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Linkage Maps of the *dwarf* and Normal Lake Whitefish (*Coregonus clupeaformis*) Species Complex and Their Hybrids Reveal the Genetic Architecture of Population Divergence

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ABSTRACT

Elucidating the genetic architecture of population divergence may reveal the evolution of reproductive barriers and the genomic regions implicated in the process. 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.

UNDERSTANDING the genetic consequences of population divergence is central to evolutionary biology (Edmands 2002; Coyne and Orr 2004; 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 genomewide perspectives are integral in working toward 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 for 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 for the mapping of gene expression profiles (expression QTL, *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 of 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

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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 that 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 (18,000–500,000 years ago; Bernatchez *et al.* 1999), whereby recession of glacial ice (150,000 years ago) 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) exhibits 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), behavioral (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 in 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 therefore was to first assemble genetic linkage maps of *dwarf* and Normal lake whitefish and their hybrids. Linkage mapping was performed on the basis of a hybrid backcross-like design for 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 compose the karyotype of this species (Phillips and Rab 2001). Because increasing evidence suggests that sex-specific chromosomal differences result 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.

Using 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; Fishman *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 that we obtained support the hypothesis that genetic divergence incurred during allopatric glacial separation and that subsequent sympatric ecological specialization has resulted in several noncomplementary genomic regions between *dwarf* and normal populations. These results 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.

MATERIALS AND 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–Mississippi glacial origin (Normal) were sampled from Témiscouata Lake (470 36′ N, 680 45′ W) and Aylmer Lake (450 50′ N, 710 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 what we considered as pure *dwarf*, pure Normal, and hybrid lake whitefish (detailed in Lu and Bernatchez 1998). Two distinct "backcross-like" crosses were used for mapping: (1) between a ♀ hybrid (♀ Normal × ♂ *dwarf*) and a ♂ *dwarf*, denoted as hybrid × *dwarf*, and (2) between a ♀ hybrid (♀ *dwarf* × ♂ Normal) and a ♂ Normal, denoted here as hybrid × 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) tags providing a barcode by which individuals could be followed throughout their life history. A biopsy (50 mg) of adipose fin tissue was sampled from each individual at the age of 1+ and

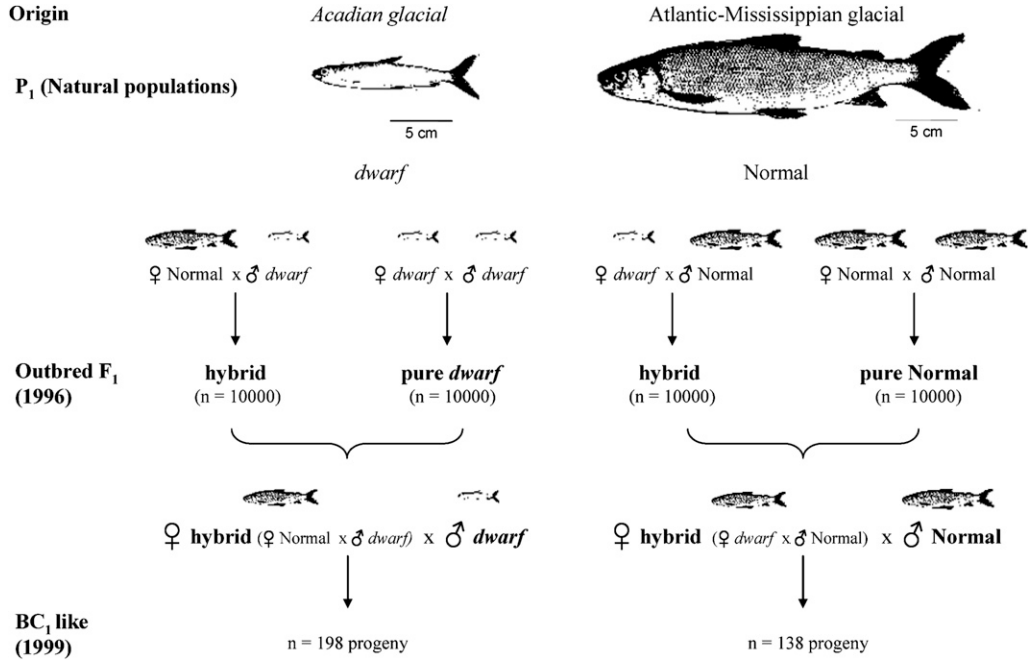


FIGURE 1.—Experimental mating scheme employed to generate the two backcross mapping families. The parental generation was sampled in the field as detailed in LU and BERNATCHEZ (1998) and as such these individuals were no longer available after spawning. The number of gametes was equilibrated in each of the F_1 groups generated for the purpose of comparing F_1 hybrid inviability.

total genomic DNA was extracted using a standard phenol–chloroform procedure.

AFLP genotyping: In total, 336 backcross progeny ($n = 198$ in the hybrid × *dwarf* cross and $n = 138$ in the hybrid × Normal cross) plus the two parents from each family were genotyped with AFLP markers. The AFLP plant mapping kit (Applied Biosystems, Foster City, CA) was used according to the protocol of VOS *et al.* (1995). Following the preselective amplification step, 15 selective primer combinations were used to amplify AFLP loci (Table 1). AFLP locus notation consisted of the dinucleotide extensions representative of their selective primer combination (*Eco*RI Axx:*Mse*I Cxx) followed by the average locus size in base pairs (to one 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). A total of 75 individuals were genotyped from each of the hybrid × *dwarf* and hybrid × Normal backcrosses. Microsatellites loci were amplified via PCR and alleles were scored from 8% polyacrylamide gels using an FMBIO II scanner (Hitachi) with the Genescan-500 size standard (Applied Biosystems) following protocols specific for each locus (APPENDIX).

Linkage analysis and genetic map construction: AFLP genotypes were scored in each family according to two patterns of segregation: (1) a 1:1 ratio resulting from the presence of a fragment in only either the female (*i.e.*, ♀*Aa*: ♂*aa*) or the male (*i.e.*, ♀*aa*: ♂*Aa*) and (2) a 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 pseudotestcross 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 (M. Lorieux, http://mapdisto.free.fr/). The significance of distorted segregation ratios was corrected for multiple comparisons [$\alpha = 0.05/k$, where k 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 an 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 among all loci were also calculated using a linkage analysis package for outcrossed families with male or female exchange of the mapping parent (LINKMFEX, Danzmann, http://www.uoguelph.ca/~rdanzmann/software/). On the basis of an 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; HUBERT and HEDGECOCK 2004; WANG and PORTER 2004). However, LANDER *et al.* (1987) acknowledged that a conservative approach is needed when the haploid number is not known or there is no preexisting data for a given system. Given the lack of preexisting 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. On the basis of 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 an LOD of 4.0. This was particularly important in determining whether

distorted markers may contribute 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, 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 fewer 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 <20 cM). Remaining loci were ordered between the framework marker intervals using the PLACE command under the threshold criterion of $2.0 < \text{LOD} < 3.0$. In some cases, when placement of loci was below this threshold, the TRY command established relative likelihoods along intervals, allowing for the ordering of these accessory loci. This frequently consisted of groups of loci separated by <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 was 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 data set 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 centimorgans; X_0 is the observed maximum distance in centimorgans 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 on the basis of 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 of homologous loci 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 female ones, we compared significant pairwise recombination estimates (an LOD threshold >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 (an LOD threshold >4.0) among linkage maps generated from both crosses. For this test we also performed an additional comparison of intermarker distances using colinear 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 $Aa - 0.5) \times 100\%$. 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 being 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 χ^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 equals 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 χ^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

TABLE 1

Summary of all AFLP loci amplified in each of the 15 primer combinations and their pattern of segregation for both backcrosses

AFLP primer combination	Hybrid × *dwarf* (1:1) ♀	Hybrid × *dwarf* (1:1) ♂	Hybrid × *dwarf* 3:1 Both	Hybrid × Normal (1:1) ♀	Hybrid × Normal (1:1) ♂	Hybrid × Normal 3:1 Both	Homologous: (1:1) In both families
AGAC	5	12	5	14	2	20	5
CAAG	34	13	9	3	16	45	5
CTAG	14	2	12	14	3	42	2
CAAT	8	39	4	28	16	29	14
ACTA	8	14	12	1	13	22	3
CATA	38	14	9	10	24	30	16
ACTC	2	16	20	10	30	5	11
AGTC	7	3	6	1	12	3	4
CCTC	9	7	10	20	9	9	6
CGTC	19	8	3	3	9	8	6
CTTC	12	6	17	7	45	14	6
CCTG	11	4	14	8	15	13	5
GGTC	18	35	7	36	54	26	23
ACTT	24	1	17	26	5	10	14
AGTT	7	2	6	28	14	8	2
Total	216	185	150	209	267	284	122

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.

combinations in the hybrid × *dwarf* and hybrid × Normal families, respectively (Table 1). Of these, 59.2% were polymorphic (551/931) in the hybrid × *dwarf* and 59.4% (760/1280) in the hybrid × Normal backcrosses (Table 1). A high percentage of the polymorphic loci were informative for mapping with 401 (72.7%) loci in the hybrid × *dwarf* and 476 (62.6%) in the hybrid × 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 × *dwarf* and hybrid × 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 × *dwarf* family while 20 were polymorphic in the hybrid × Normal family. Overall, 10 polymorphic loci were common between families (APPENDIX).

Segregation distortion of loci: Both AFLP and microsatellite loci across both families exhibited highly significant levels of segregation distortion. In the hybrid × *dwarf* family, 133 loci (32.9%) deviated from Mendelian expectations at a 0.05 table-wide 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 seven microsatellites (35%) exhibiting deviations from Mendelian expectations. In the hybrid × Normal family, 130 loci (27.8%) deviated from Mendelian expectations at the 0.05 table-wide 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 × *dwarf* family, a total of 389 AFLP and 20 microsatellites were mapped. A total of 11 AFLP remained unassigned, with 6 showing no evidence of linkage to any other markers and 5 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/female linkage group and 8.7 loci/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

TABLE 2

Summary of AFLP (denoted by their selective primer combination) and microsatellite loci segregating in both backcrosses and illustrating those markers that exhibited significant levels of segregation distortion ($P < 0.00001$) for each parent

	Hybrid × *dwarf*						Hybrid × Normal					
	♀ Hybrid			♂ *dwarf*			♀ Hybrid			♂ Normal		
		Segregation distortion			Segregation distortion			Segregation distortion			Segregation distortion	
Marker groups	Mapped	Under	Over	Mapped	Under	Over	Mapped	Under	Over	Mapped	Under	Over
AGAC	5	0	0	12	1	4	12	1	0	2	1	0
CAAG	34	0	11	13	0	4	3	1	2	16	8	1
CTAG	13	5	1	1	1	0	13	4	0	3	1	0
CAAT	8	1	1	39	1	18	27	7	4	16	5	0
ACTA	8	3	2	14	0	5	1	0	0	13	7	0
CATA	37	0	9	11	1	2	8	1	0	22	11	0
ACTC	2	0	0	16	0	4	10	4	0	30	0	3
AGTC	7	0	0	3	1	0	1	1	0	12	2	3
CCTC	9	1	0	6	1	2	19	2	4	9	2	2
CGTC	19	12	0	8	0	5	3	0	1	8	1	1
CTTC	12	5	0	6	0	0	6	1	2	43	4	1
CCTG	10	0	1	4	0	0	8	0	3	15	4	0
GGTC	18	6	7	33	9	0	36	3	0	54	10	6
ACTT	24	1	0	1	0	0	26	4	3	5	1	0
AGTT	5	1	1	2	1	1	26	3	0	12	2	0
Microsatellites	14	2	0	9	1	1	7	0	1	8	1	1
Total	211	37	33	178	17	46	199	32	20	260	60	18

Within each parent, the number of loci that exhibited significant allele-frequency distortion in either direction is also shown. under, underrepresentation of the heterozygote genotype; over, overrepresentation of the heterozyogote genotype.

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 × Normal backcross, a total 452 AFLP and 14 microsatellites were mapped. A total of 17 loci remained unassigned, with 9 exhibiting no evidence of linkage and 8 conflicting loci. A total of 23 female and 29 male linkage groups were mapped with an average number of 8.2 loci/female loci and 8.1 loci/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 >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 of the 122 total), >83% were colinear 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 × *dwarf* family and thus it was not included in the map. However, this observation as well as unpublished data from QTL in both families support 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 of the degree of homology detected between families. For the remaining loci, either a single locus was homologous across linkage groups between families (25 of 122 loci) or a conflict was observed, meaning that there was no synteny and consequently the loci were assigned to different linkage groups between families (10 of 122 loci).

Comparison of recombination frequencies between sexes and families: Pairwise recombination frequencies among significantly linked loci greater than an LOD of 4.0 (for hybrid × *dwarf* cross, $n = 3034$ female pairwise values and 2227 significant male pairwise values, while for hybrid × 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. An LSD posthoc comparison among all groups (between

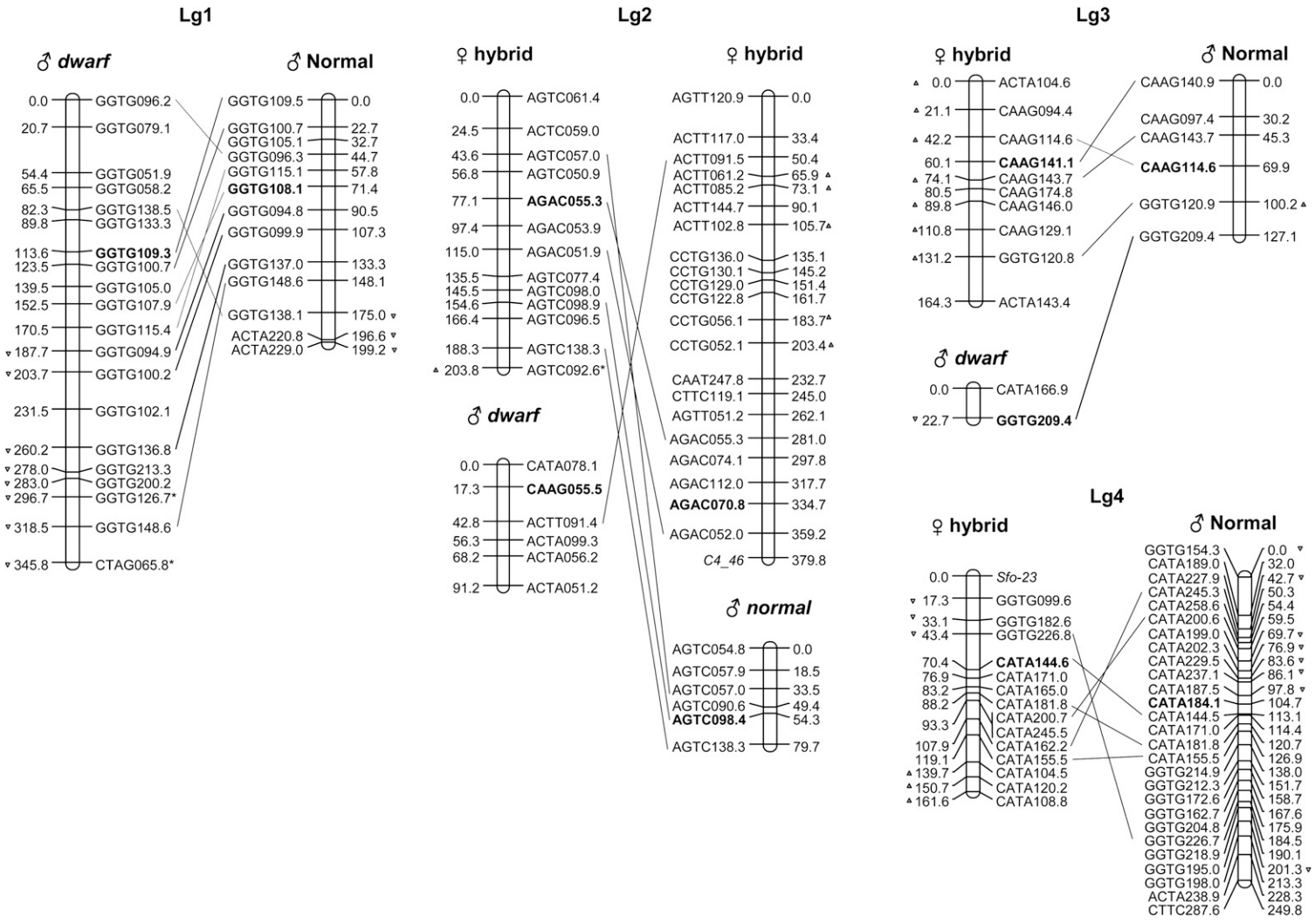


FIGURE 2.—Sex-specific genetic linkage maps of *dwarf*, Normal, and hybrid parents in the lake whitefish. Linkage groups on the left side represent the hybrid × *dwarf* backcross while linkage groups on the right side are from the hybrid × Normal backcross. Homologous loci are indicated by connecting 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 MATERIALS AND METHODS). Linkage homology between families was inferred by homologous loci that were linked in either family (noted by the lines connecting linkage groups between families). An asterisk next to the locus name indicates conflicting linkage between families. Arrowheads pointing down indicate underrepresented loci while arrowheads pointing up indicate overrepresented 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 boldface type 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.

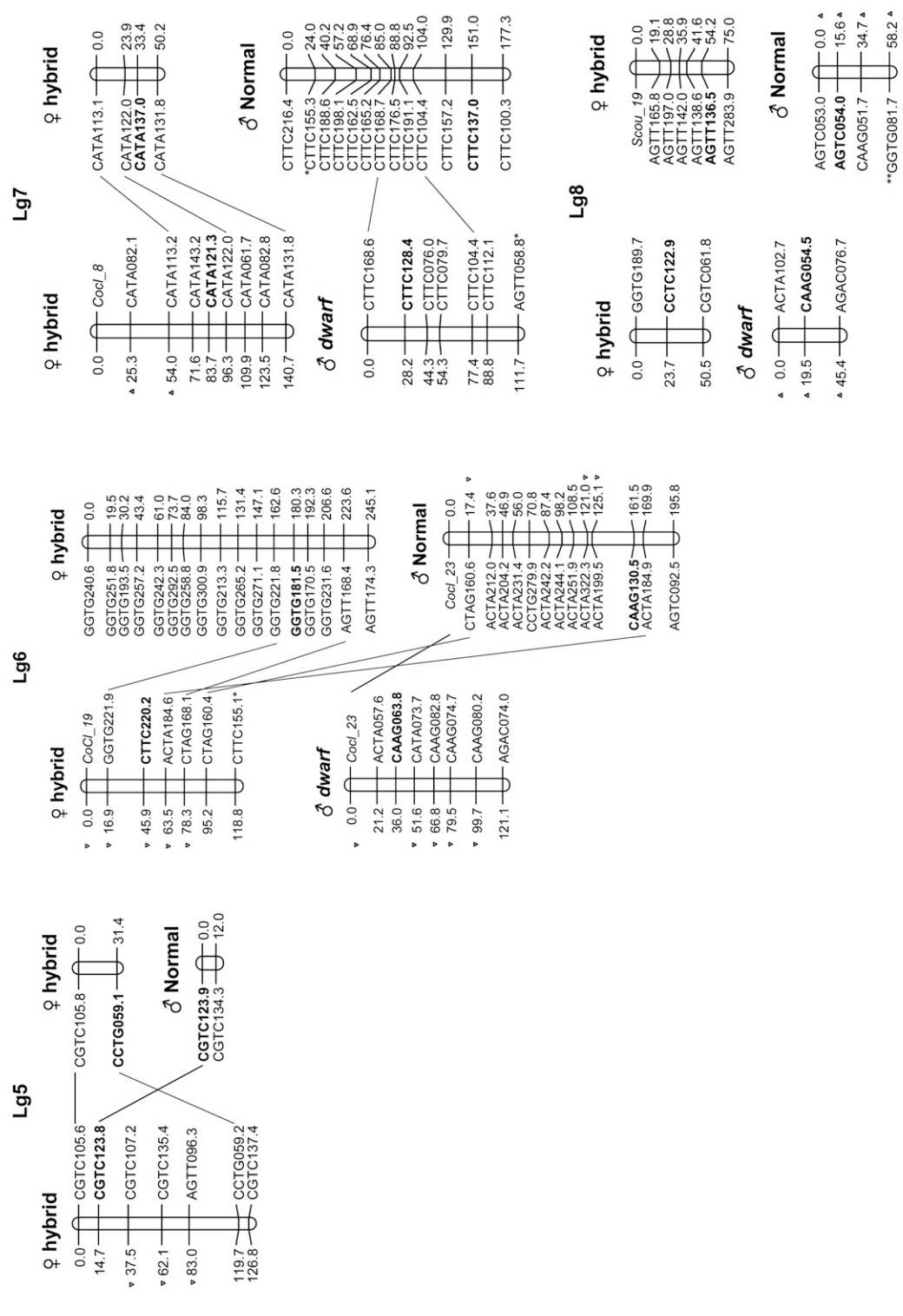
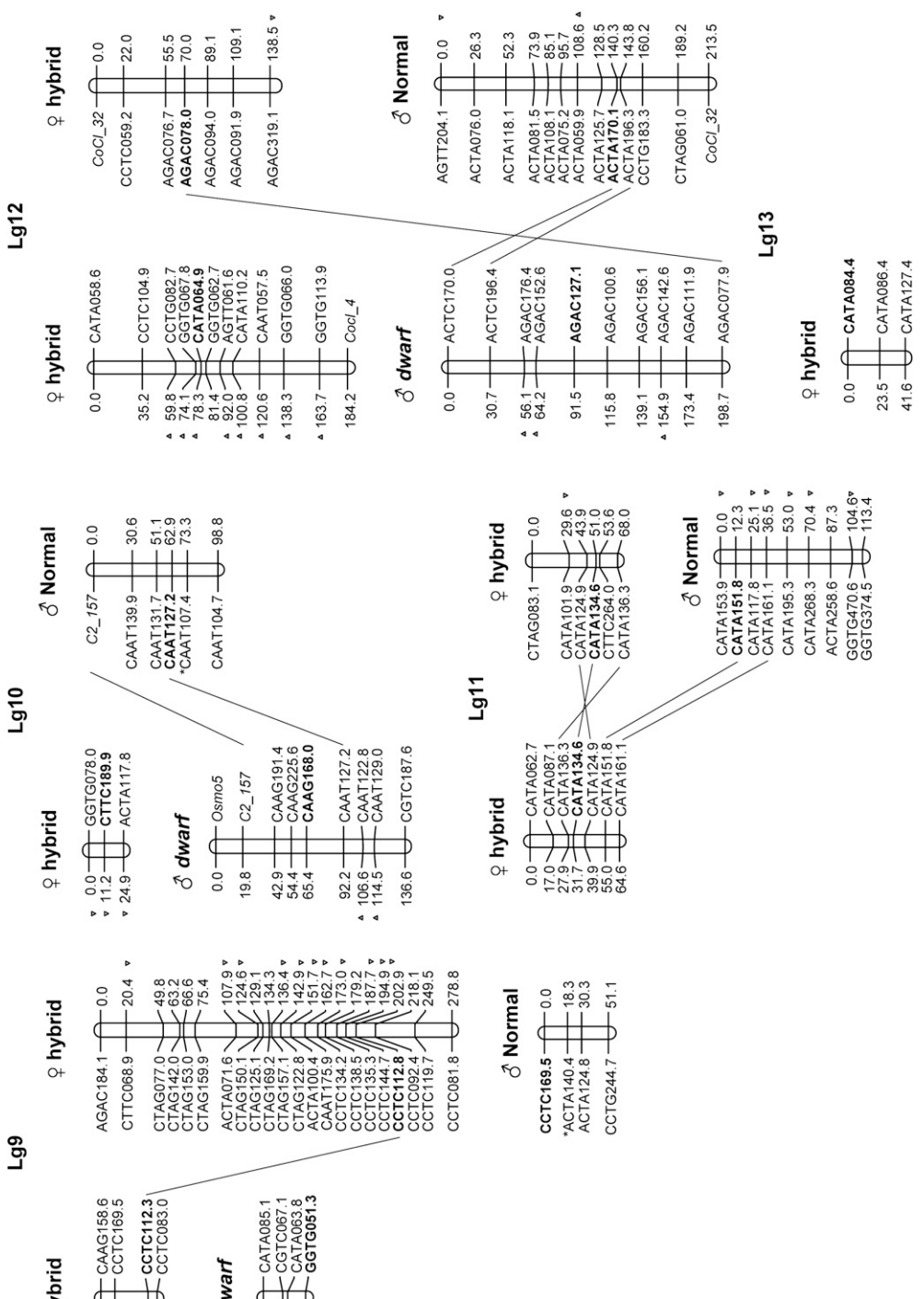


FIGURE 2.—*Continued.*



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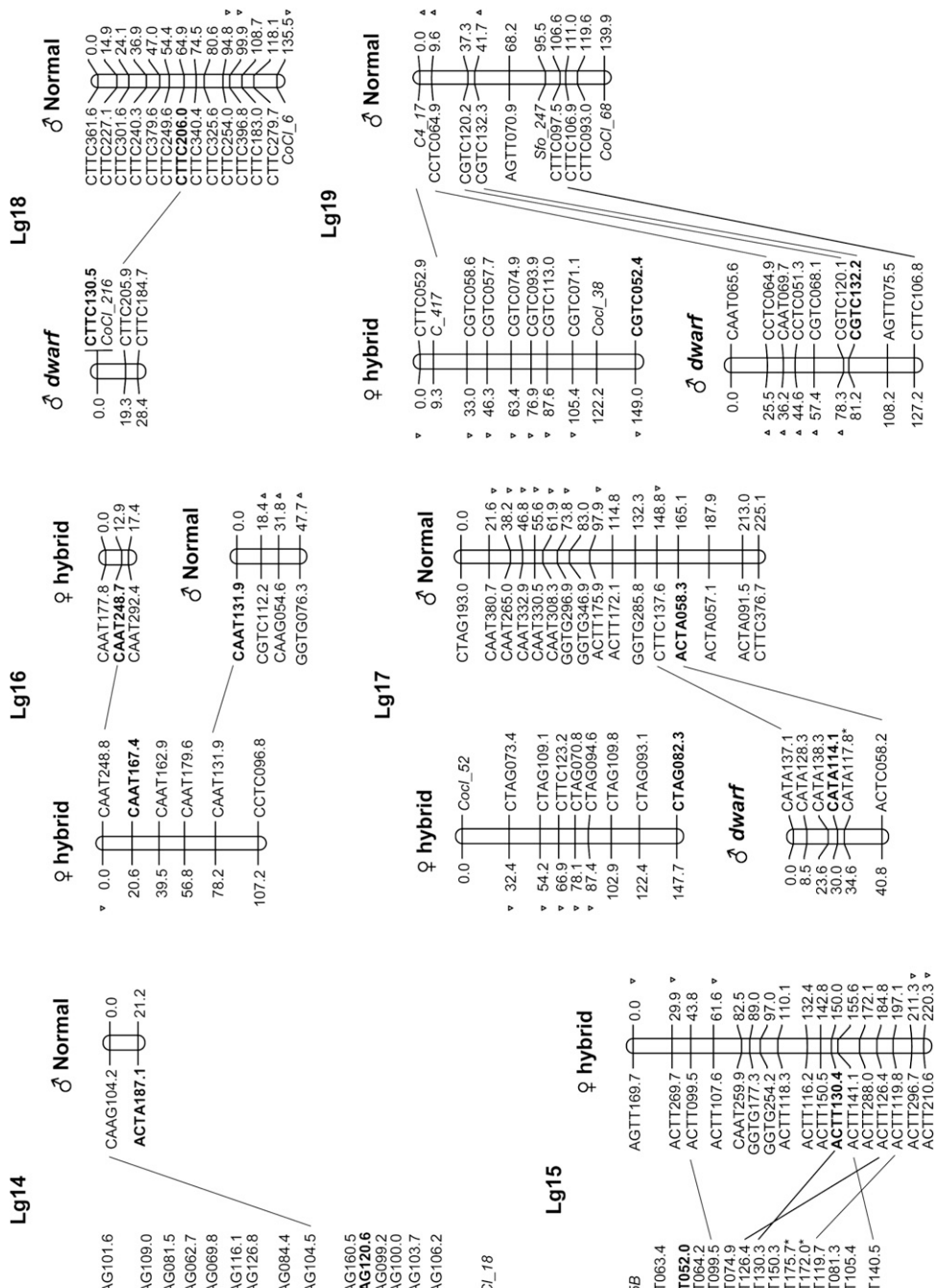

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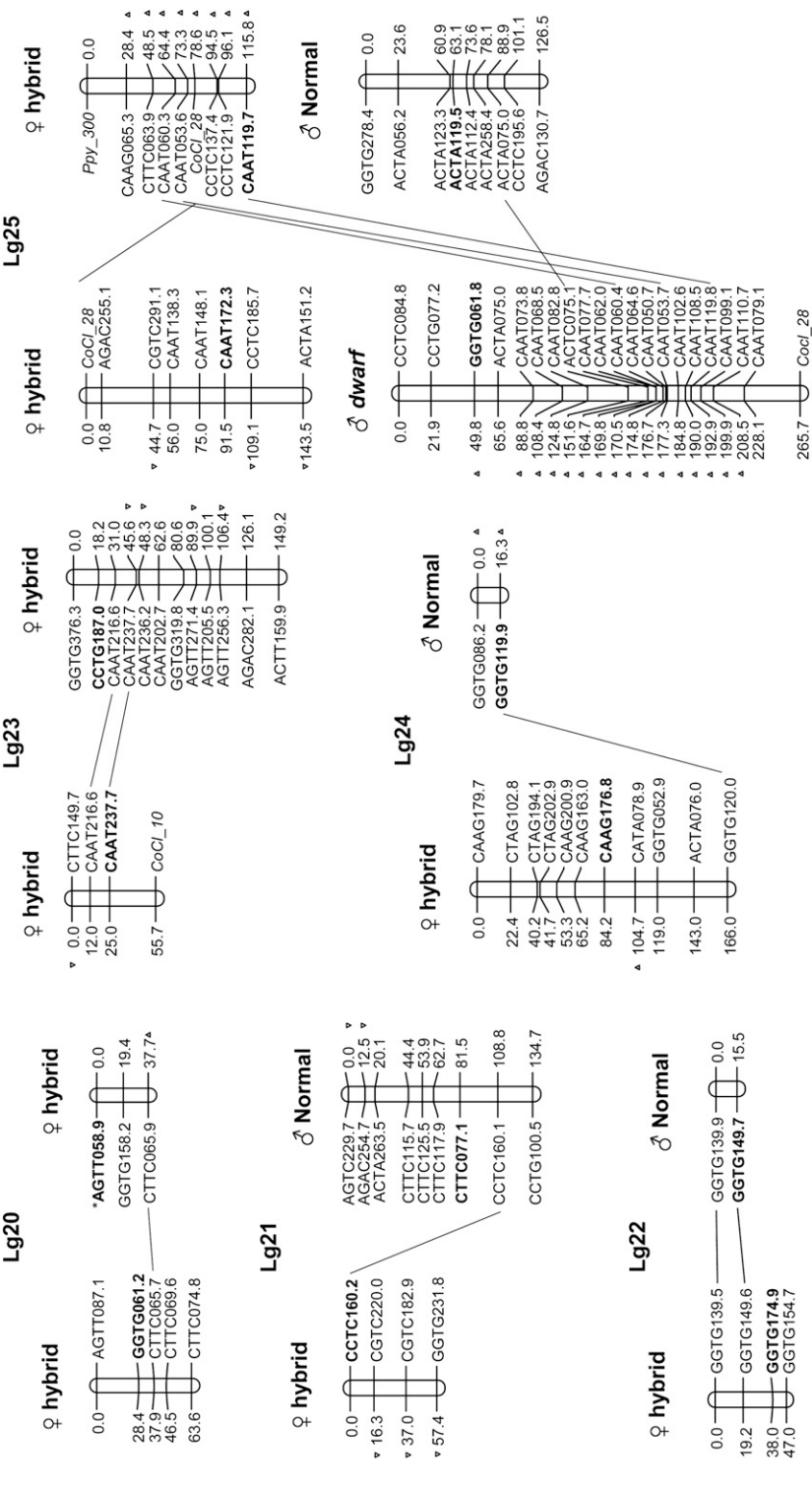

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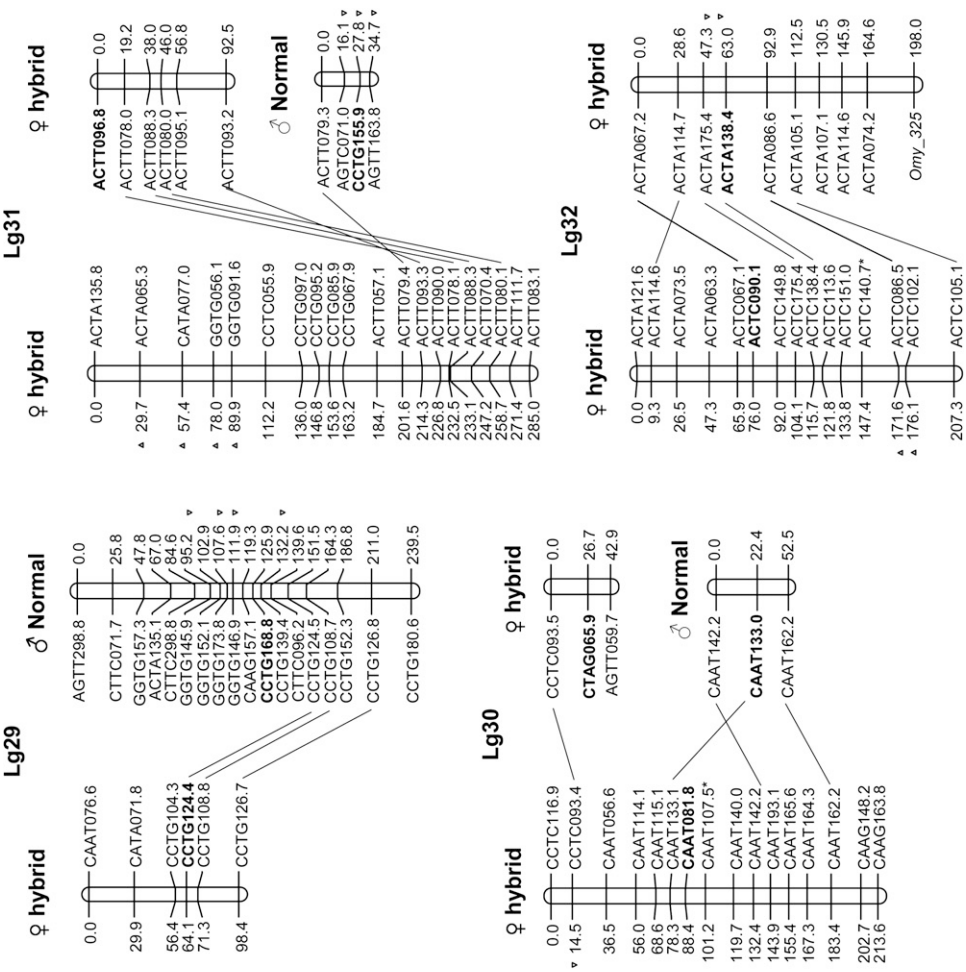

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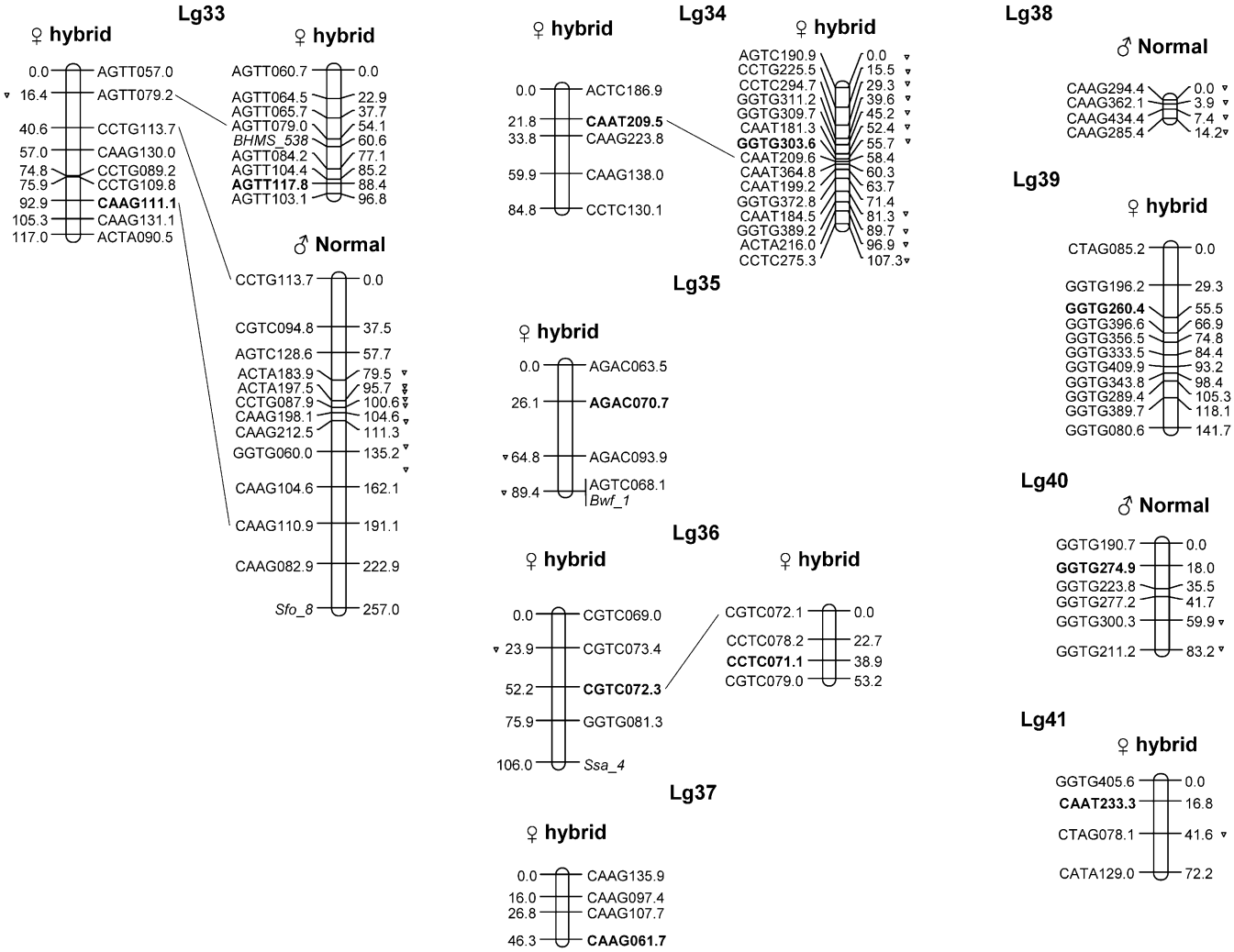

FIGURE 2.—*Continued.*

sexes and families) indicated that there was a marginal difference between the male and female recombination frequencies in the hybrid × *dwarf* (0.260 *vs.* 0.264, $P = 0.041$) family and no observable difference in the hybrid × Normal (0.231 *vs.* 0.227, $P = 0.295$). In contrast, these pairwise values were significantly reduced in the hybrid × Normal family ($P = 0.000008$) compared to the hybrid × *dwarf* (Figure 3). However, when the same comparison was performed among 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 that in 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 genomewide 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 unfavorable interactions between genomes (Table 4).

Overall there were 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 the degree of segregation distortion between families was correlated to a certain extent (Spearman $R = 0.378$, $P = 0.0021$, Figure 4).

This level of segregation distortion had only a marginal influence on the observed map distances. An

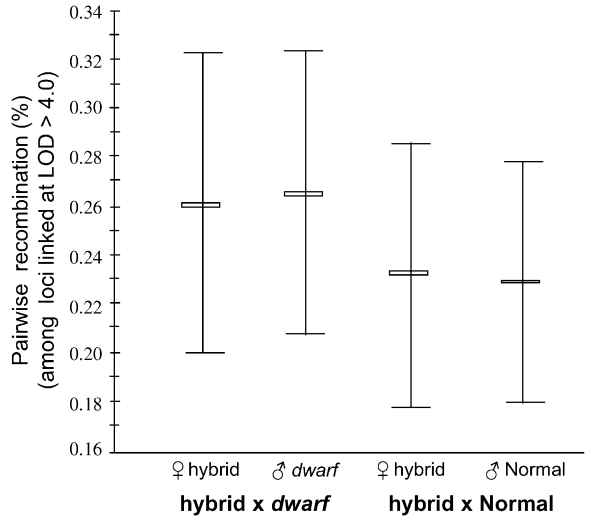

FIGURE 3.—Pairwise recombination frequencies for sex-specific markers between families when calculated among all linked loci exhibiting an LOD >4.0 (but see Table 5 for comparison of mapping distances for homologous sex-specific marker intervals between parents among families).

average change in recombination frequency of 5.7% in the hybrid × *dwarf* and 1.4% in the hybrid × 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 × 11 linkage groups = 165 tests for each family where a 5% table-wide significance threshold was inferred when $P < 0.0003$) indicated that 10 of 15 primer combinations exhibited nonrandom distribution to at least one linkage group in the hybrid × *dwarf* map ($P < 0.0001$) while 14 of 15 selective primer combos exhibited nonrandom distributions in the hybrid × Normal ($P < 0.0001$; data not shown). Of the 11 linkage groups in each family that had information for both parents, 6 were homologous, allowing comparisons between families. Of these homologous linkage groups, 4 of 6 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 5).

TABLE 3

Comparison of mapping distances for homologous sex-specific marker intervals among parents between families

LG	Marker interval	Hybrid × *dwarf*: Interval distance (cM) (♀ hybrid)	Hybrid × Normal: Interval distance (cM) (♀ hybrid)
11	CATA124.9 CATA134.6	8.2	7.1
15	ACTT130.3 ACTT150.3	5.3	5.6
23	CAAT237.7 CAAT216.6	13	14.6
31	ACTT078.1 ACTT088.3	0.6	18.8
32	ACTC175.4 ACTC138.4	11.6	15.7
LG	Marker interval	Hybrid × *dwarf*: Interval distance (cM) (♂ *dwarf*)	Hybrid × Normal: Interval distance (cM) (♂ Normal)
1	GGTG109.3 GGTG100.7	9.9	22.7
1	GGTG094.9 GGTG100.2	16	16.8
12	ACTC170.0 ACTC196.4	30.7	3.5
20	CGTC120.1 CGTC132.2	2.9	4.4
22	GGTG139.5 GGTG149.6	19.2	15.5
26	GGTG127.5 GGTG106.4	11.8	16.4
29	CCTG124.4 CCTG108.8	7.2	12.8

DISCUSSION

Linkage maps provide a powerful genomewide framework for 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 F_1 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

TABLE 4

Homologous loci exhibiting significant levels of segregation distortion in both backcrosses

		Hybrid × *dwarf*					Hybrid × Normal				
				Genotypes					Genotypes		
LG	Locus	Parent	χ^2 1:1	*aa*	*Aa*	% distortion	Parent	χ^2 1:1	*aa*	*Aa*	% distortion
3	GGTG120.8	♀ Hybrid	63.2*	28	127	31.9	♂ Normal	37.0*	33	105	25.7
12/28	CCTC104.9[a]	♀ Hybrid	36.8*	73	167	19.6	♂ Normal	65.8*	21	117	34.3
19	CCTC064.9	♂ *dwarf*	38.6*	51	136	22.7	♂ Normal	42.5*	29	105	27.9
23	CAAT237.7	♀ Hybrid	25.3*	163	84	16.0	♀ Hybrid	23.2*	59	17	27.6
25	CAAT060.4	♂ *dwarf*	98.4*	26	162	36.2	♀ Hybrid	60.5*	23	115	32.9
25	CAAT119.8	♀ Hybrid	41.2*	50	138	23.4	♀ Hybrid	29.3*	37	101	22.9
25	CAAT053.7	♂ *dwarf*	98.4*	26	162	36.2	♀ Hybrid	71.4*	19	119	35.7
28	ACTC092.7	♂ *dwarf*	36.4*	55	139	21.7	♂ Normal	46.8*	25	103	30.0

χ^2 1:1 refers to the chi-square test of 1:1 Mendelian segregation in the progeny. *aa*, homozygous for the null allele; *Aa*, heterozygous; An asterisk denotes 5% table-wide level of significance, $P < 0.00001$.

[a] Linkage conflict for CCTC104.9 where the locus was linked to LG12 in hybrid × *dwarf* and LG28 in hybrid × Normal.

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 nonrandom clusters of AFLP distribution. Overall, these results will contribute toward 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.

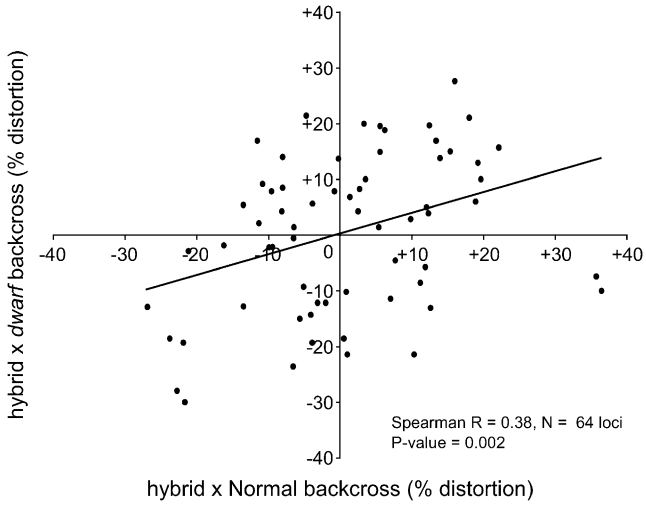

FIGURE 4.—Comparison of the percentage and direction of segregation distortion, *i.e.*, (frequency of *Aa* − expected frequency of 0.5) × 100% calculated from the observed segregation ratios for mapped, homologous loci between the hybrid × *dwarf* and hybrid × Normal backcrosses.

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; LIU *et al.* 2003; NICHOLS *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, >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 noncolinearity 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 nonrandomly 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

TABLE 5

Comparative analysis of the effect of segregation distortion on mapping order and recombination fraction as estimated between the classic backcross and Bailey's mapping function

	Hybrid × *dwarf*				Hybrid × Normal			
		RF estimate				RF estimate		
LG	Linked markers	Classic	Bailey	Change	Linked markers	Classic	Bailey	Change
1	GGTG126.7 *GGTG148.6* CTAG065.8	0.229 0.258	0.261 0.285	−0.032 −0.027	GGTG137.4 *GGTG148.6* GGTG138.1	0.164 0.257	0.174 0.280	−0.010 −0.023
1	GGTG102.1 *GGTG136.8* GGTG213.3	0.296 0.214	0.251 0.269	0.045 −0.055	GGTG099.9 *GGTG137.4* GGTG148.6	0.257 0.164	0.249 0.174	0.008 −0.010
3	CAAG94.4 *CAAG114.6* CAAG141.1	0.214 0.199	0.236 0.209	−0.022 −0.010	CAAG143.7 *CAAG114.6* GGTG120.9	0.239 0.279	0.172 0.302	0.067 −0.023
3	CAAG129.1 *GGTG120.8* ACTA143.4	0.188 0.268	0.247 0.157	−0.059 0.111	CAAG114.6 *GGTG120.9* GGTG209.4	0.279 0.253	0.302 0.156	−0.023 0.097
19	CAAT065.6 *CCTC064.9* CAAT069.7	0.219 0.12	0.224 0.126	−0.005 −0.006	C4_17 *CCTC064.9* CGTC123.9	0.099 0.132	0.124 0.075	−0.025 0.057
19	CGTC068.1 *CGTC120.1* CGTC132.2	0.208 0.054	0.26 0.061	−0.052 −0.007	CGTC134.3 *CGTC120.2* CGTC132.3	0.086 0.079	0.070 0.085	0.016 −0.006
19	CGTC120.1 *CGTC132.2* AGTT075.5	0.054 0.271	0.061 0.304	−0.007 −0.033	CGTC120.2 *CGTC132.3* AGTT070.9	0.079 0.252	0.085 0.229	−0.006 0.023
23	CoCl_10 *CAAT237.7* CAAT216.6	0.278 0.142	0.284 0.151	−0.006 −0.009	CAAT216.6 *CAAT237.7* CAAT236.2	0.171 0.092	0.191 0.111	−0.020 −0.019
23	CAAT237.7 *CAAT216.6* CTTC149.7	0.142 0.123	0.151 0.116	−0.009 0.007	CCTG187.0 *CAAT216.6* CAAT237.7	0.145 0.171	0.164 0.191	−0.019 −0.020
25	CAAT062.0 *CAAT060.4* CAAT064.6	0.053 0.08	0.069 0.124	−0.016 −0.044	CTTC063.9 *CAAT060.3* CAAT053.6	0.186 0.129	0.243 0.189	−0.057 −0.060
25	CAAT050.7 *CAAT053.7* CAAT102.6	0.09 0.117	0.121 0.098	−0.031 0.019	CAAT060.3 *CAAT053.6* CoCl_19	0.129 0.087	0.189 0.128	−0.060 −0.041
25	CAAT108.5 *CAAT119.8* CAAT099.1	0.059 0.101	0.061 0.116	−0.002 −0.015	ACTA123.3 *ACTA119.5* ACTA112.4	0.036 0.129	0.029 0.123	0.007 0.006
28	CTAG064.8 *ACTA092.7* CGTC065.2	0.141 0.164	0.159 0.157	−0.018 0.007	ACTA068.4 *ACTA092.5* CCTC104.8	0.062 0.138	0.000 0.186	0.062 −0.048

The center locus (in italics) of each marker group exhibited significant segregation distortion in both families. RF refers to the recombination fraction; "change" refers to the difference between estimates.

observed at three levels. The first level includes AFLP that map to nonrandom clusters regardless of the restriction enzymes and selective primer combinations employed, exhibiting a nonrandom distribution relative to other marker intervals typically presumed to be the result of reduced recombination around the centromere (Qi *et al.* 1998; Young *et al.* 1998; Wang and Porter 2004). The second level is AFLP loci amplified from different restriction enzymes that exhibit nonrandom distribution across linkage groups (Young *et al.* 1999; Peng *et al.* 2000; Nichols *et al.* 2003). Finally, nonrandom distribution of AFLP amplified from selective

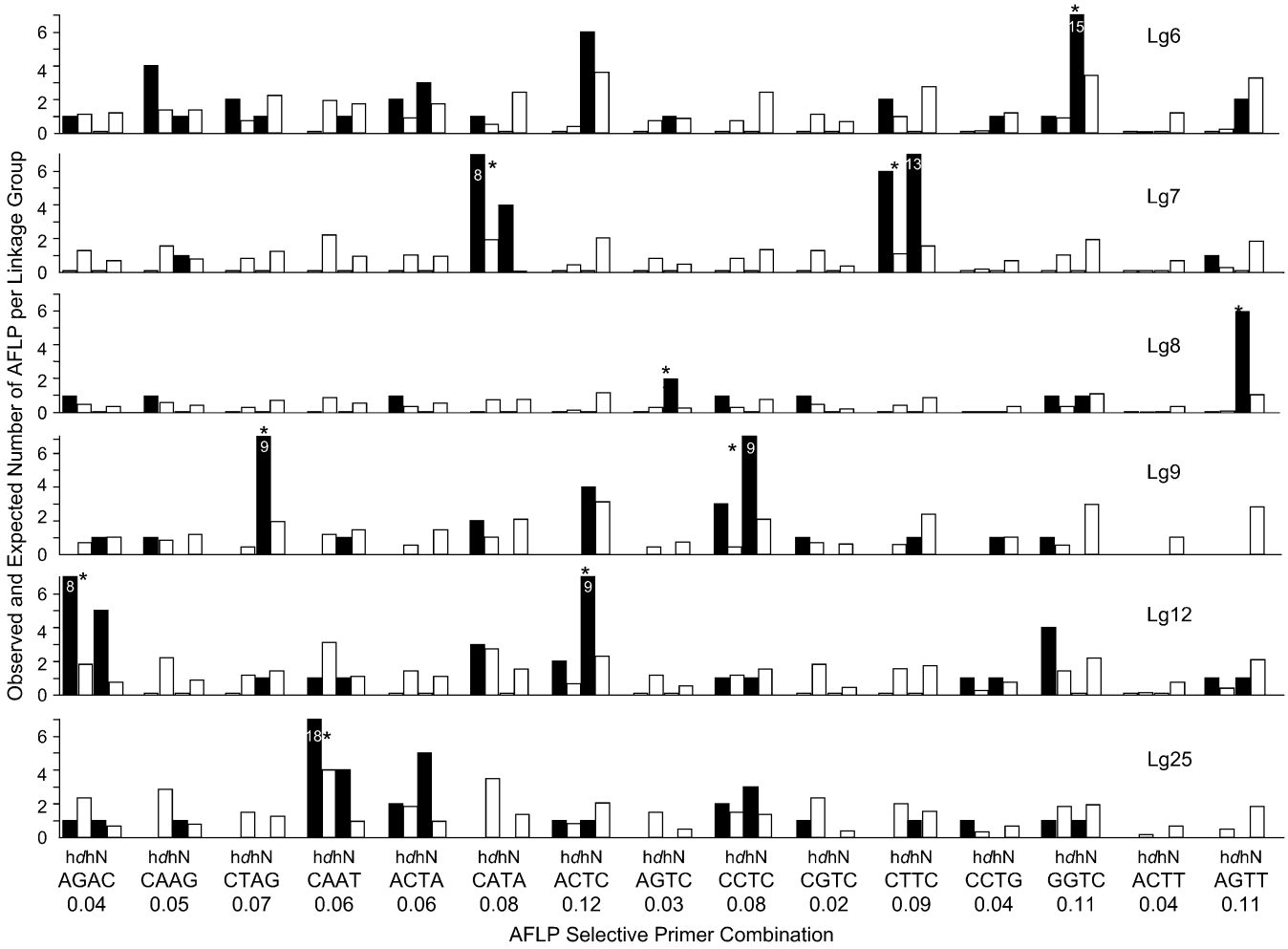

Figure 5.—Observed and expected number of AFLP for selective primer combos at homologous linkage groups containing locus information for both sexes. On the *x*-axis the primer combinations are listed for each family (h*d* = hybrid × *dwarf* and hN = hybrid × Normal) while the expected frequency of these 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 asterisk between observed columns. Numbers within bars represent absolute observed values for significant observations that were higher than the given scale.

primer combinations has also been observed (Lashermes *et al.* 2001; Schwarz-Sommer *et al.* 2003; Campbell and Bernatchez 2004; this study).

The implications of these observations for linkage mapping suggest that many selective primer combinations and restriction enzymes combined with other marker systems may be required 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, these observations 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 *Eco*RI sites cluster outside or between gene-rich regions resulting from the ability of *Eco*RI 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 nonrandom 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 nonrandom 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 *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 (DeVincente and Tanksley 1993; Otto and Lenormand 2002; Lenormand and Dutheil 2005) with the highest differences among all species being reported in salmonids (a 6.4:1 female-to-male linkage map distance ratio in the 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 (*Onchorhynkis mykiss*, Sakamoto *et al.* 2000; Nichols *et al.* 2003; O'Malley *et al.* 2003), arctic charr (*Salvelinus alpinus*, Woram *et al.* 2004), and Atlantic salmon (*Salmo salar*, Moen *et al.* 2004). However, because F_1 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 (ten-Hoopen *et al.* 1996; Chetelat *et al.* 2000; Sakamoto *et al.* 2000; Woram *et al.* 2004; Yin *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 (Plomion and O'Malley 1996; Yin *et al.* 2004). This, in turn, would have resulted in comparable levels of recombination observed between sexes, in contrast with previous studies of salmonids.

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 × Normal family when compared to the hybrid × *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 from two distinct sources: recombinant gametes or upon the formation of zygotes (Butlin 2005). While the first prediction is indirectly supported by our results between sexes, the second prediction is partially supported by our results between families, given that the degree of hybrid inviability was much higher in the hybrid × Normal family (21% survival upon hatching) compared to the hybrid × *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 (2007) found evidence for some heterozygote fitness advantages in inbred and outbred lines of Drosophila, while the linkage disequilibrium effects of these extended up to 10 Mb, 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 (2007) 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 overall could 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; Jiggins *et al.* 2005) and/or (2) decreased heterozygosity in female gametes due to outbreeding depression (*e.g.*, Ferreira and Amos 2007). 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 (>10,000 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.* 2004; Yin *et al.* 2004; Pelgas *et al.* 2006). Consequently, the number of markers segregating 3:1 was also likely inflated, particularly when considering that the parents originated from divergent sources (Jiggins *et al.* 2005; Pelgas *et al.* 2006). Overall, over one-third of the markers showed a 3:1 segregation ratio, which was much higher 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.*, Pélabon *et al.* 2005; Johansen-Morris and Latta 2006; Ferreira and Amos 2007). 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 requires 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 heterospecfic interactions between genomes due to divergence of >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 F_1 gametes or by selection on recombinant backcross (BC) zygotes (Li *et al.* 1997; Kim and Rieseberg 1999), 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 favored in hybrid genetic backgrounds might represent "selfish" genes (genes that enhance the success of the gametes that 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 F_1 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 3). 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 in contributing to 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 contribute 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 favor 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 3). It will be important for future mapping studies to 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 primarily of 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 semilethal recessive allele heterozygous in one parent must be transmitted to the F_1, and upon segregation in higher inbred crosses cause differential zygote mortality and a deficit of carrier parental homozygotes (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 F_1 parental lines, given that ~30% of AFLP loci segregated 3:1 within both families, indicating that 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, coevolved gene complexes to the extent that reduced zygote viability is 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 to increase the likelihood of correctly inferring which genetic mechanisms are responsible for incompatible interactions between genotypes.

It is equally important to consider possible nongenetic sources of segregation distortion: sampling biases, comigration, 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; Collins *et al.* 1996; Ehm *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 nonrecombinant 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; Fishman *et al.* 2001; Hackett and Broadfoot 2003).

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 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 jointly contribute 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 among 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, behavior, 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 genomewide 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 in achieving these objectives in the lake whitefish species complex.

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APPENDIX

Summary of microsatellite loci characterized by locus name, source (species specific or cross-amplified), and parental genotypes (denoted by alleles presented in numerical format) in two mapping families

			Hybrid × *dwarf*		Hybrid × Normal	
Locus	Species	Source	♀	♂	♀	♂
Cocl 4	*C. clupeaformis*	Rogers *et al.* (2004)	1,3	2,3	1,4	1,4
Cocl 6	*C. clupeaformis*	Rogers *et al.* (2004)	1,2	1,3	3,3	3,4
Cocl 8	*C. clupeaformis*	Rogers *et al.* (2004)	2,3	3,3	[a]	[a]
Cocl 10	*C. clupeaformis*	Rogers *et al.* (2004)	1,2	1,1	[a]	[a]
Cocl 18	*C. clupeaformis*	Rogers *et al.* (2004)	2,2	1,2	[a]	[a]
Cocl 19	*C. clupeaformis*	Rogers *et al.* (2004)	2,4	1,3	[a]	[a]
Cocl 23	*C. clupeaformis*	Rogers *et al.* (2004)	2,6	3,3	2,5	1,4
Cocl 28	*C. clupeaformis*	Rogers *et al.* (2004)	1,2	2,2	[a]	[a]
Cocl 32	*C. clupeaformis*	Rogers *et al.* (2004)	[a]	[a]	1,3	2,3
Cocl 38	*C. clupeaformis*	Rogers *et al.* (2004)	1,2	2,2	[a]	[a]
Cocl 52	*C. clupeaformis*	Rogers *et al.* (2004)	1,2	2,2	[a]	[a]
Cocl 68	*C. clupeaformis*	Rogers *et al.* (2004)	[a]	[a]	1,2	1,1
Cocl 80	*C. clupeaformis*	Rogers *et al.* (2004)	4,4	1,4	2,5	3,5
Cocl 216	*C. clupeaformis*	Rogers *et al.* (2004)	1,2	2,2	[a]	[a]
C2-157	*C. artedi*	Turgeon *et al.* (1999)	1,3	2,4	2,5	2,3
C2-5B	*C. artedi*	Turgeon *et al.* (1999)	1,2	2,2	1,2	2,2
C3-152	*C. artedi*	Turgeon *et al.* (1999)	1,1	1,2	2,3	2,3
C4-17	*C. artedi*	Turgeon *et al.* (1999)	1,2	1,1	2,2	1,2
C4-46	*C. artedi*	Turgeon *et al.* (1999)	[a]	[a]	2,3	3,3
BHMS-538	*Salmo salar*	B. Hoyheim (unpublished results)	[a]	[a]	2,2	1,2
BWF-1	*C. nasus*	Patton *et al.* (1997)	3,4	2,4	3,4	1,3
*Omy*325	*Oncorhynchus mykiss*	Sakamoto *et al.* (2000)	[a]	[a]	1,2	2,2
Osmo-5	*Osmerus mordax*	Taylor and Bentzen (1993)	3,3	2,4	2,5	2,3
PPY-300	*O. mykiss*	Morris *et al.* (1996)	[a]	[a]	1,2	2,2
Scou-19	*Salvelinus confluentus*	Taylor *et al.* (2001)	[a]	[a]	1,1	1,2
*Sfo*8	*S. fontinalis*	Angers *et al.* (1995)	[a]	[a]	1,2	2,2
Sfo-23	*S. fontinalis*	Angers *et al.* (1995)	1,2	2,3	[a]	[a]
Sfo-147	*S. fontinalis*	Perry *et al.* (2005)	2,2	1,2	[a]	[a]
Sfo-247	*S. fontinalis*	Perry *et al.* (2005)	[a]	[a]	1,1	2,3
Ssa-4	*S. salar*	McConnell *et al.* (1995)	1,2	1,1	1,2	2,2

[a] The locus was monomorphic for that family.