence suggest that historically contingent and adaptive trait differentiation may be jointly contributing to the differential locus-specific viability observed in dwarf -Normal hybrid crosses (Rogers et al. 2001, Rogers and Bernatchez 2006). For example, parallel locus-specific reductions in gene flow for growth QTL have been found between dwarf and Normal ecotypes among independently diverging populations, indicating that growth differences between
populations are maintained by divergent selection (ROGERS and BERNATCHEZ 2005). Evidence for parallel evolution indirectly demonstrated the role of divergent selection in maintaining differentiation between sympatric whitefish ecotypes for several traits, including morphology, behaviour, as well as gene expression associated with swimming efficiency and energy metabolism (LU and BERNATCHEZ 1999, ROGERS et al. 2002, DEROME et al. 2006). As such, understanding the consequences of population divergence in the lake whitefish will benefit from a population genomics approach that embraces a genome-wide search for the genetic basis of phenotype-environment associations (LUIKART et al. 2003, SCHÖTTERER 2003). This study demonstrates how genetic linkage maps incorporating dwarf and Normal genetic backgrounds should prove beneficial towards achieving these objectives in the lake whitefish species complex.
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Appendix 1. Summary of microsatellite loci characterized by the locus name, their source (species specific or cross-amplified) and the parental genotypes (denoted by the alleles presented in numerical format) in the two mapping families.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Species</th>
<th>Source</th>
<th>hybrid x dwarf</th>
<th>hybrid x Normal</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td>♀</td>
<td>♂</td>
</tr>
<tr>
<td>Cocl 4</td>
<td><em>C. clupeaformis</em></td>
<td>Rogers et al. 2004</td>
<td>1,3</td>
<td>2,3</td>
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<td>1,3</td>
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<td>C2-157</td>
<td><em>C. arcti</em></td>
<td>Turgeon et al. 1999</td>
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<td>BHMS-538</td>
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<td>BWF-1</td>
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<td>Sakamoto et al. 2000</td>
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<td>Osmo-5</td>
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<td>Taylor et al. 1993</td>
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<td>Species</td>
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<td>Allele 2</td>
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<td>*</td>
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<td>S. salar</td>
<td>McConnell et al. 1995</td>
<td>1,2</td>
<td>1,1</td>
</tr>
</tbody>
</table>

Note: *, indicates that the locus was monomorphic for that family.
Table 1 Summary of all AFLP loci amplified in each of the 15 primer combinations and their pattern of segregation for both backcrosses. The number of informative fragments segregating 1:1 for each parent is presented while the column 3:1 refers to the number of fragments found where both parents were heterozygotes. The final column refers to the number of homologous fragments informative for mapping in both families.

<table>
<thead>
<tr>
<th>AFLP Primer combination</th>
<th>hybrid x dwarf</th>
<th>hybrid x Normal</th>
<th>Homologous (1:1) in both families</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1:1)</td>
<td>3:1</td>
<td>(1:1)</td>
</tr>
<tr>
<td>AGAC</td>
<td>5</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>CAAG</td>
<td>34</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>CTAG</td>
<td>14</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>CAAT</td>
<td>8</td>
<td>39</td>
<td>4</td>
</tr>
<tr>
<td>ACTA</td>
<td>8</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>CATA</td>
<td>38</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>ACTC</td>
<td>2</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>AGTC</td>
<td>7</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>CCTC</td>
<td>9</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>CGTC</td>
<td>19</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>CTTC</td>
<td>12</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>CCTG</td>
<td>11</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>GGTC</td>
<td>18</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>ACTT</td>
<td>24</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>AGTT</td>
<td>7</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>216</td>
<td>185</td>
<td>150</td>
</tr>
<tr>
<td></td>
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<td>267</td>
<td>284</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>122</td>
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</table>
Table 2. Summary of AFLP (denoted by their selective primer combination) and microsatellite loci segregating in both backcrosses and illustrating those markers which exhibited significant levels of segregation distortion ($p < 0.00001$) for each parent. Within each parent, the number of loci that exhibited significant allele frequency distortion in either direction is also shown.

<table>
<thead>
<tr>
<th>Marker groups</th>
<th>hybrid x dwarf</th>
<th>hybrid x Normal</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>♀ hybrid</td>
<td>♂ dwarf</td>
</tr>
<tr>
<td></td>
<td>Mapped</td>
<td>Segregation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>distortion</td>
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<td></td>
<td></td>
<td>under</td>
</tr>
<tr>
<td>AGAC</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>CAAG</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>CTAG</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>CAAT</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>ACTA</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>CATA</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>ACTC</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>AGTC</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>CCTC</td>
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<td>1</td>
</tr>
</tbody>
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Table 2 (continued)

<table>
<thead>
<tr>
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<th>8</th>
<th>0</th>
<th>5</th>
<th>3</th>
<th>0</th>
<th>1</th>
<th>8</th>
<th>1</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGTC</td>
<td>12</td>
<td>5</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>43</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>CTTC</td>
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<td>0</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
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<td>15</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>CCTG</td>
<td>18</td>
<td>6</td>
<td>7</td>
<td>33</td>
<td>9</td>
<td>0</td>
<td>36</td>
<td>3</td>
<td>0</td>
<td>54</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>GGTC</td>
<td>24</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>26</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ACTT</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>26</td>
<td>3</td>
<td>0</td>
<td>12</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>AGTT</td>
<td>14</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Microsatellites</td>
<td>211</td>
<td>37</td>
<td>33</td>
<td>178</td>
<td>17</td>
<td>46</td>
<td>199</td>
<td>32</td>
<td>20</td>
<td>260</td>
<td>60</td>
<td>18</td>
</tr>
</tbody>
</table>

Note: under, under-representation of the heterozygote genotype; over, over-representation of the heterozygote genotype.
Table 3 Comparison of mapping distances for homologous sex-specific marker intervals among parents between families.

<table>
<thead>
<tr>
<th>Linkage Group</th>
<th>Marker Interval</th>
<th>hybrid x <strong>dwarf</strong> Interval Distance (cM)</th>
<th>hybrid x Normal Interval Distance (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>CATA124.9</td>
<td>8.2</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>CATA134.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>ACTT130.3</td>
<td>5.3</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>ACTT150.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>CAAT237.7</td>
<td>13</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>CAAT216.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>ACTT078.1</td>
<td>0.6</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>ACTT088.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>ACTC175.4</td>
<td>11.6</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>ACTC138.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>GGTG109.3</td>
<td>9.9</td>
<td>22.7</td>
</tr>
<tr>
<td></td>
<td>GGTG100.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>GGTG094.9</td>
<td>16</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>GGTG100.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>ACTC170.0</td>
<td>30.7</td>
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</tr>
<tr>
<td></td>
<td>ACTC196.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>CGTC120.1</td>
<td>2.9</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>CGTC132.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>GGTG139.5</td>
<td>19.2</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>GGTG149.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>GGTG127.5</td>
<td>11.8</td>
<td>16.4</td>
</tr>
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<td></td>
<td>GGTG106.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>CCTG124.4</td>
<td>7.2</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>CCTG108.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4 Homologous loci exhibiting significant levels of segregation distortion in both backcrosses. LG refers to linkage group, $\chi^2$ 1:1 refers to the chi-square test of 1:1 Mendelian segregation in the progeny.

<table>
<thead>
<tr>
<th>LG</th>
<th>Locus</th>
<th>hybrid x dwarf</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Parent</td>
<td>$\chi^2$ 1:1</td>
</tr>
<tr>
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<td>GGTG120.8</td>
<td>♀ hybrid</td>
<td>63.2*</td>
</tr>
<tr>
<td>12/28</td>
<td>CCTC104.9</td>
<td>♀ hybrid</td>
<td>36.8*</td>
</tr>
<tr>
<td>19</td>
<td>CCTC064.9</td>
<td>♂ dwarf</td>
<td>38.6*</td>
</tr>
<tr>
<td>23</td>
<td>CAAT237.7</td>
<td>♀ hybrid</td>
<td>25.3*</td>
</tr>
<tr>
<td>25</td>
<td>CAAT060.4</td>
<td>♂ dwarf</td>
<td>98.4*</td>
</tr>
<tr>
<td>25</td>
<td>CAAT119.8</td>
<td>♀ hybrid</td>
<td>41.2*</td>
</tr>
<tr>
<td>25</td>
<td>CAAT053.7</td>
<td>♂ dwarf</td>
<td>98.4*</td>
</tr>
<tr>
<td>28</td>
<td>ACTC092.7</td>
<td>♂ dwarf</td>
<td>36.4*</td>
</tr>
</tbody>
</table>

Note: $aa$, homozygous for the null allele; $Aa$, heterozygous; *, denotes 5% tablewide level of significance, $P<0.00001$.

*a* Linkage conflict for CCTC104.9 where the locus was linked to Lg12 in hybrid x dwarf and Lg28 in hybrid x Normal.
Table 5 Comparative analysis of the effect of segregation distortion on mapping order and distance as estimated between the classic backcross and Bailey’s mapping function. The center locus (in bold) of each marker group exhibited significant segregation distortion in both families.

<table>
<thead>
<tr>
<th>LG</th>
<th>Linked markers</th>
<th>RF Estimate</th>
<th>change</th>
<th>Linked markers</th>
<th>RF Estimate</th>
<th>change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GGTG126.7</td>
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<td>GGTG137.4</td>
<td>0.164</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GGTG148.6</td>
<td>0.258</td>
<td></td>
<td>GGTG148.6</td>
<td>0.257</td>
<td>-0.023</td>
</tr>
<tr>
<td></td>
<td>CTAG065.8</td>
<td>0.258</td>
<td>-0.027</td>
<td>GGTG138.1</td>
<td>0.280</td>
<td>-0.023</td>
</tr>
<tr>
<td>1</td>
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<td>GGTG099.9</td>
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<tr>
<td></td>
<td>GGTG136.8</td>
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<td>GGTG137.4</td>
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</tr>
<tr>
<td></td>
<td>GGTG213.3</td>
<td>0.269</td>
<td>-0.055</td>
<td>GGTG148.6</td>
<td>0.174</td>
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</tr>
<tr>
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<td>CAAG114.6</td>
<td>0.199</td>
<td></td>
<td>CAAG114.6</td>
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<tr>
<td></td>
<td>CAAG141.1</td>
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<td>-0.023</td>
</tr>
<tr>
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<td>CAAG129.1</td>
<td>0.188</td>
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<td>CAAG114.6</td>
<td>0.279</td>
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</tr>
<tr>
<td></td>
<td>GGTG120.8</td>
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<td>GGTG120.9</td>
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<tr>
<td></td>
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<td>-0.007</td>
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<td>CGTC120.2</td>
<td>CGTC132.2</td>
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<td>AGTT070.9</td>
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<tr>
<td>19</td>
<td>0.054</td>
<td>0.271</td>
<td>0.061</td>
<td>0.079</td>
<td>0.007</td>
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<td>0.289</td>
<td>0.304</td>
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</tr>
<tr>
<td></td>
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<td>-0.009</td>
<td>0.007</td>
<td>-0.009</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 5 (continued)**

Note: LG, refers to linkage group; RF refers to the recombination fraction; change refers to the difference between estimates.
FIGURE LEGENDS

Figure 1. Experimental mating scheme employed to generate the two backcross mapping families. The parental generation was sampled in the field as detailed in Lu and Bernatchez (1998) and as such these individuals were no longer available after spawning. The number of gametes was equilibrated in each of the F$_1$ groups generated for the purpose of comparing F$_1$ hybrid inviability.

Figure 2. Sex-specific genetic linkage maps of dwarf, Normal, and hybrids parents in the lake whitefish. Linkage groups on the left hand side represent the Hybrid x dwarf backcross while linkage groups on the right hand side are from the Hybrid x Normal backcross. Homologous loci are indicated by connecting lines between families. Linkage homologies within families (between male and female linkage groups) were implied upon detecting significant linkage in the repulsion phase between sex-specific markers (see methods). Linkage homology between families was inferred only by homologous loci that were linked in either family (noted by the lines connecting linkage groups between families). An * beside locus name indicates conflicting linkage between families. Downward arrows indicate under-represented loci while solid upward arrows indicate over-represented loci exhibiting segregation distortion (P < 0.00001). Sex-specific parents are denoted by female (♀) and male (♂), see cross design in Figure 1. Loci assessed as anchors are in bold within linkage groups. When possible, linkage group notation was consistent with preliminary linkage associations previously established (ROGERS ET AL. 2001). Microsatellite loci are italicized to distinguish from AFLP loci.
Figure 3. Observed and expected number of AFLP for selective primer combos at homologous linkage groups containing locus information for both sexes. On the x-axis the primer combinations are listed for each family (hd = hybrid x dwarf and hN = hybrid x Normal) while the expected frequency of these loci is listed on the bottom row. A significant cluster of AFLP in one family is denoted by an asterisk on top of the observed column while parallel clusters observed in both families are denoted by an * between observed columns. White numbers for significant observations represent absolute observed values that were higher than the given scale.

Figure 4. Pairwise recombination frequencies for sex-specific markers between families when calculated among all linked loci exhibiting an LOD greater than 4.0 (but see Table 5 for comparison of mapping distances for homologous sex-specific marker intervals between parents among families).

Figure 5. Comparison of the percentage and direction of segregation distortion, i.e., (frequency of Aa – Expected frequency of 0.5) x 100% calculated from the observed segregation ratios for mapped, homologous loci between the hybrid x dwarf and hybrid x Normal backcrosses.
Origin

\( P_1 \) (Natural populations)

\textit{Acadian glacial}\n
\[ \begin{align*}
♀ \text{Normal} & \times ♂ \text{dwarf} \\
♀ \text{dwarf} & \times ♂ \text{dwarf}
\end{align*} \]

\textit{Outbred F}_1 \text{ (1996)}

\textit{dwarf}\n
\[ \begin{align*}
♀ \text{Normal} & \times ♂ \text{dwarf} \\
♀ \text{dwarf} & \times ♂ \text{Normal}
\end{align*} \]

\[ \begin{align*}
♀ \text{hybrid} \ (♀ \text{Normal} \times ♂ \text{dwarf}) & \times ♂ \text{dwarf} \\
♀ \text{hybrid} \ (♀ \text{dwarf} \times ♂ \text{Normal}) & \times ♂ \text{Normal}
\end{align*} \]

\[ n = 198 \text{ progeny} \]

\[ n = 138 \text{ progeny} \]

\textit{Atlantic-Mississippian glacial}\n
\[ \begin{align*}
♀ \text{Normal} & \times ♂ \text{dwarf} \\
♀ \text{dwarf} & \times ♂ \text{Normal}
\end{align*} \]

\[ \begin{align*}
♀ \text{dwarf} & \times ♂ \text{Normal} \\
♀ \text{Normal} & \times ♂ \text{Normal}
\end{align*} \]

\[ \begin{align*}
♀ \text{hybrid} \ (♀ \text{Normal} \times ♂ \text{dwarf}) & \times ♂ \text{dwarf} \\
♀ \text{hybrid} \ (♀ \text{dwarf} \times ♂ \text{Normal}) & \times ♂ \text{Normal}
\end{align*} \]

\[ n = 198 \text{ progeny} \]

\[ n = 138 \text{ progeny} \]
Figure 2
Figure 2
Figure 2
Figure 2
Figure 2
Figure 3

Observed and Expected Number of AFLP per Linkage Group

AFLP Selective Primer

AGAC  0.04
CAAG  0.05
CTAG  0.07
CAAT  0.06
ACTA  0.08
ACTC  0.12
AGTC  0.03
CCTC  0.08
CGTC  0.02
CTTC  0.09
CCTG  0.04
GGTC  0.11
ACTT  0.04
AGTT  0.11

Lg6
Lg7
Lg8
Lg9
Lg12
Lg25

h/dhN
Figure 4
Figure 5