Distribution of Temperature Tolerance Quantitative Trait Loci in Arctic Charr (*Salvelinus alpinus*) and Inferred Homologies in Rainbow Trout (*Oncorhynchus mykiss*)

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ABSTRACT

We searched for quantitative trait loci (QTL) affecting upper temperature tolerance (UTT) in crosses between the Nauyuk Lake and Fraser River strains of Arctic charr (*Salvelinus alpinus*) using survival analysis. Two QTL were detected by using two microsatellite markers after correcting for experiment-wide error. A comparative mapping approach localized these two QTL to homologous linkage groups containing UTT QTL in rainbow trout (*Oncorhynchus mykiss*). Additional marginal associations were detected in several families in regions homologous to those with QTL in rainbow trout. Thus, the genes underlying UTT QTL may antedate the divergence of these two species, which occurred by ~16 MYA. The data also indicate that one pair of homeologs (ancestrally duplicated chromosomal segments) have contained QTL in Arctic charr since the evolution of salmonids from a tetraploid ancestor 25–100 MYA. This study represents one of the first examples of comparative QTL mapping in an animal polyploid group and illustrates the fate of QTL after genome duplication and reorganization.

COMPARATIVE mapping of genes is rapidly becoming an efficient method to dissect the genetic basis of quantitative trait variation (Pflieger *et al.* 2001; Doganlar *et al.* 2002). Although common among agriculturally relevant plants (Lan and Paterson 2000; Oard *et al.* 2000; Lauter and Doebley 2002) and animals (Andersson *et al.* 1998; Georges 1998; Diez-Tascon *et al.* 2001), recent comparative linkage mapping in cervids and their domesticated relatives (Slate *et al.* 2002a) has permitted quantitative trait locus (QTL) studies in a natural population of deer (Slate *et al.* 2002b), highlighting a broader applicability of this approach. However, it is becoming increasingly apparent from these studies that gene duplication is an important force in the evolution of genomes, particularly in flowering plants (Soltis and Soltis 1999; Otto and Whitton 2000). Comparative linkage mapping studies have provided important insight into the function and divergence of duplicated genes (Cronn *et al.* 1999; Schranz *et al.* 2002; Small and Wendel 2002), as shown by the identification of QTL on homeologous chromosomal segments in species of Brassica (Axelsson *et al.* 2001).

Salmonid fishes like rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), and Arctic charr (*Salvelinus alpinus*) represent good models for genomic studies following a duplication event, being derived from a tetraploid ancestor ~25–100 MYA (Allendorf and Thorgaard 1984). In addition, as commercially important species, extensive comparative linkage mapping has been undertaken, revealing both broad homology based on conservation of marker linkages (May and Johnson 1990; Sakamoto *et al.* 2000; Woram 2001), and significant divergence among species (Woram *et al.* 2003). Great inter- and intraspecific karyotypic variation, while maintaining a relatively constant number of chromosome arms (~100; Phillips and Rab 2001), suggests that genome rearrangement by Robertsonian fission-fusion events played an important role in genome evolution of extant salmonid species since their divergence from a common ancestor ~16 MYA (Pleyte *et al.* 1992; Andersson *et al.* 1995; Oakley and Phillips 1999).

Temperature tolerance is an important trait from both an economic and an evolutionary perspective in fishes, particularly among cool- and cold-water salmonids. Elevated temperatures may negatively affect fitness components, including parameters of growth, development, and reproduction (Jobling *et al.* 1995; Pankhurst *et al.* 1996). The polygenic basis of upper temperature tolerance (UTT) has recently been demonstrated in selected lines of rainbow trout by the detection of significant QTL on at least nine linkage groups (Jackson *et al.* 1998; Danzmann *et al.* 1999; Perry *et al.* 2001; our unpublished data). Some of these QTL exhibit unpredictable epistatic effects with the genomic background in which they are expressed (Danzmann *et al.* 1999), as well as sex-specific effects (Perry 2001), highlighting the complexity of this trait in salmonids.

Arctic charr, which extend into boreal circumpolar

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regions (Scott and Crossman 1985), include three putative subspecies in North America representing the Laurentian, Arctic, and Labradorean mitochondrial lineages (Brunner *et al.* 2001), which have undergone different thermal selection pressures. We used information on known locations of QTL for UTT in rainbow trout to test for similar effects in homologous chromosomal regions of Arctic charr on the basis of the current genetic maps for these species (Sakamoto *et al.* 2000; Woram *et al.* 2003, 2004). Arctic charr have higher numbers of acrocentric chromosomes relative to metacentric chromosomes compared to rainbow trout (Hartley 1987; Phillips and Rab 2001) so the comparison will provide important information on the fate of duplicated genes in the face of significant genome reorganization. We also incorporated knowledge of homeologous (duplicated) chromosome arm relationships in rainbow trout to test for the possible conservation of duplicated QTL effects in Arctic charr. We used survival analysis, a novel approach to QTL detection, which tests the relative survival through time of individuals inheriting either allele from a parent when subjected to a thermal challenge.

MATERIALS AND METHODS

Strain and family history: The aquaculture strains used in this study were derived from the Nauyuk Lake (Northwest Territories, Canada) and Fraser River (Labrador, Canada) populations approximately four generations ago. These populations not only are separated by large geographic distances, but also are characterized by differences in life history and thermal selection regimes. Nauyuk Lake fish, found in the Canadian sub-Arctic (68° N; Gyselman 1994), are considered to be much less temperature tolerant than Fraser River fish (56° N; Dempson and Green 1984). The optimal temperatures for Nauyuk Lake brood stock are up to 3° lower than those for the Labrador strain of charr (Tabachek 1991). Furthermore, Nauyuk Lake and Fraser River fish possess distinct mitochondrial genomes and belong to the Arctic and Labradorean lineages of Arctic charr, respectively (Brunner *et al.* 2001).

Arctic charr gametes were collected from adults in spawning condition on October 22 and 27, 1998, at Coldwater Hatcheries (Coldwater, Ontario, Canada). Eggs and milt from each individual were transported on ice to the Hagen Aqualab facilities (University of Guelph, Guelph, Ontario, Canada). Crosses were produced by mixing the gametes of charr derived from Fraser River, Nauyuk Lake, and F_1 hybrids between the two strains, yielding four F_1 and one backcross families (Table 1). Incubation of embryos took place at 4° until exogenous feeding was achieved, at which time progeny were transferred to raceways ($\sim 1 \times 3$ m). The water source originated from an aquifer (underground spring), whose temperature fluctuated between 10° and 12°. The families were pooled and selectively genotyped to ascertain their family origins following the thermal challenge trials. All rearing practices and thermal challenge experiments followed the University of Guelph Aqualab standard operating procedures for holding salmonid fishes and the Canadian Council for Animal Care guidelines.

Upper temperature tolerance trials: Progeny were subjected to upper temperature tolerance trials 13 months post-fertilization. Trials were conducted within a single week beginning at 17:00 to minimize effects of seasonal or diurnal changes in physiology. Furthermore, to ensure maximum control of temperature, a stand-alone tank (closed system) was set up that could be programmed and monitored via computer. Feeding was terminated 4 days prior to the thermal challenge, and a random subset of fish was transferred to the experimental tank the preceding evening.

TABLE 1

Background information on five Arctic charr families used to search for QTL

Cross	*N*	Dam	Sire	Family
12-111	44	Fraser	F_1	B_1
12-114	37	Fraser	Nauyuk	F_1
21-114	32	Fraser	Nauyuk	F_1
27-139	56	Nauyuk	Fraser	F_1
30-136	42	Nauyuk	Fraser	F_1

The cross name is composed of the code for the dam used, followed by that for the sire. Two parental strains (Fraser and Nauyuk) and interstrain hybrids (F_1) were used to produce backcross, F_1, and pure strain families. *N*, the number of progeny included in each family on the basis of pedigree analysis. Note that 12-114 and 21-114 were selectively genotyped (15–25% of the least- and most-temperature-tolerant fish).

A pilot trial (lot I), where 100 fish were taken randomly from a tank containing individuals from all families, indicated that these particular charr possessed a higher incipient lethal temperature than that suggested by the literature (22.5°; Baroudy and Elliott 1994). As a result, a modification of traditional thermal challenges was employed (Jackson *et al.* 1998). Temperature was increased from the ambient temperature to the published incipient lethal temperature (22.5°) over a period of 60 min and then kept constant for 30 min. Subsequently, the temperature was raised by 0.5° every 30 min until the end of the trial, resulting in a stepped profile (Figure 1). Air stones were inserted into the tank to aerate and evenly distribute the heated water. Fish were considered to have died when they lost equilibrium and could not right themselves; at this point they were euthanized with an overdose of clove oil (Keen *et al.* 1998), placed on ice, and given individual tags indicating their time of death. Thermal profiles and temperature at death were recorded by two probes placed at either end of the experimental tank that collected data every 10 sec (BoxCar Pro 3.5). The trial continued until all fish had succumbed to the thermal challenge. Three such thermal challenges (lots II, III, and IV) were required to test all the fish. Body weight (wet weight to the nearest tenth of a gram) and fork length (in millimeters) were recorded and muscle and branchial tissue were sampled. All tissues were frozen at −20° until genetic analyses could be undertaken.

Genetic analysis: DNA was extracted from 25–50 mg of muscle or branchial tissue using the standard phenol chloroform method (Bardakci and Skibinski 1994) as well as a QIAGEN DNEasy tissue extraction kit. Microsatellite loci were screened in all parents using PCR to detect polymorphisms within families (Tables 2 and 3). When marker loci were monomorphic (a single band in all fish) or did not amplify (no scoreable product), the next closest alternative was screened on the basis of its proximity on the linkage group. Loci on additional linkage groups were also analyzed. Linkage groups are designated with the prefix "RT-" when referring to rainbow trout and "AC-" when referring to Arctic charr to differentiate between the linkage maps constructed for the two species (Sakamoto *et al.* 2000; Nichols *et al.* 2003; Woram *et al.*

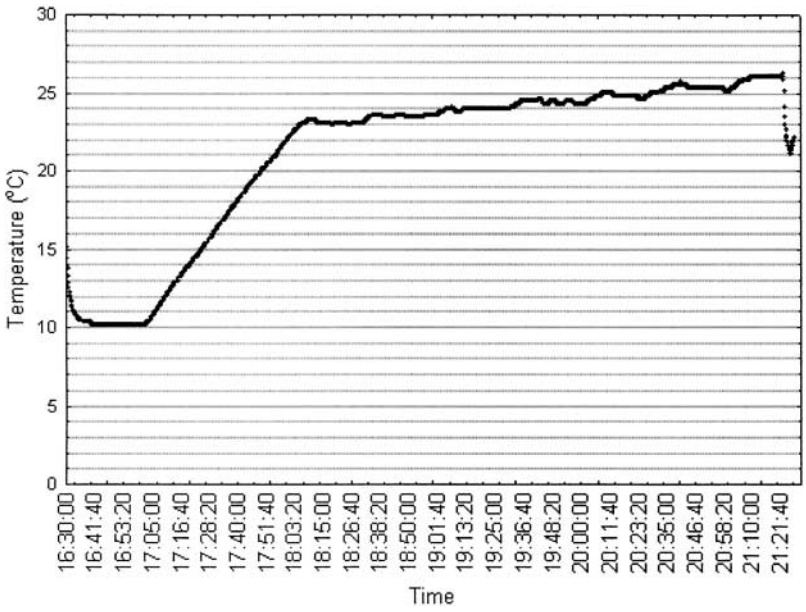

FIGURE 1.—Temperature profile representing the thermal challenge to which lot II Arctic charr were exposed. Water temperature and time from 17:00 are indicated on the *y*-axis and *x*-axis, respectively. Fish were held at 10° prior to the trial. The ramping zone represents the 60-min period when the temperature was increased to the theoretical incipient lethal temperature of 22° for Arctic charr (BAROUDY and ELLIOTT 1994). Subsequently, temperature was increased every 30 min by 0.5° until the end of the trial. When an individual lost equilibrium and was no longer responsive to external stimuli, its time of "death" was recorded and it was euthanized.

2004). We are adopting the rainbow trout linkage group nomenclature of NICHOLS *et al.* (2003) because those assignments are based upon a larger number of genetic markers, including known marker positions from the SAKAMOTO *et al.* (2000) map.

Loci were chosen on the basis of previous knowledge of polymorphism in other Arctic charr families (WORAM *et al.* 2004) and known association with QTL in rainbow trout, so that multiple linkage groups would be represented. In particular, all loci linked to UTT QTL in rainbow trout were screened in Arctic charr first. Specifically, significant QTL for UTT have been found on linkage groups RT-21, formerly designated RT-B (SAKAMOTO *et al.* 2000), RT-14 (formerly RT-D), and RT-6 (formerly RT-S; (JACKSON *et al.* 1998; DANZMANN *et al.* 1999; PERRY *et al.* 2001). We have also detected significant QTL effects on RT-1 (formerly RT-18) and RT-15 (formerly RT-8; our unpublished data), although the latter designation is tentative as it is based upon data derived from only 48 progeny in one of the backcross families. Furthermore, suggestive QTL have been found on RT-2, RT-3, RT-8, RT-10, RT-12, RT-16, RT-24, RT-20, and RT-31. In the above set, linkage groups RT-12 and RT-16 represent homeologous pairs, and the identified UTT QTL on both linkage groups map to similar locations, suggesting a conservation of QTL effect (our unpublished data). In addition, homeologies have been identified between RT-2/9, RT-3/25, RT-14/26, RT-23/24, RT-9/20, RT-9/13, RT-27/31, and RT-10/18 (SAKAMOTO *et al.* 2000; NICHOLS *et al.* 2003). We attempted to screen markers from known homeologs of the three significant QTL regions in rainbow trout [*i.e.*, markers from RT-26 (homeologous to RT-14)]. Unfortunately, the homeologous affinities for RT-6 and RT-21 are unknown. Randomly chosen markers from additional linkage groups in Arctic charr that are homologous to rainbow trout homeologs with QTL (*i.e.*, RT-9, RT-13, and RT-27) were also analyzed.

The following PCR program, with slight locus-specific modifications, was used: an initial denaturation cycle of 5 min at 95°, followed by 35 cycles of 1 min at the locus-specific annealing temperature, 1 min at 72°, 1 min at 95°, and a final extension time of 10–20 min at 72°. All loci used in this study, annealing temperatures, and known repeat sequences are presented in Table 2. Alleles were separated on a 6% polyacrylamide denaturing gel and visualized with a fluorescence imaging system (Hitachi FMBIOII). Fragment size was estimated by adding 2 ml of GeneScan 350 (Tamra) size standard (PE Applied Biosystems) to each of several lanes of the gel.

Statistical analysis: PROBMAX (DANZMANN 1997) was used to confirm the familial identity of the progeny through pedigree analysis (families 12-111, 12-114, 21-114, 30-136, and 27-139) using genotypes of up to 38 loci per family. Due to time constraints, families 12-114 and 21-114 were selectively genotyped, with 15–25% of the most- and least-temperature-tolerant fish chosen for analysis (*i.e.*, the tails of the distribution). Selective genotyping is a powerful method for QTL detection, although it may result in biased estimates of allelic effects (*e.g.*, DARVASI and SOLLER 1992).

Normality of temperature tolerance data was tested within each family prior to quantitative trait analysis using a Kolmogorov-Smirnov test, which is appropriate for samples sizes used here (Table 4). In addition, the Pearson product-moment correlation was used to determine whether fork length or body weight was associated with upper temperature tolerance in each family.

Due to the potential variability of the temperature profiles across lots (II, III, and IV) and the uneven representation of families within lots, temperature profiles of individual families across lots were compared using the Welch statistic for unequal sample size and variance. For all families except 12-114, mean time until death ("Time"), cumulative temperature profile from 10° acclimation temperature at time of death ("Area"), and "knockdown" temperature ("Temp") were not significantly different across lots (Table 4). Knockdown temperature is defined as the maximal temperature to which an individual fish can survive before it loses equilibrium. Mean temperatures differed across lots for family 12-114. However, regression of "lot" onto temperature tolerance in each family showed that it contributed a negligible amount to the total variance of the model (no increase or a decrease in R^2; data not shown). Therefore, progeny from families tested in different lots were pooled for analysis, and "Time" was used as the response variable.

Progeny of heterozygous parents (Table 3) were tested for the expected 1:1 segregation of alleles using the chi-square goodness-of-fit test statistic. Sequential Bonferroni correction for multiple tests was used to ensure an experiment-wide error rate of $P < 0.05$ within each family (RICE 1989). To compen-

TABLE 2

Microsatellite loci, comparative linkage groups, and sequence data used in this study

Locus name[a]	Rainbow trout	Arctic charr	Repeat sequence	T_A[b]	Primer source
Ssa77NUIG	—	1	—	48	GenBank accession no. AF420542
Ssa17/iNVH	—	3	Various GT	56	GenBank accession no. AF256668 using clone name BHMS161
Ssa17/iiNVH	—	24	Various GT	56	GenBank accession no. AF256668 using clone name BHMS161
Ots516/iNWFSC	—	23	$(GA)_{14}(CA)$	48	GenBank accession no. AY042706; Naish and Park (2002)
Ots516/iiNWFSC[c]	—	4, 5	$(GA)_{14}(CA)$	50	GenBank accession no. AY042706; Naish and Park (2002)
SSOSL32/I	26?	4	—	56	GenBank accession no. Z69642; Slettan *et al.* (1997)
SSOSL32/ii	26?	25	—	56	GenBank accession no. Z69642; Slettan *et al.* (1997)
OmyFGT19TUF	1	27, dupl.	$(GT)_{27}$	50	Tokyo University of Fisheries; Sakamoto *et al.* (1996)
Ssa3NUIG	—	34	—	50	GenBank accession no. AF420494
Ssa14DU	14	9	$(TC)_{10}(N)_{15}(TC)_3(N)_2$ $(AC)_{12}(TC)_3(N)_5(CA)_4$	48	Dalhousie University; Morris *et al.* (1996)
OmyOGT5TUF	12 & 16	UNA	—	54	Tokyo University of Fisheries; Sakamoto *et al.* (1996)
Ssa30/iNVH	3?	8	$(CA)_{42}CG(CA)_7$	50	GenBank accession no. AF256680 using clone name BHMS206
Ssa30/iiNVH	3?	6	$(CA)_{42}CG(CA)_7$	50	GenBank accession no. AF256680 using clone name BHMS206
Ots501/iNWFSC	27?	6	$(GA)_{22}$	48	GenBank accession no. AY042691; Naish and Park (2002)
Ots501/iiNWFSC	27?	12	$(GA)_{22}$	48	GenBank accession no. AY042691; Naish and Park (2002)
Omy301UoG	21	7	$(GT)_{20}$	55	University of Guelph; Jackson *et al.* (1998)
Ssa105NVH	—	8	$(CA)_{25}$	57	GenBank accession no. AF256748 using clone name BHMS330
OmyRGT39TUF	26	25	$(CA)_{12}$	54	Tokyo University of Fisheries; Sakamoto *et al.* (1996)
Ssa119NVH	12 & 16	12	$(CA)_3AA(CA)_6CG\ (CA)_4$	55	GenBank accession no. AF256759 using clone name BHMS219
Ssa185NVH	20	13	$(GT)_7GA(GT)_3$	57	GenBank acession no. AF256816 using clone name BHMS437
Ssa189NVH	—	13	$(CA)_4$	57	GenBank accession no. AF256820 using clone name BHMS491
OmyPuPuPyDU	24	13	—	54	Dalhousie University; Morris *et al.* (1996)
Ssa85DU	24	13	$(GT)_{14}$	55	Dalhousie University, Morris *et al.* (1996)
Sco19UBC	—	14	—	50	University of British Columbia; Taylor *et al.* (2001)
OmyRGT2TUF	10	15, dupl.	—	58	Tokyo University of Fisheries; Sakamoto *et al.* (1996)
Omy18INRA	8[d]	16	—	55	Institut National de la Recherche Agronomique; K. Gharbi and R. Guyomard
Ssa121NVH	13	19	$(CA)_{52}$	52	GenBank accession no. AF256761 using clone name BHMS411
OmyJTUF	13	19	—	56	Tokyo University of Fisheries; Sakamoto *et al.* (1996)
OmyRGT46TUF	13	19	$(CA)_{10}$	57	Tokyo University of Fisheries; Sakamoto *et al.* (1996)
Omy38DU	10	46	—	50	Dalhousie University; Morris *et al.* (1996)
Sfo23LAV	—	UNA	—	50	Laval University; Angers *et al.* (1995)
OmyRGT30TUF	9	20	—	52	Tokyo University of Fisheries; Sakamoto *et al.* (1996)
OmyRGT4TUF	9	20	$(CA)_6TA(CA)_{13}$	56	Tokyo University of Fisheries; Sakamoto *et al.* (1996)
TRCARR(INRA)	9	20	—	48	Institut National de la Recherche Agronomique; K. Gharbi and R. Guyomard
Cocl3LAV	6	26	$(GT)_{35}$	50	Laval University; L. Bernatchez
Ssa132NVH	—	25	$(CA)_7$	53	GenBank accession no. AF256769 using clone name BHMS121
SsaF43NUIG	—	26	$(CA)_{18}$	50	National University of Ireland; R. Powell
One10ASC	10	26	$(CA)_{25}$	50	GenBank accession no. U56710; Scribner *et al.* (1996)
Str7INRA	6, dupl.	25, dupl.	—	53	Institut National de la Recherche Agronomique; K. Gharbi and R. Guyomard
SSOSL456	—	29	$(AC)_{12}AG(AC)_{10}$	60–50	GenBank accession no. Z69645; Slettan *et al.* (1997)

Markers were mapped to linkage groups in rainbow trout and Arctic charr backcross families (Sakamoto *et al.* 2000; Woram *et al.* 2004). —, no data currently available; dupl., single primer pair amplifies duplicated loci but only one marker is mapped (*i.e.*, the other marker is monomorphic); UNA, the marker is currently unassigned to any known linkage group; ?, a single marker assigned to the linkage group shown for rainbow trout. Because of the duplicated status of the marker in Arctic charr, it was not possible to assess the exact cross homologies.

[a] The locus name is composed of a three-letter acronym designating the species in which the primer set was designed, followed by a lab-specific clone number and a suffix indicating the source of the primers: Ssa, *S. salar*; Str, *S. trutta*; Omy, *O. mykiss*; Ocl, *O. clarki*; One, *O. nerka*; Ots, *O. tshawytscha*; Sco, *Salvelinus confluentus.*

[b] The annealing temperature used to amplify microsatellite loci.

[c] Ots516/iiNWFSC is linked to a different linkage group in each of two sires.

[d] The marker was mapped in rainbow trout mapping family 41. All other rainbow trout markers were mapped in families 25 and 44.

TABLE 3

Polymorphisms detected in the parents of the five Arctic charr families

	Parent							
Primer name	M-111[a]	F-12	M-114	F-21	F-27	M-139	F-30	M-136
Ssa77NUIG	Y	N	Y	N	Y	N	N	Y
Ssa17/iNVH	Y	Y	Y	Y	Y	Y	N	Y
Ots516/iNWFSC	Y	N	Y	N	Y	N	N	N
SSOSL32/i	Y	N	Y	N	N	Y	N	Y
OmyFGT19/iTUF	Y	N	N	Y	N	N	N	N
OmyFGT19/iiTUF	N	N	N	Y	N	N	Y	N
Ssa3NUIG	Y	N	Y	N	N	N	N	N
Ssa14DU	N	Y	N	Y	N	Y	N	Y
OmyOGT5TUF	N	N	N	Y	Y*	Y*	N	Y
Ssa30/iNVH	Y	N	N	N	N	N	N	Y
Ssa30/iiNVH	Y	Y	Y	Y	Y	Y	Y	Y
Ots501/iNWFSC	Y	N	Y	Y	N	Y	Y	N
Ots501/iiNWFSC	Y	N	Y	N	N	N	N	N
Omy301UoG	Y	Y	Y	Y	Y	Y	Y	N
Ssa105NVH	N	N	N	Y	Y	N	N	N
OmyRGT39TUF	Y*	Y*	N	Y	Y	N	Y*	Y*
Ssa119NVH	N	Y*	Y*	Y*	N	N	N	Y
Ssa185NVH	Y	N	Y	N	N	N	Y*	Y*
Ssa189NVH	Y	N	N	N	Y	N	Y	N
OmyPuPuPyDU	Y	N	Y	N	N	N	N	Y
Ssa85DU	Y	Y	Y	Y	N	Y	Y	N
Sco19UBC	Y	Y	Y	Y	Y	Y	N	Y*
OmyRGT2TUF	Y*	Y*	Y	N	N	Y	Y	Y
Omy18INRA	N	Y	Y	N	N	Y	Y	Y
Ssa121NVH	N	N	Y*	Y*	N	Y	Y	N
OmyJTUF	Y	N	Y	N	N	N	N	N
OmyRGT46TUF	N	Y	N	Y	Y	Y	Y	Y
Omy38DU	Y*	Y*	Y*	N	N	Y	Y	Y
Sfo23LAV	Y	Y	Y	Y	Y	Y	Y	N
OmyRGT30TUF	Y	N	Y	N	N	N	N	N
OmyRGT4TUF	Y*	Y*	Y*	Y*	Y	N	Y	Y
TRCARR	Y*	Y*	Y*	Y*	N	N	N	Y
Cocl3LAV	Y	Y	Y	Y	Y	Y	Y	Y
Ssa132NVH	Y	N	Y	N	Y	N	Y	N
SsaF43NUIG	Y	N	Y	Y	Y	N	Y	N
One10ASC	Y	Y	N	Y	N	N	Y	N
Str7INRA	Y	N	Y	N	Y*[b]	Y*[b]	Y	N
SSOSL456	Y	Y*	Y*	N	N	N	N	N
Total polymorphic[c]	30	18	28	21	17	17	20	19

Y, the parent is heterozygous for the marker shown; N, the parent is not heterozygous; *, the parents are heterozygous for identical alleles at the respective marker.

[a] Parents are aligned such that the parents for the same families are adjacent to one another. Female (F) 12 was mated to male (M) 111 and 114, while male 114 was also mated to female 21. Similarly, female 27 was mated to male 139 and female 30 to male 136.

[b] Presence of a null allele requires the treatment of this locus as genotypic data for family 27-139.

[c] The total number of polymorphic loci for that parent. Note that this number does not correspond to the number of loci tested, since accurate scoring of alleles in progeny was impossible at some loci even if the parents were informative (*e.g.*, Table 5).

sate for the increased likelihood of generating type II errors when this correction is applied to large number of tests, we considered that a correction based upon the number of linkage groups examined was appropriate. Thus, our experiment-wide a $\alpha = 0.05$ level was defined as 0.05/16 and $P \leq 0.003$, since we examined markers located on 16 Arctic charr linkage groups.

QTL analysis was performed on the maternal and paternal component separately using survival analysis on "Time" to compare the allele classes. The Kaplan Meier product limit measure was employed because it is a nonparametric (or distribution-free) method (Kleinbaum 1996), and the thermal profiles for each allele class had a nonconstant slope. To account for the change in slope midway through the thermal chal-

TABLE 4

Descriptive statistics for five Arctic charr (*S. alpinus*) families

Family	*N*	Trait	Normality	Mean	Standard deviation
2-111	44	Time	NS	492.64	36.41
		Area	NS	2135.03	531.92
		Temp	NS	24.91	0.49
		BW	NS	18.80	8.65
		FL	NS	11.88	1.71
		K	NS	1.04	0.016
12-114	37	Time	NS	491.70	23.25
		Area	$P < 0.1$	2116.57	350.15
		Temp	$P < 0.15$	24.84	0.44
		BW	NS	19.13	6.74
		FL	NS	12.12	1.56
		K	NS	1.02	0.073
21-114	32	Time	$P < 0.2$	507.91	24.28
		Area	$P < 0.15$	2364.55	367.7
		Temp	NS	25.14	0.445
		BW	NS	17.68	8.10
		FL	NS	11.65	1.77
		K	NS	1.04	0.061
27-139	56	Time	NS	533.14	16.75
		Area	$P < 0.2$	2811.57	284.91
		Temp	NS	25.60	0.345
		BW	NS	20.35	4.94
		FL	NS	12.37	1.08
		K	NS	1.05	0.066
30-136	42	Time	NS	530.02	13.43
		Area	NS	2749.11	211.80
		Temp	$P < 0.15$	25.52	0.25
		BW	NS	18.89	4.55
		FL	NS	12.08	1.05
		K	NS	1.05	0.072

N, the number of progeny in each family. Normality was tested using the Kolmogorov-Smirnov test statistic. Time, time until death during thermal trials (minutes); Area, cumulative temperature tolerance from 10° acclimation (degree minutes); Temp, temperature of death; BW, body weight (grams); FL, fork length (millimeters); K, condition factor; see text for details.

lenge, a censoring variable was included whereby the first 50% of fish to have died were considered "uncensored" (exponent value of 1) and the last 50% "censored" (exponent value of 0). Thus, the survival rate data are included for all individuals up to the censoring point, while individuals surviving past this point are "alive." This parallels the method by which the critical thermal maximum is calculated. The survival analysis performed in this study therefore compares the following survival function of each allele class at a single locus:

$$S(t) = \prod_{j=1}^{t}[(n - j)/(n - j + 1)]^{\delta(j)}.$$

In this equation $S(t)$ represents the survival function, n is the total number of cases, Π denotes the geometric product across all cases less than or equal to t, j is the individual of interest, t is the time interval, and $\delta(j)$ is a constant that is either 1, if the jth case is uncensored, or 0, if it is censored. This estimate of the survival function is the *product limit estimator* (Statistica for Windows 1995; Kleinbaum 1996).

This equation compares the proportion of censored and uncensored individuals in each allele class while taking into account the rate of death ("hazard function"); the *P*-value is derived using Cox's *F*-test, a test statistic specific to survival analysis, which is appropriate for limited sample sizes (Statistica for Windows 1995). We dealt with the potential confounding effect of body weight by regressing time of death onto body weight in the families for which body weight significantly correlated with survivorship (12-111, 21-114; data not shown). Thus, we calculated "body weight-corrected time of death" by the formula $\hat{Y} = \bar{Y} + residuals$; this corrected measure of time was then used in the survival analysis for these two families. However, it is important to note that the results did not change whether body weight was taken into account or not (data not shown).

Genotypic classes were similarly compared in progeny when both the sire and the dam were heterozygous for the same alleles. This was accomplished by scoring homozygotes as 11 or 22 (depending on whether they inherited small or large alleles) and heterozygotes as 12. Then each pairwise comparison was performed (*i.e.*, 11 *vs.* 22, 11 *vs.* 12, and 12 *vs.* 22).

RESULTS

A single locus (Ssa3NUIG in family 12-111) deviated significantly from Mendelian proportions ($\chi^2_{adj\ (0.05,\ 1)} = 9.82$, $P < 0.05$). Loci for which significant deviations from Mendelian segregation were detected were further tested for conformation to 1:1:1:1 genotypic ratios across both parents and did not deviate significantly from expectations ($P > 0.05$; data not shown).

Marker-UTT associations were detected using survival analysis (Table 5). Significant associations between marker alleles and UTT were detected for Ssa189NVH on linkage group AC-13 in the dam of 27-139 ($P < 0.003$), as well as for SsaF43NUIG (AC-26) in the sire of 12-111 ($P < 0.001$). The localization of QTL on linkage group AC-13 is further supported in 12-111 by the detection of suggestive associations ($0.003 < P < 0.05$) in alleles derived from the male at Ssa85DU ($P < 0.046$) and at Ssa185NVH ($P < 0.021$). Similarly, suggestive associations at One10ASC in 12-111 (sire; $P < 0.005$) and 12-114 (dam; $P < 0.021$) corroborate the QTL on linkage group AC-26.

The sex-specific distribution of markers on AC-13 suggests that QTL effects are localized in two different regions (region marked by Ssa189NVH in female 27 and a second marked by Ssa85DU, Ssa185NVH, and OmyPuPuPyDU in male 111). It appears that two different QTL exist on AC-13 because the effect in female 27 is confined to a marker (Ssa189NVH) that is unlinked to the region containing the other three markers. Moreover, the effect in male 111 is strongest in the region marked by Ssa85DU, Ssa185NVH, and OmyPuPuPyDU and less so in the region marked by Ssa189NVH (10 cM distant).

Additional suggestive associations between microsatellite loci and UTT were detected in different families

on linkage groups AC-4 (sire effect; SSOSL32/i), AC-9 (sire; Ssa14DU), AC-12 (genotypic data; Ssa119NVH), AC-15 (sire; OmyRGT2/iiTUF), AC-19 (dam; OmyRGT-46TUF), AC-20 (dam; OmyRGT4TUF; genotypic data; OmyTRCARR), AC-25 (dam; OmyRGT39TUF), and one unassigned marker (genotypic data; OmyOGT5-TUF). The QTL on AC-4 and AC-25 are in homeologous regions. The QTL region on AC-4 marked by SSOL32/i appears homeologous to a QTL region on AC-25 marked by RGT39TUF.

In summary, 1 significant and 3 marginal associations were detected out of a total of 37 independent tests across both parents in family 12-111; 2 marginal QTL in 12-114 and 21-114, out of 29 and 27 tests, respectively; no associations in 29 tests in 30-136; and 1 significant and 4 putative associations from a total of 29 tests in 27-139. This corresponds to 2 significant (1%) and 11 marginal (7%) associations between microsatellite loci and UTT detected in 151 tests across five families, when dam and sire components and genotypic data are considered (see Tables 3 and 6 for more details).

The data suggest that there may be a heterozygote advantage at OmyTRCARR (Table 5). Heterozygous 120/126 individuals showed greater survival relative to the 120/120 homozygote in half-sib family 12-114. Heterozygotes tend to be more temperature tolerant than their homozygous siblings, as assessed by mean survival time and mortality rate. This phenomenon would not have been detected had both parents not been heterozygous for the same alleles (*i.e.*, QTL analysis could not be performed on the whole data set).

DISCUSSION

Our study is one of the first to undertake comparative QTL mapping in an animal polyploid group. More importantly, our findings highlight the complexities when the taxa have undergone significant genomic reorganization after the polyploid event and have been subjected to very different evolutionary selection pressures. Salmonid fishes like rainbow trout and Arctic charr have long been accepted as important animal models for chromosomal and genetic divergence following a polyploid event (Allendorf and Thorgaard 1984; May and Johnson 1990; Phillips and Rab 2001) but only recently have studies started to address how this molecular architecture relates to phenotypic expression and evolution (Perry 2001; Perry *et al.* 2001; O'Malley *et al.* 2003; Woram *et al.* 2003). We have detected two significant QTL and seven suggestive QTL for UTT in Arctic charr. Two of the suggestive QTL are found on homeologous linkage groups, indicating functional conservation across duplicated chromosomes. Moreover, comparative mapping suggests that as many as six of these chromosomal regions also have detectable effects in rainbow trout. Thus, some of the genes underlying temperature tolerance QTL may antedate the divergence of Arctic charr and rainbow trout from a common ancestor ~16 MYA (Andersson *et al.* 1995).

Determining the homologies of regions between the species, and thus testing if a QTL effect appears to be conserved at the chromosomal level, is complex because of differences in the composition of the marker sets, the karyotypic divergence between the species (Phillips and Rab 2001), and the sex-specific recombination rates in salmonid fishes (Sakamoto *et al.* 2000). Reduced recombination in males results in inheritance of entire chromosome segments (except in telomeric regions) and leads to an increased ability to detect QTL but a decreased ability to localize the QTL to a particular segment. In contrast, marker-trait associations are less likely to be detected in females because of large interlocus distances. However, once a potential linkage group has been targeted, significant marker-trait associations in females tend to be more representative of true QTL location.

Rainbow trout have UTT QTL in regions that are homologous to those containing the two significant QTL in Arctic charr (Jackson *et al.* 1998; Danzmann *et al.* 1999; Perry *et al.* 2001; Figure 2). First, the QTL marker on RT-24 (Ssa85DU) is in close proximity to the QTL markers on AC-13 (OmyPuPuPyDU and Ssa185NVH; Figure 2a). However, we cannot determine whether the second QTL region on AC-13 shows similar effects across species because Ssa189NVH has yet to be mapped in rainbow trout. Second, the proximity of the QTL marker on AC-26 (SsaF43NUIG) to Cocl3LAV, which in turn maps proximally to the QTL marker on RT-6 (Ssa20.19NUIG), suggests conservation across species (Figure 2b).

Four of the suggestive UTT QTL in Arctic charr may also show homologies in rainbow trout and Arctic charr. First, the marker Ssa14DU (AC-9 and RT-14) is associated with differential thermal challenge survival in both species (Figure 2c). Second, the QTL marker on AC-12 (Ssa119NVH) maps syntenically to Omy77DU in males (Woram *et al.* 2004; Figure 2d). This region on RT-16 also has a suggestive UTT effect in rainbow trout (Jackson *et al.* 1998; Danzmann *et al.* 1999). The third case is not as straightforward because of complexities in linkage homologies between the species. The QTL marker (OmyRGT2/iiTUF) on AC-15 falls in the same cluster of markers as the QTL marker (Omy105DU) on RT-10 (Jackson *et al.* 1998; Danzmann *et al.* 1999) in the male, suggesting conservation across species (Figure 2e). However, other markers showing zero recombination to Omy105DU on RT-10 map to other linkage groups in Arctic charr (*e.g.*, One10ASC on AC-26). Thus, we cannot be sure of homology until we resolve the linkage arrangements in both sexes. Fourth, the chromosomal region surrounding TRCARR on RT-20 (our unpublished results) and on AC-20 (QTL marker shows zero recombination with TRCARR in males) both contain UTT QTL (Figure 2f).

The detection of QTL in orthologous regions of Arc-

TABLE 5

Putative QTL for UTT detected in five families of Arctic charr (*S. alpinus*) using survival analysis

AC[a]	RT[b]	Locus	Family	Parent	Allele	*N*	% cen.	Cox's *F*	*P*-value
3	—	Ssa17NVH	30-136	M	191	24	37.5	2.75	0.068
					195	15	73.3		
4	26	SSOSL32/i	27-139	M	84	26	26.9	2.93	0.009
					94	30	70.0		
UNA	—	Ssa3NUIG	12-111	M	246	6	33.3	2.53	0.054
					250	25	68.0		
9	14	Ssa14DU	27-139	M	151	29	62.1	2.12	0.024
					155	27	37.0		
8	—	Ssa105NVH	21-114	F	103	14	64.3	2.18	0.062
					107	11	38.9		
UNA	12/16	OmyOGT5TUF[c]	27-139	N/A	11	15	80.0	4.25	0.010
					22	13	38.5		
12	12/16	Ssa119NVH[c]	12-114	N/A	11	8	75.0	3.72	0.022
					22	14	35.7		
14	—	Sco19UBC	27-139	F	190	26	61.5	1.67	0.089
					228	30	40.0		
14	—	Sco19UBC	12-111	F	190	14	42.9	2.22	0.071
					228	21	66.7		
14	—	Sco19UBC	21-114	M	174	18	61.1	2.28	0.058
					217	13	51.6		
13	—	Ssa189NVH	27-139	F	123	31	67.7	2.83	0.003[d]
					125	25	28.0		
13	—	Ssa189NVH	12-111	M	123	17	64.7	1.99	0.066
					125	25	44.0		
13	20	Ssa185NVH	12-111	M	123	18	72.2	3.34	0.005
					125	23	34.8		
13	24	OmyPuPuPyDU	12-111	M	438	22	63.6	2.07	0.049
					460	21	38.1		
13	24	Ssa85DU	12-111	M	180	23	39.1	1.95	0.081
					240	21	61.9		
15	10	OmyRGT2/iiTUF	27-139	M	320	26	61.5	2.02	0.035
					360	29	41.4		
15	10	OmyRGT2/iiTUF	12-114	F	280	20	60.0	1.89	0.083
					320	18	38.9		
19	13	OmyJTUF	21-114	M	122	17	35.3	2.60	0.060
					132	15	66.7		
19	13	OmyRGT46TUF	21-114	F	165	19	68.4	3.77	0.004
					171	13	23.1		
20	9	OmyRGT30TUF	12-111	M	84	23	39.1	2.02	0.071
					88	20	60.0		
20	9	OmyRGT4TUF	27-139	F	139	21	33.3	2.35	0.014
					143	35	60.0		
20	9/20	*TRCARR*,[c] 11 *vs.* 12	21-114	N/A	11	7	71.4	2.47	0.072
					12	20	40.0		
20	9/20	*TRCARR*,[c] 11 *vs.* 12	12-114	N/A	11	9	11.1	6.72	0.005
					12	17	76.5		
20	9/20	*TRCARR*,[c] 22 *vs.* 12	12-114	N/A	12	17	76.5	4.26	0.023
					22	11	36.4		
25	26	OmyRGT39TUF	21-114	F	109	19	68.4	3.75	0.006
					22	11	36.4		
25	6	Str7INRA	12-114	M	323	14	64.3	1.93	0.088
					350	23	43.5		
25	—	Ssa132NVH	12-111	M	122	14	64.3	1.87	0.094
					130	24	41.7		

(*continued*)

TABLE 5

(Continued)

AC[a]	RT[b]	Locus	Family	Parent	Allele	*N*	% cen.	Cox's *F*	*P*-value
26	6	Cocl3LAV	12-114	F	236	17	58.8	1.92	0.083
					278	18	38.9		
26	—	SsaF43NUIG	12-111	M	100	25	68.0	3.37	0.002[d]
					110	19	26.3		
26	10	One10ASC	12-111	M	160	22	68.2	3.01	0.007
					166	19	31.6		
26	10	One10ASC	12-114	F	164	19	68.4	2.71	0.021
					166	17	35.3		

Pairs of linkage groups that are underlined may show conservation of QTL. *N*, the number of alleles; "% cen.," the percentage of individuals that are censored (alive) within each allele class when 50% of the fish have succumbed to the thermal challenge (see text for details); "Cox's *F*" is the value of the test statistic and the *P*-value represents its significance level (underlined, $P < 0.05$). —, the locus is unassigned in that species.

[a] Linkage groups on which loci are found in Arctic charr (Sakamoto *et al.* 2000; Woram *et al.* 2004). When more than one rainbow trout linkage group is shown, this indicates that the marker is duplicated in this species.

[b] Linkage groups on which the loci are found in rainbow trout (Sakamoto *et al.* 2000; Woram *et al.* 2004). When more than one rainbow trout linkage group is shown, this indicates that the marker is duplicated in this species.

[c] UTT of genotypic classes (11 *vs.* 22 unless noted) was compared when both parents were heterozygous for the same alleles.

[d] Loci passing Bonferroni threshold for 16 tests.

tic charr and rainbow trout supports the findings from many recent comparative QTL studies. Homologies are detectable across both closely and distantly related species (Paterson *et al.* 2000; Lahbib-Mansais *et al.* 2003). Interestingly, some analyses suggest a remarkable conservation of genetic architecture in that the domestication of the Solanaceae has involved a limited number of loci in the different species (Doganlar *et al.* 2002). Our results also support the recurring theme that either gene duplication at the level of entire genomes (polyploidization) and subsequent gene loss (diploidization; Bowers *et al.* 2003) or intrachromosomal segmental duplication (Locke *et al.* 2003) affects the propensity of conservation in gene order and location.

We have limited evidence for the apparent functional retention of duplicate QTL regions in Arctic charr as only one pair of ancestral homeologs had detectable QTL. Marginal evidence that three pairs of ancestral homeologs contained detectable QTL for either spawning date or body weight has been found in rainbow

TABLE 6

Summary of associations between allelic variation at microsatellite loci and upper temperature tolerance in five families of Arctic charr

Family name	No. of loci tested[a]	Dam component[b]	Sire component[b]	Genotypic data[c]	Significant associations[d]	Marginal associations[d]
12-111	30	12	21	4	1	3
12-114	22	11	14	4	0	2
21-114	22	11	13	3	0	2
30-136	24	12	15	2	0	0
27-139	23	12	15	2	1	4

[a] The number of different microsatellite loci tested in each family in which either one or both parents were heterozygous. Since it was possible to analyze both the dam and the sire components separately for some loci, the number of loci tested is not equal to the sum of dam component + sire component + genotypic data.

[b] The number of loci where the female was heterozygous and both female alleles could be distinguished from the male alleles. Similarly, under the sire component, the number of loci indicated are those heterozygous in the male parent where both male alleles could be distinguished from the female alleles.

[c] The number of loci where both parents were heterozygous but shared identical alleles. Only pairwise tests among the three genotypic classes were performed.

[d] For each family, the number of significant ($P < 0.003$) and marginal associations with UTT ($0.003 < P < 0.05$) detected are indicated.

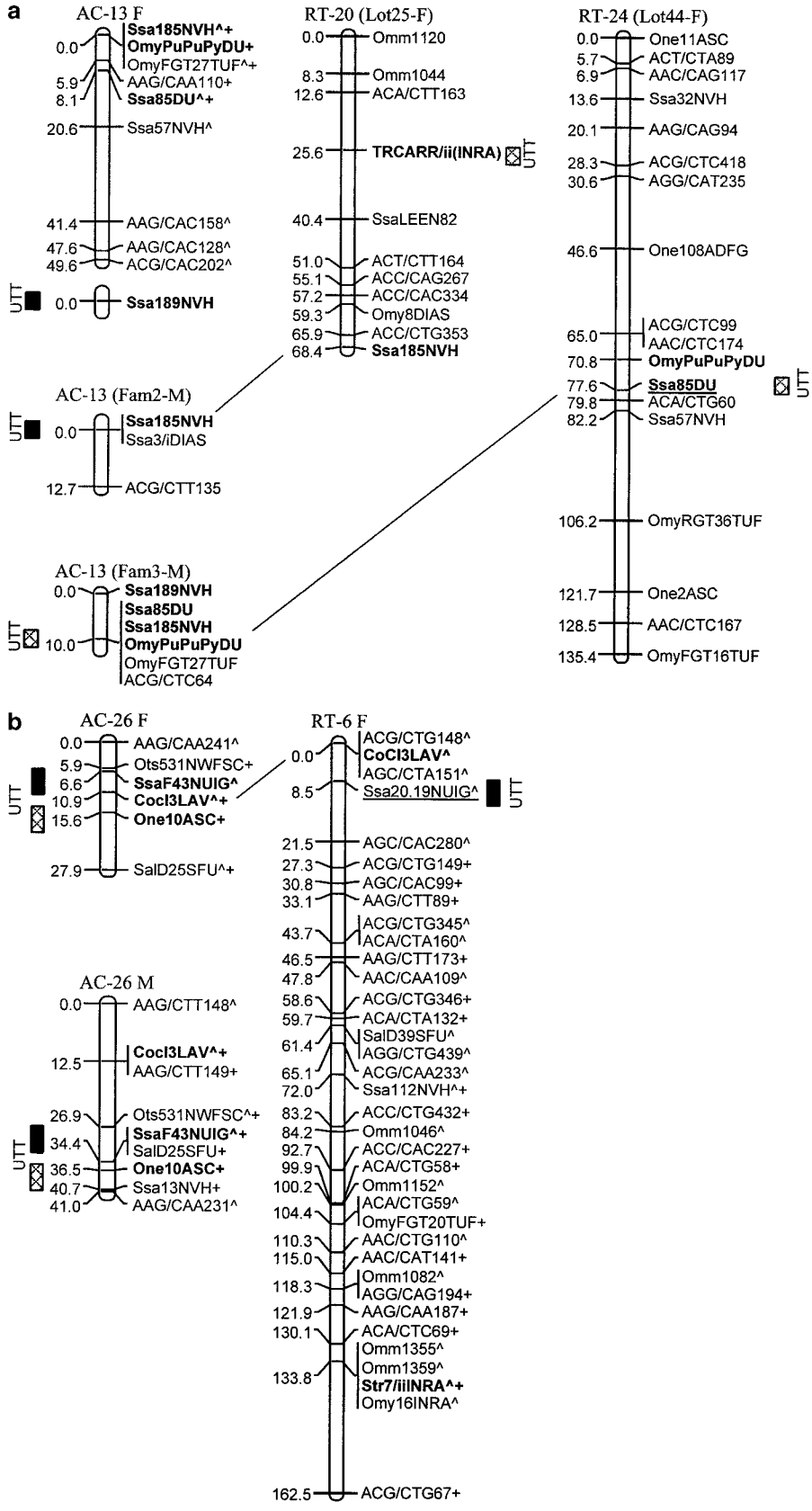

FIGURE 2.—Comparative homologies between Arctic charr and rainbow trout chromosomes possessing UTT QTL (a solid box indicates UTT regions with $P < 0.01$ and a cross-hatched box indicates UTT regions where $0.05 > P > 0.01$). Male (M) and female (F) specific maps for each species are indicated following the linkage group designation and preceding the mapping panel used. For Arctic charr, two mapping families were used: Family 2 (Fam2) and Family 3 (Fam3). The markers analyzed in the present study are in boldface type. Similarly, in rainbow trout, two main mapping families, lot 25 and lot 44, were used for linkage map construction. For composite maps (involving combined data from both mapping families), the marker polymorphism sources are as follows: Fam2, ^; Fam3, +; lot 25, ^; lot 44, +. Details for the map construction and marker sources are presented in WORAM *et al.* (2004).

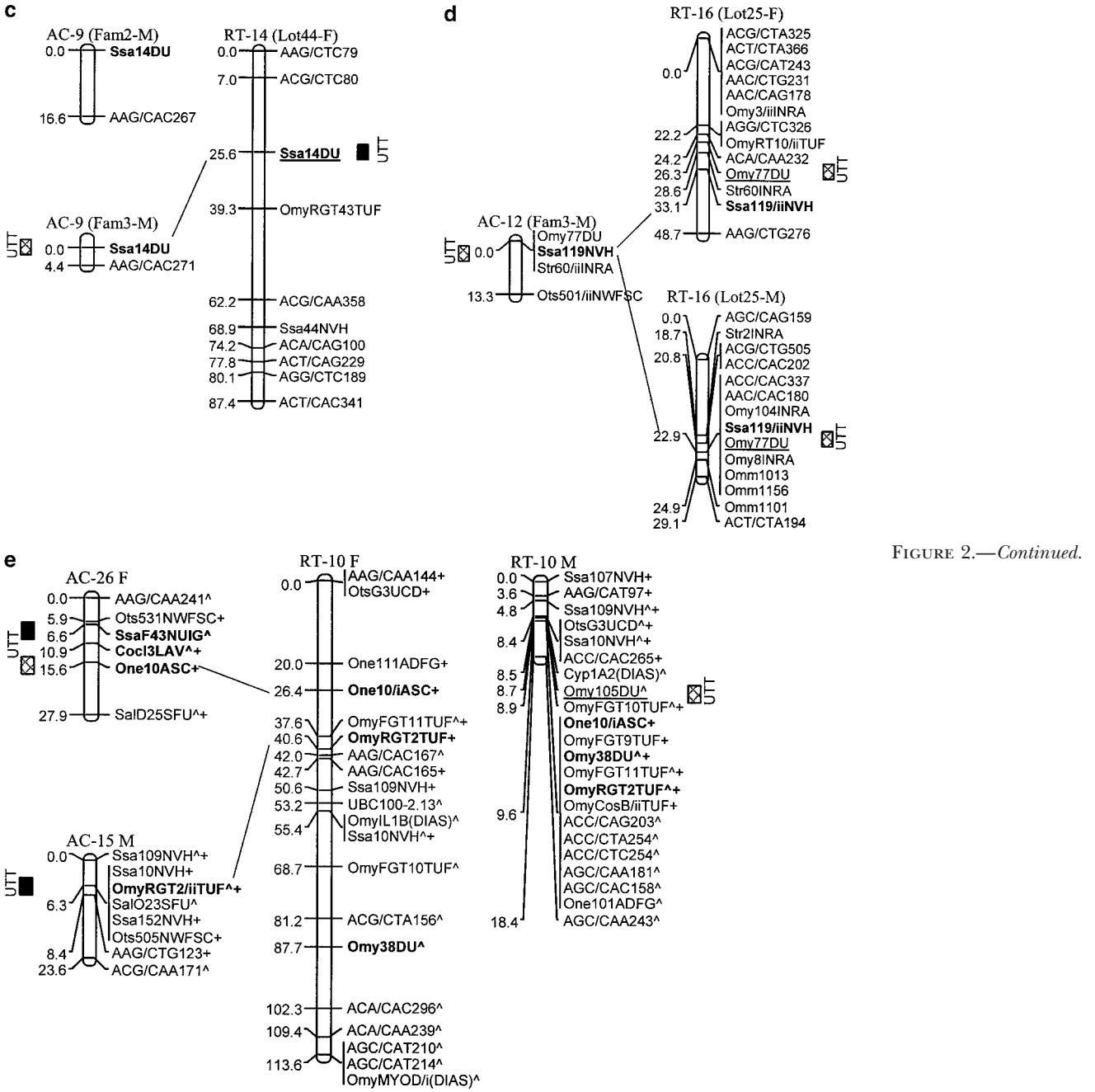

FIGURE 2.—*Continued.*

trout out of eight homeologs tested (O'MALLEY *et al.* 2003). In one of the three pairs of rainbow trout homeologs, the duplicated QTL regions mapped to the same relative chromosomal location, while the exact localization of the QTL position in one of the other pairs was difficult to infer since it was based upon data from a male-derived map. In addition, the mapping of body weight QTL to four pairs of homeologous segments in Atlantic salmon (D. REID, A. SZANTO, B. GLEBE, R. DANZMANN and M. FERGUSON, unpublished observations) provides some evidence that ancestrally duplicated chromosomes may retain similar gene function.

The importance of gene duplication and polyploidy in the evolution of phenotypic diversity is more readily apparent when considering plants, of which a large proportion are thought to be polyploid in origin (SOLTIS and SOLTIS 1999; OTTO and WHITTON 2000). Studies have found a high degree of observed duplication of QTL in autopolyploids (*e.g.*, sugarcane; MING *et al.* 2002) and possibly less so in allopolyploids (*e.g.*, cotton; CRONN *et al.* 1999). The loss or divergence of gene function may also relate to the length of the diploidization process (APARICIO 2000). Unfortunately, there are few animal systems in which to test these ideas. Nevertheless, the salmonids are expected to show more divergence of gene function (less conservation of QTL across homeologs) compared to sugarcane where the polyploid event is thought to have occurred within the last few million

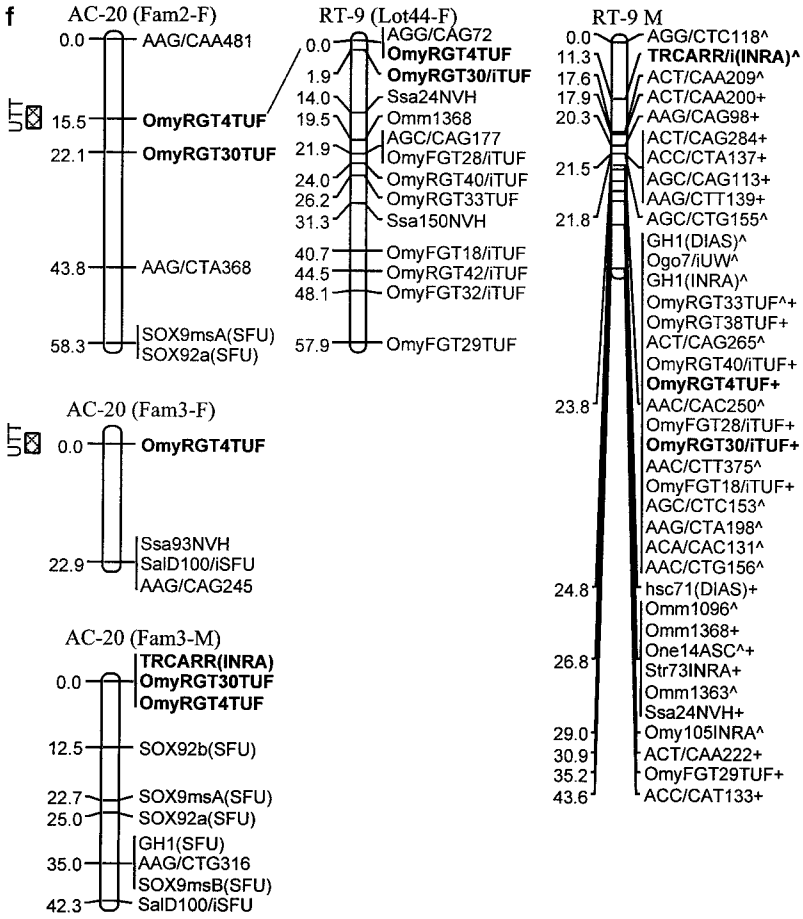

FIGURE 2.—*Continued.*

years (PATERSON *et al.* 2000). The existing salmonid QTL data certainly support this prediction. However, allozyme studies suggest that salmonids exhibit as much as 50% retention of duplicate allozyme expression (ALLENDORF and THORGAARD 1984) and thus some conservation of QTL across ancestral homeologs is expected. High rates in the frequency of duplicate preservation have been attributed to relaxed selection or accelerated evolution at replacement sites early on, followed by a gradual increase in selective constraints (LYNCH and CONERY 2000), with genes involved in the adaptive environmental and stress responses being significantly overrepresented (KONDRASHOV *et al.* 2002). Thus, we might expect to see greater rates of QTL conservation across homeologs for certain traits, but not others, once the data become available.

The observation that multiple QTL were detected in pure strain parents (Fraser River and Nauyuk Lake) was unexpected. It was predicted that greater effects would have been detected in the male F_1 hybrid parent due to segregation of QTL alleles, under the assumption that pure strains were almost fixed for alternate alleles. This was inferred because these strains are descended from populations that are adapted to very different thermal regimes as mentioned previously. While the majority of QTL effects were detected in the F_1 male parent, QTL effects were also detected in all the other parents, with the exception of the female parent in one family. Such cryptic variation for temperature tolerance within "pure" strains may have been uncovered upon disruption of the genetic background, as suggested for various invariant phenotypic characters in teosinte when crossed to maize (LAUTER and DOEBLEY 2002). The observation that generally more effects were found for the alleles inherited from Fraser strain parents than for those from Nauyuk Lake fish may relate to the numbers of founding individuals used initially to produce the different strains, such that there was more genetic variation in the Fraser than in the Nauyuk Lake fish (LUNDRIGAN 2001).

The genetic basis of UTT QTL is not presently known. Evidence in Fugu and Ictalurus indicates that many microsatellites are present in untranscribed regions of genes and even in coding regions (EDWARDS *et al.* 1998; LIU *et al.* 1999), suggesting that microsatellite-based QTL studies across species may be informative with respect to detecting close linkages to functional genes. Although a variety of candidate genes for stress response are known in vertebrates, very few of these have been mapped in salmonid genomes. For example, the only heat-shock cognate mapped in salmonids is localized to linkage group RT-9 in rainbow trout (SAKAMOTO *et al.* 2000), which is homologous to the QTL-containing re-

gion on AC-20 in four families of Arctic charr. The exact localization of these markers to the putative candidate gene is difficult to ascertain since the *hsc71* gene in rainbow trout was mapped in the male. The expression of heat-shock protein (*hsp*) families is directly related to thermotolerance (Coleman *et al.* 1995), and thus *hsp* may be primary candidate genes for UTT QTL on AC-20. Future research on candidate genes will provide important insights into the genetic basis of QTL affecting traits of evolutionary interest.

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