# QUANTITATIVE TRAIT LOCI FOR FITNESS TRAITS IN ARCTIC CHARR: CONSERVATION IN RAINBOW TROUT AND CORRELATIONS AMONG TRAITS.

A Thesis

Presented to

The Faculty of Graduate Studies

of

The University of Guelph

by

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In partial fulfillment of requirements

for the degree of

Master of Science

June, 2001

# ABSTRACT

## QUANTITATIVE TRAIT LOCI FOR FITNESS TRAITS IN ARCTIC CHARR: CONSERVATION IN RAINBOW TROUT AND CORRELATIONS AMONG TRAITS

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University of Guelph, 2001

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I searched for quantitative trait loci (QTL) affecting fitness traits in crosses between the Nauyuk Lake and Fraser River strains of Arctic charr (*Salvelinus alpinus*). A comparative mapping approach using microsatellite loci demonstrated that several chromosomal regions influencing upper thermal tolerance and body size have been conserved in Arctic charr based on previous work with rainbow trout (*Oncorhynchus mykiss*). Thus, genes underlying fitness QTL may antedate the divergence of these two species. QTL for condition factor and relative gonad size (gonadosomatic index) were also identified. In addition, pairs of homeologues (ancestrally duplicated chromosomal segments) have maintained similar functions in Arctic charr since the evolution of salmonids from a tetraploid ancestor 100-25MYA. However, co-occurrence of body size and thermal tolerance QTL on the same linkage group, and high phenotypic correlations between the two traits, suggest that associated genes control a more basic physiological function like metabolism.

**ACKNOWLEDGEMENTS**

There are so many people I would like to thank that I don't even know where to start. I suppose I should thank my advisor first, Dr. M. M. Ferguson, for taking me on three years ago when I didn't know why QTL or Arctic charr mattered to anyone. Although we didn't necessarily always agree, her continuous editing efforts resulted in a thesis that, I hope, everyone was happy with in the end. I would also, of course, like to thank my committee members, Drs. R. G. Danzmann and J. A. Robinson for helpful comments and agreeing to last minute meetings.

My lab mates have been a constant source of entertainment and support throughout my research, and after seven semesters, there have been a lot of them. In no particular order, I would like to thank Chris Martyniuk, Alastair Wilson, Gavin McDonald, Kathleen O'Malley, Guy Perry, Tony Fishback, Dabbi Gíslason, Mike Robinson, Tricia Lundrigan, Jake Stout, Darrin Reid and Heather Allen, to each of whom I am deeply grateful. I would especially like to thank Rachael Woram for so many little things, not the least of which were her valiant efforts to finish the Arctic charr linkage map. So many other people in the department have provided assistance or friendship at some time, I can't cite all of them; I just hope you know who you are!

This work would not have been possible without the help and dedication of members of the aquaculture industry who provided gametes, invaluable advice, and technical support, including Coldwater Hatchery, Alma Research Station and the Hagen Aqualab. Bob Frank and Paul Matsumoto not only taught me a lot about the proper care and maintenance of fish, but they were also no more than a phone call or a shout away when catastrophe struck or I needed a coffee.

Finally, I would like to thank both my real and adopted families for providing support, advice or perspective when it seemed like I would never finish this degree. I probably would not have had any clue what I was getting into when I went into science if not for my parents. The Royces' home was a haven of calm in Toronto when I needed to get away from Guelph...and let's be honest: Jon put up with a lot.

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# CHAPTER 1:

# GENERAL INTRODUCTION

Quantitative variation in both morphological traits (e.g. body size) and aspects of life history (e.g. spawning time) is common. Chromosomal regions affecting the expression of quantitative traits, but for which the underlying genetic architecture is not necessarily known, have been termed quantitative trait loci (QTL). Multiple QTL may exhibit additive, epistatic or environmentally dependent effects on the expression of complex phenotypes (Tanksley 1993; Poompuang and Hallerman 1997). The study of the evolutionary genetics of such traits is becoming possible due to continued development of quantitative theory, and recent advances in molecular technologies (reviewed by Cheverud and Routman 1993). However, the majority of QTL have been detected in livestock and crop species mainly due to their economic importance, but also as a result of well-established selection programmes and known pedigree structure in those taxa. QTL have been identified for a multitude of production traits, among which milk yield and composition in cattle (Georges 1998), growth and fatness in swine (Andersson et al. 1998), grain yield in maize (Austin and Lee 1998) and seed production and dormancy in rice (Oard et al. 2000). Characters of evolutionary interest for which QTL have been identified include antler pubertal and seasonality traits in deer interspecific crosses (Goosen et al. 2000), honey bee stinging behaviour (Hunt et al. 1998), spawning time in rainbow trout (Sakamoto et al. 1999) and floral morphology in monkeyflower (Bradshaw et al. 1998). The latter traits are of importance due to their potential as reproductive isolating factors influencing speciation.

The detection of QTL has been greatly facilitated by the construction of dense linkage maps, which are a representation of the genetic association and physical ordering of molecular markers on chromosomes (or linkage groups). Statistical associations between

alleles at polymorphic marker loci and the trait of interest may reveal the presence of a QTL in the region, and does not require knowledge of the underlying gene, its function or mode of action. Ideally, many polymorphic markers must be mapped to each linkage group at intervals of <5 cM in order to cover the entire genome. This allows the use of interval mapping to further refine the localization of the QTL, and eventually sequencing can be employed to identify potential candidate genes (Cheverud and Routman 1993; Tanksley 1993). Alternatively, in the event that only a sparse or low density map is available, point-analysis might be more appropriate, whereby each locus is tested independently for association with the trait. Frequently, this approach is used to identify regions of interest, or when markers are sparsely or unevenly distributed on linkage groups, while interval mapping is performed once the region of interest is better-characterized (Doerge et al. 1997). In contrast to the marker locus approaches, the candidate gene approach allows direct measurement of genotypic values, the interpretation of results in relation to function and may be more easily applied to natural populations (Cheverud and Routman 1993; Tanksley 1993).

The mapping effort is greatly dependent upon the types of markers used, such that studies of quantitative traits have only become possible with recent molecular advances. Although isozymes are inexpensive and represent functional products, they are the least effective marker type due to low levels of detectable polymorphism (Jarne and Lagoda 1996). RAPDs (randomly amplified polymorphic DNA), on the other hand, are more polymorphic, but are not codominant, and have poor repeatability. AFLPs (amplified fragment length polymorphisms) are time-efficient and amplify multiple loci simultaneously, but may exhibit dominant expression and uneven genomic distribution

patterns (Ferguson and Danzmann 1998; Young et al. 1998). Microsatellites, a class of VNTR (variable number of tandem repeat loci), are currently one of the most common marker type used for linkage mapping. Microsatellites are composed of repeat units between one and five base pairs in length, are highly polymorphic, and exhibit codominant inheritance. Further, they are widely distributed in most eukaryotic genomes and are relatively easy to visualize and amplify using PCR. These advantages outweigh the high development costs and reported scoring difficulties (O'Reilly and Wright 1995; Jarne and Lagoda 1996; Ferguson and Danzmann 1998).

In Canada, commercially important aquaculture species include salmonid fishes like rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*). In order to improve breeding programmes, as well as to study evolutionary genetics, salmonid linkage maps have been constructed using allozymes (May and Johnson 1990), AFLPs (Young et al. 1998) and most recently, microsatellites (Sakamoto et al. 2000). QTL have already been detected in rainbow trout for temperature tolerance and spawning time using microsatellites, (Jackson et al. 1998; Danzmann et al. 1999; Sakamoto et al. 1999; Perry et al. 2001), and current work is focusing on growth and age at maturity (Martyniuk 2001; O'Malley, 2001). Difficulties in interpreting the genetic basis of these traits arise due to the autotetraploid ancestry of salmonids (Allendorf and Thorgaard 1984), which affects genome organization via peculiar chromosome pairing in males during meiosis. This results in the phenomenon of pseudolinkage, whereby ancestrally duplicated (homeologous) regions allow pairing of chromosomes in multivalent formations (Allendorf and Thorgaard 1984), in some cases involving metacentrics formed by centric fusion of non-homologous acrocentric

chromosomes (May et al. 1980; May et al. 1982; Wright et al. 1983). This model of meiosis explains the discrepancy between linkage maps constructed in male and female salmonids, in which the female map may represent more accurately the true distance between markers. The apparent compression of linkage groups in males reflects reduced recombination. Since tetravalent formations may allow the formation of chiasmata distally, increased recombination may occur near the telomeres (Johnson et al. 1987; Sakamoto et al. 2000). Similar sex-specific differences in recombination rate have been reported in other fish (Matsuda et al. 1999; Agresti et al. 2000), humans (Broman et al. 1998), cattle (Knott et al. 1998), and fowl (Groenen et al. 2000), with the heterospecific sex exhibiting a general reduction in recombination along tracts of the genome.

Mapping in related species provides an additional means of comparing genome organization and evolution, as well as QTL detection. Extensive efforts are under way in crosses of wild boar and commercial pigs to find wild type QTL and genes that might enhance production in domestic stocks (Knott et al. 1998). Among salmonids, crosses between rainbow trout and lake charr (*Salvelinus namaycush*), brook charr (*Salvelinus fontinalis*) and lake charr, and brook charr and Arctic charr were initially produced for comparative genome mapping using allozymes (May et al. 1980; Hollister et al. 1984; Johnson et al. 1987; May et al. 1989), suggesting genomic conservation of marker order among the Salmonidae. Currently, linkage maps are being constructed using microsatellites in rainbow trout, brown trout (*Salmo trutta*), Atlantic salmon and Arctic charr as part of an international mapping effort (Sakamoto et al. 2000; O' Malley 2001; R. Woram, pers. comm.; Gharbi, pers. comm., Danzmann pers. comm.).

Comparative mapping in rainbow trout, brown trout and Arctic charr has revealed extensive homology, based on conservation of marker linkages. Sakamoto et al. (2000) have identified twenty-nine linkage groups in rainbow trout, of which five confirm previous linkages based on allozymes (May and Johnson 1990). Although the Arctic charr linkage map is much less well developed, at least twenty linkage groups appear to be similar in both species (see figure 2-2 for several linkage groups). Six additional chromosomal segments have been identified in Arctic charr whose homology with known rainbow trout linkage groups is unclear (R. Woram, pers. comm.). These discrepancies may be the result of many factors, including the lack of polymorphism at cross-species amplifying markers, the limited number of markers designed in Arctic charr, and the paucity of markers used relative to salmonid genome size (e.g. genome coverage). Further, Arctic charr possess an ancestral genome structure, with a high proportion of acrocentric chromosomes (58 acrocentrics, Phillips and Ihssen 1985; 2n=78-82, Hartley 1991) relative to rainbow trout (2n=58-60) due to a greater number of Robertsonian fusions in the latter. As a result, not only do we expect to detect a greater number of linkage groups in Arctic charr, but markers may also be more difficult to localise on small acrocentric chromosomes (statistical probability of detected linkage). Great inter- and intra-specific karyotypic variation, with a relatively constant number of chromosome arms (Allendorf and Thorgaard 1984; Phillips and Ihssen 1985; Hartley 1991), suggests that different evolutionary mechanisms may have acted on the genomes of salmonid species since their divergence from a common ancestor 100-25 million years ago (Allendorf and Thorgaard 1984).

Arctic charr are perhaps the most highly physiologically and morphologically polymorphic, and the least well understood, of all salmonid species. Like brook charr, it is a coldwater salmonid, and has the most northern distribution of any freshwater fish, occurring in boreal circumpolar regions (Scott and Crossman 1985). Current distributions most likely reflect a combination of strict thermal preferenda and local adaptation, historical glacial processes and colonisation potential (Hocutt and Wiley 1986). In North America, three mitochondrial lineages have been characterised, including Laurentian, Arctic and Labrador stocks (Wilson et al. 1996). These strains differ in many physiological and morphological characters, of which fitness traits, such as growth rate, fecundity, spawning time and maturation age may be most important (Dempson and Green 1984; Gyselman 1994; Beddow et al. 1998). Dramatic inter- and intrapopulation variability have also been documented, in particular in the choice of life history strategy adopted (residency or anadromy). Such plasticity is not only temporally and spatially variable, but may also lead to sympatric speciation when tradeoffs between alternative strategies exist (Skúlason et al. 1996; Gíslason et al. 1999).

Due to genomic conservation in Arctic charr and rainbow trout, QTL mapping in Arctic charr should be a feasible approach to the examination of genome evolution in polyploid-derived salmonid species. In addition, the existence of aquaculture strains derived from populations diverging in multiple evolutionarily important characters (Krieger 1987; Tabachek 1991) provides suitable genetic material for QTL studies in a species for which experimental selected lines have not been developed. The identification of functional chromosomal regions for various traits in rainbow trout, including upper temperature tolerance and growth related traits (Jackson et al. 1998; Danzmann et al.

1999; Perry et al., 2001; O'Malley, 2001; Martyniuk, 2001) provides a good basis for searching for QTL in Arctic charr.

This thesis aims to determine whether or not functional chromosomal regions (QTL) are conserved in salmonids despite genome reorganizations. Chapter 2 outlines a search for QTL for upper temperature tolerance in Arctic charr using a comparative mapping approach. In Chapter 3, QTL for several measures of life history including growth-related (body size and condition factor) and reproduction parameters (gonadosomatic index) are presented, and correlations among fitness characters are discussed. Finally, chapter 4 summarizes the evolutionary implications of this work.

**Strain History and Arctic Charr Crosses**

The Arctic charr (*Salvelinus alpinus*) aquaculture industry is relatively new in Canada, but Canadian producers are fast becoming the lead exporters to Europe and Asia of market-sized fish. Two strains of Arctic charr are currently in production, including Labrador and Nauyuk strains, as well as a third strain (Tree River) which may be composed of Arctic charr and closely related Dolly Varden (*Salvelinus malma*). The Labrador strain is derived from three collections of anadromous fish made in the Fraser River, Labrador in 1980, 1981 and 1984. The 1984 stock was first certified (disease free and appropriate for distribution) at the Rockwood Aquaculture Research Centre in Winnipeg and distributed to producers across Canada, and is probably derived from at most 12 families. In 1993, fish from the 1980 and 1981 collections were certified, and distributed mainly in Manitoba and by special permit in Ontario. At most 19 families, derived from 29 individuals in 1981, and 40 parents of which at least 6 families were certified in the 1980 collection, were included in the latter certified stocks, although fish from fewer families were typically sent to producers (deMarch and Baker 1990; deMarch 1992). The Nauyuk strain (Northwest Territories), on the other hand, is derived from at most four families, produced by crossing anadromous and resident populations of fish (at most 17 families initially) collected from Nauyuk Lake in 1978 (Papst and Hopky 1984). Arctic charr from Nauyuk Lake typically exhibit lower fecundity, higher early growth rate, later age at maturity and later spawning date than Fraser River fish (Dempson and Green 1984; Gyselman 1994; deMarch 1997 and others). Furthermore, Labrador strain fish tend to breed over longer periods than Nauyuk fish (M. Burke, pers. comm.). These population differences appear to translate to differences in aquaculture strains, which

exhibit variation in hatchability, survival to the eyed stage, and growth (Krieger 1987; de March 1992; de March 1997). All strains now exhibit low fertilisation and viability, and it has been difficult to maintain genetic variability due to breeding constraints (deMarch and Baker 1990).

Arctic charr gametes were collected from broodstock in spawning condition on October 22$^{nd}$ and 27$^{th}$ 1998 at Coldwater Hatcheries, (Coldwater, Ontario). Eggs and milt from each individual were transported on ice to the Hagen Aqualab facilities (University of Guelph) where fertilisation was achieved through mixing of gametes in tank water. Crosses were produced by mating charr derived from Fraser River, Nauyuk Lake and F1 hybrids between the two strains, yielding F1, F2, backcross and "pure" strain families. Incubation of embryos took place at 4°C until exogenous feeding was achieved, at which time progeny were transferred to raceways (approx. 1x3 meters). The water source originated from an aquifer (underground spring), whose temperature fluctuated between 10°C and 12°C. Twelve families (Table 1-1) were tagged with fluorescent elastomer dyes and pooled 10 months post-fertilisation. Of these twelve families, only five were used for this study as a result of random mortality of several entire families. All rearing practices followed University of Guelph Aqualab standard operating procedures for holding salmonid fish (unpublished 1997).

Table 1.1. Background information on twelve surviving Arctic charr families produced in October 1998. 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 (F1) were used to produce backcross, F1 and pure strain families. Five families were used to search for QTL (Chapters 2 and 3) and are indicated by a *. N indicates the number of progeny included in each family that was used, based on pedigree analysis. Note that 12-114 and 21-114 were selectively genotyped (15-25% of the least and most temperature tolerant fish, see chapter 2).

| Cross | N | Dam | Sire | Family |
|---|---|---|---|---|
| 12-111* | 44 | Fraser | F1 | Backcross |
| 12-114* | 37 | Fraser | Nauyuk | F1 |
| 21-111 | | Fraser | F1 | Backcross |
| 21-114* | 32 | Fraser | Nauyuk | F1 |
| 27-126 | | Nauyuk | Nauyuk | Nauyuk |
| 27-139* | 56 | Nauyuk | Fraser | F1 |
| 29-136 | | Nauyuk | Fraser | F1 |
| 30-136* | 42 | Nauyuk | Fraser | F1 |
| 32-135 | | Nauyuk | Fraser | F1 |
| 32-137 | | Nauyuk | Fraser | F1 |
| 5-110 | | Fraser | F1 | Backcross |
| 5-115 | | Fraser | F1 | Backcross |

# CHAPTER 2:

# CONSERVATION OF QTL FOR UPPER TEMPERATURE TOLERANCE IN ARCTIC CHARR, *SALVELINUS ALPINUS*

## INTRODUCTION

Temperature is one of the most critical features of the environment affecting fish biology (Crawshaw and O'Connor 1997). Deviations beyond the optimal range of thermal tolerance may affect aspects of fitness, as temperature becomes a physiological stressor. Among cool and coldwater species like salmonid fishes, high temperatures are an important limiting factor in species distributions; heat stress may prevent the survival of marginal populations lacking the genetic variability to adapt (Jenkins et al. 1997). The ranges of thermotolerance differ markedly among species, with Arctic charr (*Salvelinus alpinus*) being the least resistant to high temperature and the most resistant to low temperatures at all life stages (Rombough 1997). The ultimate upper lethal temperature for four races (phenotypically differentiated stocks) of *S. alpinus* parr (0+ and 1+) has been shown to increase with acclimation temperature, and was estimated to be approximately 25°C (Baroudy and Elliott 1994). In contrast, thermal maxima for Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) parr were assessed at 32°C and 30°C, respectively (Elliott and Elliott 1995). These levels of upper temperature tolerance correspond to the geographic distributions and thermal history of salmonid species, and are modulated by recent thermal conditions (acclimation temperature).

Local thermal conditions may also affect species distributions at the population level. Studies of systems exhibiting thermal stratification and inhabited by two species of salmonid fish indicate that species tend to segregate according to their thermal preferenda, and that they behaviourally regulate when temperatures become too high (Matthews et al. 1993; Nielsen and Lisle 1993; Bonneau and Scarnecchia 1996). In Fjellfrøsvatn, Norway, variation in temperature tolerance between dwarf and normal

morphs of Arctic charr has further been implicated in the speciation process through its control on developmental rates (Klemetsen et al. 1997).

The direct negative effects of increased temperature on fitness components in salmonids, including reproduction, growth and development, are thought to result from alteration of secretion and action of reproductive hormones (reviewed in Van der Kraak and Pankhurst 1997). In particular, maintenance of broodstock at elevated temperatures has been shown to negatively affect gonadal growth, ovulation and spermiation, gamete viability and embryonic survival (Pankhurst et al. 1996). Jobling et al. (1995) hypothesized that temperature-dependent inhibition of ovulation in Arctic charr was the result of changes in phospholipid biosynthesis and fatty acid incorporation, crucial to the proper development of embryos. Growth rate, on the other hand, may be impeded due to increased metabolic costs at high temperatures. Further, growth rate compensation at high latitudes may reflect countergradient variation, in which the fastest growing genotypes are selected in environments with the most negative effect on growth (Jobling 1997). Thus, differential selection intensities in response to temperature in populations along a latitudinal cline may result in local adaptation or extinction (McCarthy and Houlihan 1997).

The importance of temperature as a stressor, and of individuals' ability to cope with it, has led to extensive study of the molecular basis of upper temperature tolerance. In response to heat shock, organisms as diverse as plants, bacteria, yeast, sea urchins and various vertebrates have the ability to synthesize a group of proteins known as stress or heat shock proteins (*hsp*) (Dean and Atkinson 1985; Heikkila et al. 1986; Currie and Tufts 1997). Heat shock proteins possess both inducible forms that repair and protect

cells from stress damage, and constitutive forms that are involved in protein folding, assembly, aggregate removal and translocation and activation of other proteins (Dyer et al. 1991; Dietz 1994). *In vitro* studies have shown that synthesis patterns are tissue and species-specific for *Hsp 70* and *90* protein families (Dyer et al. 1991; Airaksinen et al. 1998), the threshold-induction temperature of which increases with acclimation temperature (Dietz and Somero 1993; Dietz 1994). This implies a direct correlation between heat shock protein synthesis in a stress challenge and thermotolerance (reviewed in Coleman et al. 1995). Further, biochemical diversity in *hsp* protein families among *Poeciliopsis* species not only suggests that selection may act in different thermal environments, but also that individual variation in *hsp* expression may affect fitness (White et al. 1994).

The polygenic basis of upper temperature tolerance has been demonstrated by the detection of quantitative trait loci (QTL) for upper temperature tolerance in rainbow trout (Jackson et al. 1998; Danzmann et al. 1999; Perry et al. 2001). In particular, QTL have been detected on at least ten linkage groups, including 18, B, D, E, Fii, G, H, Oi, N and S. Furthermore, several chromosomal regions were shown to explain a significant portion of the variance in the response; loci Omy325UoG (linkage group B) and Ssa14DU (D) contribute approximately 22% of the variation in thermal tolerance in three backcrosses of rainbow trout. In addition, locus Ssa20.19NUIG (S) was associated with a QTL with effects ranging from 9-16% of the phenotypic variance in both outbred and backcross families (Danzmann et al. 1999; Perry et al. 2001). These loci may exhibit unpredictable epistatic effects with the genomic background in which they are expressed (Danzmann et al. 1999). Perry et al. (2001; submitted) also suggest that the sex locus on linkage group

18 and several QTL on the autosomes interact to influence thermal tolerance in outbred rainbow trout. These studies illustrate the complex nature of the genetic architecture underlying upper temperature tolerance.

Measurement of upper temperature tolerance (UTT) in the laboratory has employed several methodologies because of the complexity of the response. The ultimate lethal temperature has been defined as the highest temperature to which fish can acclimate. For practical purposes, the incipient lethal temperature is frequently used in studies of fish temperature tolerance, and represents the temperature at which all individuals will eventually die (Fry 1971), given a particular stage of development and acclimation temperature (reviewed in Lyytikäinen et al. 1997). Loss of equilibrium corresponds to the endpoint of the critical thermal maximum (Ctmax) and is considered to be an ecologically valuable method for studying thermal resistance (Diaz and Buckle 1999). This point can be referred to as "ecological death" since the individual's physiology has been so disrupted that continued exposure to the temperature would eventually lead to death. The aforementioned methods are employed in order to compare the thermal tolerances of populations or species. However, when undertaking studies for QTL analysis, it is necessary, or often desirable, to know individual thermal tolerances such that families may be characterised. In this case, the time to death (or loss of equilibrium) of each individual at the temperature coinciding with the populations' UTT is recorded, giving a normal distribution of thermal tolerances. This is the " individual knockdown time" used in some *Drosophila* studies (Berrigan et al.. 2000), and has the advantage that it allows for the comparison of resistance to multiple kinds of stress. "Knockdown temperature" has been similarly used, whereby thermal stress is administered as a

constant rate of temperature increase, and may be more useful when trying to mimic conditions in nature (Berrigan et al. 2000). An analogous measure in fish is the "Effective Time" in the zone of thermal tolerance, and is calculated relative to the incipient lethal temperature (Fry 1971). Much debate surrounds the issue of whether these measures of temperature tolerance produce equivalent results, or in fact they represent different traits. Recent work in *Drosophila* species suggests that results of comparison of UTT between species pairs selected in different thermal environments are highly correlated, and that LT50, individual knockdown time and knockdown temperature are in fact the same traits (Berrigan et al. 2000). This implies that QTL studies employing different measures of temperature tolerance in different species may be comparable.

A comparative approach to QTL detection may be feasible in salmonid fishes due to the general cross-amplification of microsatellite loci across even the most distantly related taxa (Morris et al. 1996; Condrey and Bentzen 1998; Olsen et al. 1998). Linkage mapping in rainbow trout (Sakamoto et al. 2000), Arctic charr (R. Woram, pers. comm.), and other salmonid species (K. Gharbi, pers. comm.; Danzmann, pers. comm.) suggests that chromosomal blocks are generally conserved in salmonid genomes despite extensive translocation events. Thus, it should be possible to determine if QTL for UTT in rainbow trout are conserved in Arctic charr as a result of ancestral climatic adaptation. Further, local climatic adaptation might permit identification of unique segregating genes influencing thermal tolerance in Arctic charr. Alternatively, the detection of additional loci might highlight chromosomal regions in which to focus future efforts in salmonid species.

This chapter examines the conservation of QTL for UTT in Arctic charr by targeting markers from chromosomal regions linked to UTT in rainbow trout (Jackson et al. 1998; Danzmann et al. 1999; Perry et al. 2001; Perry et al. submitted). When particular loci were invariant in Arctic charr, loci in close proximity were used to evaluate QTL effects. Loci from additional linkage groups were also surveyed for a better representation of the genome. Thus, markers from at least sixteen chromosomal arms were tested, based on linkage groups characterised in rainbow trout (Sakamoto et al. 2000) and Arctic charr (R. Woram, pers. comm.).

## MATERIALS AND METHODS

### 1. Strain and family history

The aquaculture strains used in this study were derived from the Nauyuk Lake, Northwest Territories and Fraser River, Labrador populations approximately four generations ago. These populations are not only separated by large geographic distances, but they are also characterised by differences in life history and thermal selection regimes. Furthermore, Nauyuk Lake and Fraser River fish possess distinct mitochondrial genomes, and belong to the Arctic and Labrador lineages of Arctic charr, respectively (Wilson et al. 1996). The detection of QTL for various traits may be facilitated by the use of families derived from such differentiated lineages since they may contain different alleles. Thus, families were produced by crossing fish from Coldwater Hatchery in all possible combinations, yielding pure strain, interstrain (F1) hybrids and backcrosses (pure X F1). Low fertilisation success and systems failure (cut-off of aeration for entire

families) at the rearing facility (Hagen Aqualab, University of Guelph) resulted in the survival of twelve families out of sixty-four at the time of the experiments (Table 1-1).

2. Upper Temperature Tolerance Trials

Progeny from all families (Table 1.1) were pooled and subjected to upper temperature tolerance trials 13 months post fertilisation. Trials were conducted within a single week beginning at 17:00 in order to minimise 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. A pilot trial (Lot I) indicated that these particular charr possessed a higher incipient lethal temperature than that suggested by the literature (22.5°C, 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°C) over a period of 60 minutes, and then kept constant for 30 minutes. Subsequently, the temperature was raised by 0.5 °C every 30 min until the end of the trial, resulting in a stepped profile (Figure 2.1). Air-stones were inserted into the tank in order to aerate and evenly distribute the heating water. Fish were considered to have experienced "ecological death" when they lost equilibrium and could not right themselves; at this point they were euthanised with an overdose of clove oil (Keen et al. 1991), 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 and set to collect data every 10 seconds (BoxCar Pro 3.5). Three such

thermal challenges (Lots II, III and IV) were required to test all the fish. Body weight, fork length and gonad weight were recorded and muscle and branchial tissue were sampled. All tissues were frozen at –20 degrees C until genetic analyses could be undertaken.

3. 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 the Polymerase Chain Reaction (PCR; MJ Research Inc. PTC-100 Programmable Thermal Cycler) in order to detect polymorphisms within families. Loci were chosen based on previous knowledge of polymorphism in other Arctic charr families, known association with or close proximity to QTL in rainbow trout, and 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. When these loci were monomorphic (a single band in all fish) or did not amplify (no scoreable product), the next closest alternative was screened, based on its proximity on the linkage group. In Arctic charr, linkage groups are designated as 1 or 2 (i.e. S1 and S2) when markers on two segments are homologous to those on a single linkage group in rainbow trout, but loci on each segment are not linked. Loci on additional linkage groups were randomly chosen.

The following PCR programme, with slight locus-specific modifications, was used: an initial denaturation cycle of 5 min at 95°C, followed by 35 cycles of 1 min at the locus-specific temperature, 1 min at 72°C, 1 min at 95°C and a final extension time of 10-20 min at 72°C. All loci used in this study, annealing temperatures and known repeat

sequences are presented in Table 2-1. Alleles were separated in a 6% polyacrylamide denaturing gel and visualised with a fluorescence imaging system (Hitachi FMBIOII). Fragment size was estimated by adding 2 µl of GeneScan 350 [Tamra] size standard (PE Applied Biosystems) to each of several lanes of the gel.

4. Statistical analysis

a) Detection of QTL for upper temperature tolerance (UTT)

PROBMAX (Danzmann 1997) was used to confirm the familial identity of the progeny through pedigree analysis. This was necessary because a system failure resulted in mixing of family lots, and differential mortality of individuals comprising the twelve initial families was observed (see introduction and Table 1-1). As a result, five families were used for the QTL analysis, and were comprised of one backcross (12-111) and four F1 hybrids (12-114, 21-114, 30-136, and 27-139). 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 small samples sizes (Table 2-2). In addition, the Pearson Product-Moment correlation was used to determine whether or not fork length or body weight was associated with upper temperature tolerance in each family

As a result of 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°C acclimation temperature at time of death (=Area) and 'knockdown' temperature (=Temp) were not significantly different across lots. 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 were tested for the expected 1:1 segregation of alleles using the Chi-Square goodness of fit test statistic (Table 2-3). Sequential Bonferroni correction for multiple tests was used to ensure an experimentwise error rate of $p<0.05$ within each family (Rice 1987).

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. In order to account for the change in slope midway through the thermal challenge, a censoring variable was included whereby the first 50% of fish to have died were considered "uncensored" (exponent value=1), and the last 50% "censored" (exponent value=0, see below and appendix for an example). 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. Thus the survival analysis performed in this study compares the following survival function of each allele class at a single locus:

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

In this equation, S (t) is the survival function, n is the total number of cases, $\prod$ 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 $j^{th}$ case in uncensored, or 0 if it is censored. This estimate of the survival function is termed the *product limit estimator* (Statistica, 1995; Kleinbaum 1996).

This equation effectively compares the proportion of censored and uncensored individuals in each allele class; the p-value is derived using Cox's F test, which is appropriate for small sample sizes. Covariates cannot be included in non-parametric approaches, but this was not a concern here because, 1) censoring may alter the relationship between the covariate and the response (because all individuals that died after the midpoint are considered to be equally temperature tolerant), and 2) body weight and the locus are not necessarily independent, potentially compromising the results by the inclusion of the covariate.

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).

Since nonparametric survival analysis does not allow estimation of the magnitude of the allele effect, loci with marginal or significant associations using survival analysis were tested ***post factum*** using a general linear model of the form

$$\gamma_i = \mu + \alpha_i + \varepsilon_i$$

where $\gamma_i$ is the UTT response for individual i, $\mu$ is the mean, $\alpha_i$ is the effect of the allele inherited from the sire or the dam, and $\varepsilon_i$ is the error term. Body weight was included as a covariate when necessary in order to parallel previous studies (families 12-111 and 21-114). The important factors in the model were selected by backward elimination. Outliers greater than $\pm 2\ \sigma$ were removed if they improved the model fit. Then, the allelic effect was estimated by determining the $R^2$ for the model at each locus (estimated as genotype sums of squares/total sums of squares). This was appropriate since the experimental designs used in this study were simple (full sib families), and backward elimination was used to choose the significant parameters for the reduced model (locus effect, including body weight as a covariate when necessary). In the case of genotypic classes, all were regressed together first, and subsequently allele classes were compared. This parallels simple linear regression methods by which the phenotypic effects of candidate genes have been estimated (Haley and Knott 1992; Gerbens et al. 1999). Regression analysis was performed using Statistic software (1995).

Sequential Bonferroni was applied to the number of linkage groups tested (k=16), yielding a threshold for significance of $p<0.003$ (experimentwise $\alpha=0.05$).

## RESULTS

A single locus deviated significantly from Mendelian proportions (Table 2-3). Segregation distortion at Ssa3NUIG in family 12-111 could be explained by the presence of a null allele in dam 12 (non-amplifying allele due to primer site sequence divergence), with reduced viability of the null/146 genotype. However, there was no evidence of a null allele segregating at this locus in 12-114, or any other family. 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 2-4). Significant associations between marker alleles and UTT were detected for SsaBHMS491 (linkage group G) in the dam of 27-139 and SsaF43NUIG (S2) in the sire of 12-111.

There may be a heterozygote advantage at OmyTRCarr (Table 2-4). Heterozygotes (120/126) showed greater survival relative to the 120/120 homozygote in half-sib family 12-114. Heterozygous individuals 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).

The variance explained by the models for loci showing the most significant associations with UTT (based on survival analysis) are 11% for SsaBHMS491 and 9% for SsaF43NUIG. In general, loci accounted for 4% to 35% of the phenotypic variance with the inclusion of marginal associations. Comparison of heterozygotes (12) and

homozygotes (11) at TRCarr in 12-114 yielded an $R^2$ of 61%, which was probably inflated by the unbalanced numbers of the genotypic classes (Table 2-4).

## DISCUSSION

Comparative mapping of markers on two homologous chromosomal regions in both rainbow trout suggests that QTL for UTT have been conserved in the two species. QTL have been identified on linkage groups G and S in both species. However, it is difficult to determine the exact location of the QTL on linkage group S in Arctic charr given that the linkage group is composed of two unlinked segments that are linked in rainbow trout. The strongest association with thermal tolerance was detected with loci on segment S2 in Arctic charr, whereas the chromosomal block affecting UTT in rainbow trout (Perry et al. 2001) is homologous to S1 in Arctic charr (see figure 2-2). Testing of additional loci on this linkage group in rainbow trout will be required in order to more accurately pinpoint the location of the QTL. It is possible that this linkage group is composed of two fused segments in rainbow trout.

Loci from a number of other linkage groups showed marginal associations with UTT in this study (D, E, F, H, N, and Oi) (Table 2-4). Multiple markers on Oi showed marginal associations. These results, combined with the knowledge of previously reported associations for homologous regions in rainbow trout (Figure 2.2; Jackson et al. 1998; Danzmann et al. 1999), suggests the detection of similar QTL in Arctic charr. In particular, variation at Ssa14DU on linkage group D is associated with temperature tolerance in both species. The marginal nature of these associations may reflect the

distance between the marker and the QTL (higher recombination) rather than lack of a QTL per se.

The existence of a QTL for UTT with moderate effect (~10%) on linkage group B in rainbow trout (Jackson et al. 1998; Perry et al. 2001) is not supported by the Arctic charr data. However, the analysis of linkage group B was based on a different marker (Omy301UoG) than that used in rainbow trout (Omy325UoG). Unfortunately, Omy325UoG could not be resolved in this analysis. Thus, the distance between these markers may preclude QTL detection with Omy301UoG if the QTL is located near Omy325UoG (O'Malley 2001; Woram, pers. comm). All other associations detected in this study were found at markers that are in relatively close proximity to those associated with UTT in rainbow trout (Figure 2.2).

The ability to detect associations between UTT and variation at marker loci may be effected by the sex of the segregating parent. Reduced recombination in males results in inheritance of entire chromosome segments (Sakamoto et al. 2000), leading to amplification of the QTL signal in these regions, but a decreased ability to localise the QTL. In contrast, marker-trait associations are less likely to be detected in females in a random genome survey 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. For instance, since a weak UTT QTL was detected on D with segregating sire alleles, it is likely that the QTL is in fact some distance from that region (Table 2-4; Figure 2-2). Similarly, it is not possible to infer QTL location from genotypic data, such that the true location of the QTL on linkage group F remains unknown. In contrast, the QTL on linkage group G may be in close

proximity to SsaBHMS491 based on data from the dam. Fine scale mapping and QTL studies in additional families will be required in order to resolve QTL location more accurately.

The marginal association of UTT with linkage group C/L in Arctic charr deserves further attention. Ozaki et al. (2000) found two QTL for IPN disease resistance on chromosomes A and C, which may be homeologous (ancestrally duplicated) in Arctic charr (as inferred by duplicated markers). This suggests a (partial) common underlying genetic basis for resistance to multiple forms of stress. Since Ozaki et al. (2000) do not report having taken into account the physical condition (health, condition factor, body size) of their fish, the QTL for disease resistance that they detected on linkage group A may in fact be associated with body size. This is suggested by the detection of QTL for fork length in Arctic charr on linkage group A (chapter 3). This may relate to the significant correlation between temperature tolerance and body size variables in certain families (Table 3-5).

This study suggests that a major sex-linked marker is associated with temperature tolerance in both rainbow trout and Arctic charr, despite genome reorganisation. On linkage group 15, SSOSL32/i shows a highly suggestive association ($p = 0.009$) with temperature tolerance in Arctic charr (Table 2-4), and is also associated with phenotypic sex (R. Woram, pers. comm.). In rainbow trout, the sex locus is on linkage group 18, and is closely associated with UTT marker OmyFGT19TUF (Sakamoto et al. 2000; Perry et al. 2001). Klein (2000) suggests that the severity of the stress response may be sex-dependent, possibly due to the effects of sex steroids on the immune system. Males may exhibit reduced disease resistance and stress tolerance due to the immunosuppressive

effects of androgens. This may be particularly severe in polygynous species (like salmonids) for which males rely on high testosterone levels for development of secondary sexual characteristics. Although the fish in this study were not sexually mature, it is interesting to note that the 94 allele was associated with improved temperature tolerance at SSOSL32/i (Table 2-4), and appears to be tightly linked to male phenotypic sex in Arctic charr (Somorjai, unpublished).

Marginal associations of both SSOSL32/i and OmyRGT39TUF with UTT in Arctic charr suggests that ancestrally duplicated (homeologous) chromosomal segments have maintained similar functions despite extensive translocation events. The existence of homeologous regions within a species may be inferred by the presence of duplicated markers (Sakamoto et al. 2000). The mapping of SSOSL32/i on 15 and SSOSL32/ii on E suggests that these linkage groups are homeologous in Arctic charr (R. Woram, unpublished) (Figure 2-2). Further, OmyRGT39TUF is linked to SSOSL32/ii on E (Woram, pers. comm). Thus, QTL effects are detectable on both homeologues in this study. Functional conservation across homeologues has also been demonstrated for body size and spawning time QTL in rainbow trout (O'Malley 2001). Cronn et al. (1999) suggest that functional conservation of duplicated regions may not be uncommon in polyploid-derived taxa. Further, loss or divergence of gene function may relate to the length of the diploidisation process (Aparicio 2000). Since their divergence from a common tetraploid ancestor 100-25 MYA, salmonids exhibit as much as 50% loss of duplicate allozyme expression (Allendorf and Thorgaard 1984). It is therefore surprising that homeologous genes have maintained similar or overlapping expression patterns in Arctic charr.

The use of linear regression to estimate QTL effects in Arctic charr reveals interesting patterns. Across all families, the variance in UTT explained by the model was as low as 4%, and as high as 61% (Table 2-4). Significant QTL on G and S2 account for 9 and 11%, respectively, to the phenotypic variance in UTT, which represent moderate QTL effects (Bradshaw et al. 1998; Danzmann et al. 1999). Marginal associations detected in backcross 12-111 explained from 4 to 14% of the variance in the trait. A similar range of QTL effects was detected in F1 hybrid 27-139, although SSOSL32/i may contribute as much as 22% of the variation in UTT, which approximates a large QTL effect. If this delineates the range in Arctic charr, then more large effect QTL have been detected in this study relative to values reported for rainbow trout. In particular, QTL on S account for up to 35% of the variance in UTT in Arctic charr (One10ASC on segment S2), but only 9-16% in rainbow trout (Ssa20.19NUIG; Danzmann et al. 1999; Perry et al. 2001).

Despite the generalities discussed above, it is necessary to include a *caveat* when reporting the QTL effects found in this study. First, the degree of significance for marker-trait associations determined by survival analysis does not necessarily represent values determined by regression, or inferred QTL effect. Second, QTL detected using data from selectively genotyped progeny may exhibit biased effects (Darvasi and Soller 1992), so QTL effects estimated from families 12-114 and 21-114 may be somewhat inflated (for example 35% at One10ASC). Other large values occur when using genotypic data, especially when comparing two genotypic classes (Table 2-4). Furthermore, the use of full-sib families and intercross hybrids may incorporate dominance effects as well as additive effects (Lynch and Walsh 1998), which may inflate the phenotypic variance

explained by the QTL. The latter are particularly important factors when attempting to explain the strength of the effects at TRCarr in 12-114, which appears to exhibit overdominace. Finally, the removal of outliers, in combination with the small sample sizes of the families, could increase the $R^2$ due to improved model fit.

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 F1 hybrid parent of backcross 12-111 due to segregation of QTL alleles, under the assumption that pure strains were fixed for alternate alleles. This was inferred because these strains are descended from populations that were adapted to very different environmental regimes, in particular temperature. Nauyuk Lake fish, found in the Canadian sub-Arctic (68° N, Gyselman 1994), were expected to be much less temperature tolerant than Fraser River fish (56° N, Dempson and Green 1984), which belong to the Labrador lineage (Wilson et al. 1996). Some empirical evidence for this lies in the observation that optimal temperatures for Nauyuk Lake broodstock were up to 3 °C lower than for Labrador strain charr (Tabachek 1991). Fixation of QTL alleles in each of the parents (i.e. no segregation of alleles within parents) of interstrain hybrid 30-136 due to recent culture history may help to explain the detection of only a single, weak association in this family. In contrast, the detection of UTT QTL in the Nauyuk parent of the other F1 crosses may be due to the use of fish from anadromous and resident populations to produce the broodstock. These "Nauyuk" parents may therefore effectively represent F1 hybrids themselves.

Consistent effects of alleles at particular loci were observed in multiple families. In half-sibs 12-111 and 12-114, allele 166 at One10ASC is associated with low temperature

tolerance irrespective of parental sex, or the background in which it is expressed. This allele was deleterious when inherited from Fraser dam 12 or F1 male 111, and when expressed in Nauyuk sire 114 or Fraser dam 12 (Table 2-4). Similarly, at SsaBHMS491 allele 123 performs better than allele 125 in a Fraser background (male 139 or female 12) whether detected in a Nauyuk or an F1 hybrid fish. The cause of these effects must remain speculative since they may be confounded with sex or cross-type (F1 vs. B1) effects. A severe bottleneck in the Nauyuk strain may simply have reduced the genetic variation present relative to Fraser strain fish, thereby fixing the most beneficial alleles. However, preliminary data suggest that Nauyuk fish are as variable as Fraser fish in the hatchery, and that "temperature tolerance alleles" are present in both populations (Somorjai, unpublished).

Some support for epistatic interactions between allele and genomic background exists for One10ASC. Although the 166 allele tends to be associated with lowered thermal tolerance, it is interesting to note that this QTL effect was detected in dam 12 when crossed to a Nauyuk male, but not when crossed to an F1 hybrid. Similar phenomena have been observed in outbred rainbow trout for UTT QTL (Danzmann et al. 1999). Breakdown of favourable gene complexes may occur in successive hybridisation events in outbred genomes (Armbruster et al. 1999).

The genetic basis of UTT QTL is not presently known. Recent 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 the use of QTL studies to study the evolution of functional regions across species may be informative. At this time, the only heat shock cognate mapped in salmonids is localised to

linkage group Oi in rainbow trout (Sakamoto et al. 2000). QTL were identified in this region in four families of Arctic charr, as well as in rainbow trout (Danzmann et al. 1999). As discussed in the introduction, the expression of heat shock protein (*hsp*) families is directly related to thermotolerance, and thus *hsp* may be primary candidate genes for UTT QTL. A protein complex including several integral proteins and *hsp70* mediates the temperature-sensitive transport of proteins across the mitochondrial inner membrane (Rassow et al. 1999), providing a mechanism by which membrane fluidity may affect thermotolerance in eukaryotes. Furthermore, expression of *hsp*71 in trout erythrocytes following heat shock, but not anoxia, may indicate that the induction of this protein at least is stress-type-dependent (Airaksinen et al. 1998).

The fact that microsatellites with functional properties are frequently associated with transcription factors (reviewed in Liu et al. 1999) lends support to the idea that this UTT QTL may in fact be linked to the expression of heat shock elements (HSE). HSE mediate the induction and activation of heat shock proteins, via metal-sensitive heat shock factors (HSF). Although the metallothionein gene is not itself activated by heat shock, common metal ion-sensitive intermediates may affect transcriptional activation of HSF during thermal stress (Gedamu and Zafarullah 1993). In addition, it is possible that prolonged exposure to increased temperature would result in leaking of metal ions (like zinc) that are detrimental to cellular functioning, thereby activating metallothionein expression.

The metallothionein gene was recently mapped to linkage group 15 in Arctic charr (McGowan, pers. comm.; R. Woram pers. comm.; figure 2-2), and may represent another candidate gene implicated in stress tolerance. Its known functions include mobilisation of metal ions like zinc and copper, which are implicated in reproduction and early ovary

development in trout, as well as capture stress (Olsson 1993). These functions are supported by the localisation of the metallothionein gene near sex-locus marker SSOSL32/i, a putative UTT QTL. Therefore, this may provide indirect evidence for the role of sex-specific steroids in the stress response (Klein 2000).

This chapter illustrated the usefulness of comparative mapping for detection of chromosomal regions with conserved function across taxa. In addition, UTT QTL were detected in Arctic charr that highlight regions in which to focus future mapping and QTL detection efforts in other species, in particular rainbow trout. The effects of strain history on upper temperature tolerance are discussed, as are potential candidate genes and underlying mechanisms for thermotolerance.

Table 2-1. Microsatellite loci, comparative linkage groups and sequence data used in this study. Markers were mapped to linkage groups in rainbow trout ('b') and Arctic charr ('c') backcross families (Sakamoto et al. 2000; R. Woram pers. comm.). The locus name ('a') 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 ('e'): Ssa=Salmo salar; Str=S. trutta; Omy=*Oncorhynchus mykiss*; Ocl=*O. clarki*; One=*O. nerka*; Ots=*O. tshawytscha*; Sco=*Salvelinus confluentus*. 'd' indicates the annealing temperature in (°C) employed to amplify microsatellite loci * ': Otsd3/ii is linked to a different linkage group in each of two sires; ' - ' indicates that no data are currently available; 'dupl.'=single primer pair amplifies duplicated loci; one or both of which have not been mapped. Note that primers with the 'BHMS' designation have been given new designations ('NVH'; Hoyheim 2000).

| Locus name[a] | RT[b] | AC[c] | Repeat sequence | $T_A$(°C)[d] | Primer source[e] |
|---|---|---|---|---|---|
| Ssa77NUIG | - | 2 | - | 48 | National University of Ireland, R. Powell |
| SsaBHMS161 | - | 8 | VARIOUS GT | 56 | unpublished |
| Otsd3/iiNWFSC* | - | 15, 18L | $(GA)_{14}(CA)$ | 50 | unpublished |
| SSOSL32/I | E | 15 | - | 56 | Norwegian college of Veterinary Medicine, Slettan et al., 1997 |
| OmyFGT19/iTUF | 18 | - | $(GT)_{27}$ | 50 | Tokyo University of Fisheries, Sakamoto et al. 1996, Sakamoto 1997 |
| Ssa3NUIG | - | - | - | 50 | National University of Ireland, R. Powell |
| Ssa14DU | D | - | $(TC)_{10}(N)_{15}(TC)_3(N)_2$ $(AC)_{12}(TC)_3(N)_5(CA)_4$ | 48 | Dalhousie University, Morris et al. 1996 |
| OmyOGT5TUF | F, dupl. | - | - | 54 | Tokyo University of Fisheries, Sakamoto et al. 1996, Sakamoto 1997 |
| SsaBHMS206/ii | - | A | $(CA)_{42}CG(CA)_7$ | 50 | unpublished |
| Otsa5/iNWFSC | - | A | $(GA)_{22}$ | 48 | unpublished |
| Omy301UoG | B | B | $(GT)_{20}$ | 55 | University of Guelph; Jackson et al. 1998 |
| SsaBHMS206/i | - | C/L | $(CA)_{42}CG(CA)_7$ | 50 | unpublished |
| SsaBHMS330 | - | C/L | $(CA)_{25}$ | 57 | unpublished |
| OmyRGT39TUF | E | E | $(CA)_{12}$ | 54 | Tokyo University of Fisheries, Sakamoto et al. 1996, Sakamoto 1997 |
| Otsa5/iiNWFSC | - | Fii | $(GA)_{22}$ | 48 | unpublished |
| SsaBHMS219 | - | Fii | $(CA)_3AA(CA)_6CG$ $(CA)_4$ | 55 | unpublished |
| SsaBHMS437 | - | G | $(GT)_7GA(GT)_3$ | 57 | unpublished |
| SsaBHMS491 | - | G | $(CA)_4$ | 57 | unpublished |
| OmyPuPuPyINRA | G | G | - | 54 | Institut National de la Recherche Agronomique, K. Gharbi and R. Guyomard |
| Ssa85DU | G | G | $(GT)_{14}$ | 55 | Dalhousie University, Morris et al. 1996 |
| Sco19 | - | G2 | - | 50 | University of British Columbia; Taylor et al. 2001 |
| OmyRGT2/iiTUF | H | H | - | 58 | Tokyo University of Fisheries, Sakamoto et al. 1996, Sakamoto 1997 |
| Omy18INRA | J | J | - | 55 | Institut National de la Recherche Agronomique, K. Gharbi and R. Guyomard |

Table 2-1 continued

| Locus name[a] | RT[b] | AC[c] | Repeat sequence | $T_A$(°C)[d] | Primer source[e] |
|---|---|---|---|---|---|
| SsaBHMS411 | - | N | $(CA)_{52}$ | 52 | unpublished |
| OmyJTUF | N | N | - | 56 | Tokyo University of Fisheries, Sakamoto et al. 1996, Sakamoto 1997 |
| OmyRGT46TUF | N | N | $(CA)_{10}$ | 57 | Tokyo University of Fisheries, Sakamoto et al. 1996, Sakamoto 1997 |
| Omy38DU | H | H2 | - | 50 | Dalhousie University, Morris et al. 1996 |
| Sfo23LAV | - | - | - | 50 | Laval University, Angers et al. 1995 |
| OmyRGT30TUF | Oi | Oi | - | 52 | Tokyo University of Fisheries, Sakamoto et al. 1996, Sakamoto 1997 |
| OmyRGT4TUF | Oi | Oi | $(CA)_6TA(CA)_{13}$ | 56 | Tokyo University of Fisheries, Sakamoto et al. 1996, Sakamoto 1997 |
| OmyTRCarrINRA | Oi | Oi | - | 48 | Institut National de la Recherche Agronomique, K. Gharbi and R. Guyomard |
| Otsd3/iNWFSC | - | R | $(GA)_{14}(CA)$ | 48 | unpublished |
| Cocl3LAV | S | S2 | $(GT)_{35}$ | 50 | Laval University, L. Bernatchez |
| SsaBHMS121 | - | S | $(CA)_7$ | 53 | unpublished |
| SsaF43NUIG | - | S2 | $(CA)_{18}$ | 50 | National University of Ireland, R. Powell |
| One10ASC | H | S2 | $(CA)_{25}$ | 50 | Alaska Science Centre, Scribner et al. 1996 |
| Str7INRA | S, dupl. | S, dupl. | - | 53 | Institut National de la Recherche Agronomique, K. Gharbi and R. Guyomard |
| SSOSL456 | - | - | $(AC)_{12}AG(AC)_{10}$ | 60-50 | Norwegian college of Veterinary Medicine, Slettan et al., 1997 |

Table 2-2. Descriptive Statistics for five Arctic charr (*Salvelinus alpinus*) families. 'N' represents number of progeny in each family. Normality was tested using the Kolmogorov-Smirnov test statistic. Time = time until death during thermal trials (min); Area = cumulative temperature tolerance from 10°C acclimation (°min); Temp = temperature of death (°C); BW = body weight (g); FL= fork length (mm); see text for details.

| Family | N | Trait | Normality | Mean | Std. Dev. |
|---|---|---|---|---|---|
| 12-111 | 44 | Time | n.s. | 492.64 | 36.41 |
| | | Area | n.s. | 2135.03 | 531.92 |
| | | Temp | n.s. | 24.91 | 0.49 |
| | | BW | n.s. | 18.80 | 8.65 |
| | | FL | n.s. | 11.88 | 1.71 |
| | | K | n.s. | 1.04 | 0.016 |
| | | GW | n.s. | .013 | .015 |
| | | RI | p<0.01 | 0.106 | 0.095 |
| 12-114 | 37 | Time | n.s. | 491.70 | 23.25 |
| | | Area | p<0.1 | 2116.57 | 350.15 |
| | | Temp | p<0.15 | 24.84 | 0.44 |
| | | BW | n.s. | 19.13 | 6.74 |
| | | FL | n.s. | 12.12 | 1.56 |
| | | K | n.s. | 1.02 | 0.073 |
| 21-114 | 32 | Time | p<0.2 | 507.91 | 24.28 |
| | | Area | p<0.15 | 2364.55 | 367.7 |
| | | Temp | n.s. | 25.14 | 0.445 |
| | | BW | n.s. | 17.68 | 8.10 |
| | | FL | n.s. | 11.65 | 1.77 |
| | | K | n.s. | 1.04 | 0.061 |
| 27-139 | 56 | Time | n.s. | 533.14 | 16.75 |
| | | Area | p<0.2 | 2811.57 | 284.91 |
| | | Temp | n.s. | 25.60 | 0.345 |
| | | BW | n.s. | 20.35 | 4.94 |
| | | FL | n.s. | 12.37 | 1.08 |
| | | K | n.s. | 1.05 | 0.066 |
| | | GW | p<0.01 | 0.010 | 0.014 |
| | | RI | p<0.01 | 0.052 | 0.066 |
| 30-136 | 42 | Time | n.s. | 530.02 | 13.43 |
| | | Area | n.s. | 2749.11 | 211.80 |
| | | Temp | p<0.15 | 25.52 | 0.25 |
| | | BW | n.s. | 18.89 | 4.55 |
| | | FL | n.s. | 12.08 | 1.05 |
| | | K | n.s. | 1.05 | 0.072 |
| | | GW | p<0.01 | 0.015 | 0.036 |
| | | RI | p<0.01 | 0.047 | 0.095 |

Table 2-3. Segregation distortion in five families of Arctic charr (*Salvelinus alpinus*) using the Chi-Square goodness-of-fit test. 'Parent' indicates whether alleles were inherited from the sire (M) or the dam (F). 'N' is the number of alleles in each allele class. '$X^2_{adj}$' indicates the test statistic Values in bold signify that the test is significant at that locus at $p<0.05$ (after Bonferroni correction for multiple tests).

| Family | Locus | Parent | Allele | N | $X^2_{adj\ (0.05,\ 1)}$ |
|---|---|---|---|---|---|
| 12-111 | Ssa3NUIG | M | 246 | 7 | **9.82**** |
| | | | 250 | 26 | |
| | OtsD3/iNWFSC | M | 188 | 24 | 4.97 |
| | | | 192 | 10 | |
| 12-114 | OmyRGT46TUF | F | 145 | 12 | 4.48 |
| | | | 165 | 26 | |
| | Ssa14DU | F | 151 | 25 | 4.69 |
| | | | 155 | 11 | |
| 21-114 | SsaBHMS437 | M | 123 | 21 | 4.03 |
| | | | 125 | 9 | |
| 27-139 | Omy301UoG | M | 94 | 36 | 4.02 |
| | | | 108 | 20 | |
| 30-136 | OmyOGT5TUF | M | 164 | 29 | 5.36 |
| | | | 170 | 13 | |
| | Omy18INRA | M | 178 | 9 | 4.03 |
| | | | 180 | 21 | |
| | Sco19LAV | M | 212 | 13 | 5.03 |
| | | | 228 | 26 | |
| | SsaBHMS219 | M | 132 | 12 | 6.56 |
| | | | 148 | 27 | |

Table 2-4. Putative QTL for upper temperature tolerance (UTT) detected in five families of Arctic charr (*Salvelinus alpinus*) using survival analysis. Linkage groups on which the loci are found in Arctic charr and rainbow trout (*Oncorhynchus mykiss*) are denoted by superscripts 'a' and 'b', respectively (Sakamoto et al. 2000, R. Woram pers. comm.).'N' represents the number of alleles and '% 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 (bold=p<0.05). "*' indicates that UTT of genotypic classes (11 vs. 22 unless noted) was compared when both parents were heterozygous for the same alleles. '–' indicates the locus is unassigned in that species '**': loci passing Bonferroni threshold for 16 tests; '$R^2$':variance (in %) contributed by the model using linear regression ('Reg').

| AC[a] | RT[b] | Locus | Family | Parent | Allele | N | % cen | Cox's F | p value | Reg($R^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| 8 | - | SsaBHMS161 | 30-136 | M | 191 | 24 | 37.5 | 2.75 | 0.068 | n.s. |
| | | | | | 195 | 15 | 73.3 | | | |
| 15 | E | SSOSL32/i | 27-139 | M | 84 | 26 | 26.9 | 2.93 | **0.009** | **0.000**** |
| | | | | | 94 | 30 | 70.0 | | | (22%) |
| E | E | OmyRGT39TUF | 21-114 | F | 109 | 19 | 68.4 | 3.75 | **0.006** | **0.003**** |
| | | | | | 113 | 12 | 25.0 | | | (20%) |
| - | - | Ssa3NUIG | 12-111 | M | 246 | 6 | 33.3 | 2.53 | 0.054 | n.s. |
| | | | | | 250 | 25 | 68.0 | | | |
| - | D | Ssa14DU | 27-139 | M | 151 | 29 | 62.1 | 2.12 | **0.024** | **0.049** |
| | | | | | 155 | 27 | 37.0 | | | (5%) |
| C/L | - | SsaBHMS330 | 21-114 | F | 103 | 14 | 64.3 | 2.18 | 0.062 | **0.020** |
| | | | | | 107 | 11 | 38.9 | | | (13%) |
| - | F | OmyOGT5TUF* | 27-139 | N/A | 11 | 15 | 80.0 | 4.25 | **0.010** | **0.009** |
| | | | | | 22 | 13 | 38.5 | | | (22%) |
| Fii | - | SsaBHMS219* | 12-114 | N/A | 11 | 8 | 75.0 | 3.72 | **0.022** | **0.010** |
| | | | | | 22 | 14 | 35.7 | | | (27%) |
| G2 | - | Sco19LAV | 27-139 | F | 190 | 26 | 61.5 | 1.67 | 0.089 | **0.032** |
| | | | | | 228 | 30 | 40.0 | | | (7%) |
| G2 | - | Sco19LAV | 12-111 | F | 190 | 14 | 42.9 | 2.22 | 0.071 | n.s. |
| | | | | | 228 | 21 | 66.7 | | | |
| G2 | - | Sco19LAV | 21-114 | M | 174 | 18 | 61.1 | 2.28 | 0.058 | **0.047** |
| | | | | | 217 | 13 | 51.6 | | | (11%) |

Table 2-4, continued

| AC[a] | RT[b] | Locus | Family | Parent | Allele | N | % cen | Cox's F | p value | Reg($R^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| G | - | SsaBHMS491 | 27-139 | F | 123 | 31 | 67.7 | 2.83 | **0.003**** | **0.010** |
| | | | | | 125 | 25 | 28.0 | | | (11%) |
| G | - | SsaBHMS491 | 12-111 | M | 123 | 17 | 64.7 | 1.99 | 0.066 | **0.026** |
| | | | | | 125 | 25 | 44.0 | | | (8%) |
| G | - | SsaBHMS437 | 12-111 | M | 123 | 18 | 72.2 | 3.34 | **0.005** | **0.000**** |
| | | | | | 125 | 23 | 34.8 | | | (14%) |
| G | G | OmyPuPuPyINRA | 12-111 | M | 438 | 22 | 63.6 | 2.07 | **0.049** | **0.017** |
| | | | | | 460 | 21 | 38.1 | | | (10%) |
| G | G | Ssa85DU | 12-111 | M | 180 | 23 | 39.1 | 1.95 | 0.081 | 0.088 |
| | | | | | 240 | 21 | 61.9 | | | (5%) |
| H | H | OmyRGT2/iiTUF | 27-139 | M | 320 | 26 | 61.5 | 2.02 | **0.035** | 0.083 |
| | | | | | 360 | 29 | 41.4 | | | (4%) |
| H | H | OmyRGT2/iiTUF | 12-114 | F | 280 | 20 | 60.0 | 1.89 | 0.083 | 0.098 |
| | | | | | 320 | 18 | 38.9 | | | (9%) |
| N | N | OmyJTUF | 21-114 | M | 122 | 17 | 35.3 | 2.60 | 0.060 | **n.s.** |
| | | | | | 132 | 15 | 66.7 | | | |
| N | N | OmyRGT46TUF | 21-114 | F | 165 | 19 | 68.4 | 3.77 | **0.004** | **0.042** |
| | | | | | 171 | 13 | 23.1 | | | (11%) |
| Oi | Oi | OmyRGT30TUF | 12-111 | M | 84 | 23 | 39.1 | 2.02 | 0.071 | n.s. |
| | | | | | 88 | 20 | 60.0 | | | |
| Oi | Oi | OmyRGT4TUF | 27-139 | F | 139 | 21 | 33.3 | 2.35 | **0.014** | **0.013** |
| | | | | | 143 | 35 | 60.0 | | | (11%) |
| Oi | Oi | TRCarr* 11vs12 | 21-114 | N/A | 11 | 7 | 71.4 | 2.47 | 0.072 | n.s. |
| | | | | | 12 | 20 | 40.0 | | | |
| Oi | Oi | TRCarr*, 11vs12 | 12-114 | N/A | 11 | 9 | 11.1 | 6.72 | **0.005** | **0.000**** |
| | | | | | 12 | 17 | 76.5 | | | (61%) |
| Oi | Oi | TRCarr*, 22vs12 | 12-114 | N/A | 12 | 17 | 76.5 | 4.26 | **0.023** | **0.016** |
| | | | | | 22 | 11 | 36.4 | | | (24%) |

Table 2-4, continued 2

| AC[a] | RT[b] | Locus | Family | Parent | Allele | N | % cen | Cox's F | p value | Reg($R^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| S1 | S | Str7INRA | 12-114 | M | 323 | 14 | 64.3 | 1.93 | 0.088 | 0.059 |
| | | | | | 350 | 23 | 43.5 | | | (9%) |
| S1 | - | SsaBHMS121 | 12-111 | M | 122 | 14 | 64.3 | 1.87 | 0.094 | 0.080 |
| | | | | | 130 | 24 | 41.7 | | | (6%) |
| S2 | S | Cocl3LAV | 12-114 | F | 236 | 17 | 58.8 | 1.92 | 0.083 | **0.031** |
| | | | | | 278 | 18 | 38.9 | | | (12%) |
| S2 | - | SsaF43NUIG | 12-111 | M | 100 | 25 | 68.0 | 3.37 | **0.002**** | **0.024** |
| | | | | | 110 | 19 | 26.3 | | | (9%) |
| S2 | H | One10ASC | 12-111 | M | 160 | 22 | 68.2 | 3.01 | **0.007** | n.s. |
| | | | | | 166 | 19 | 31.6 | | | |
| S2 | H | One10ASC | 12-114 | F | 164 | 19 | 68.4 | 2.71 | **0.021** | **0.000**** |
| | | | | | 166 | 17 | 35.3 | | | (35%) |

Figure 2-1. Temperature profile representing the thermal challenge to which lot II Arctic charr (*Salvelinus alpinus*) were exposed. Water temperature (°C) and Time from 17:00 are indicated on the ordinate and abscissa, respectively. Fish were acclimated to 10°C prior to the trial. The ramping zone represents the 60-minute period during which temperature was increased to the theoretical incipient lethal temperature of 22°C for Arctic charr (Baroudy and Elliott 1994). Subsequently, temperature was increased every 30 minutes by 0.5°C 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 euthanised.

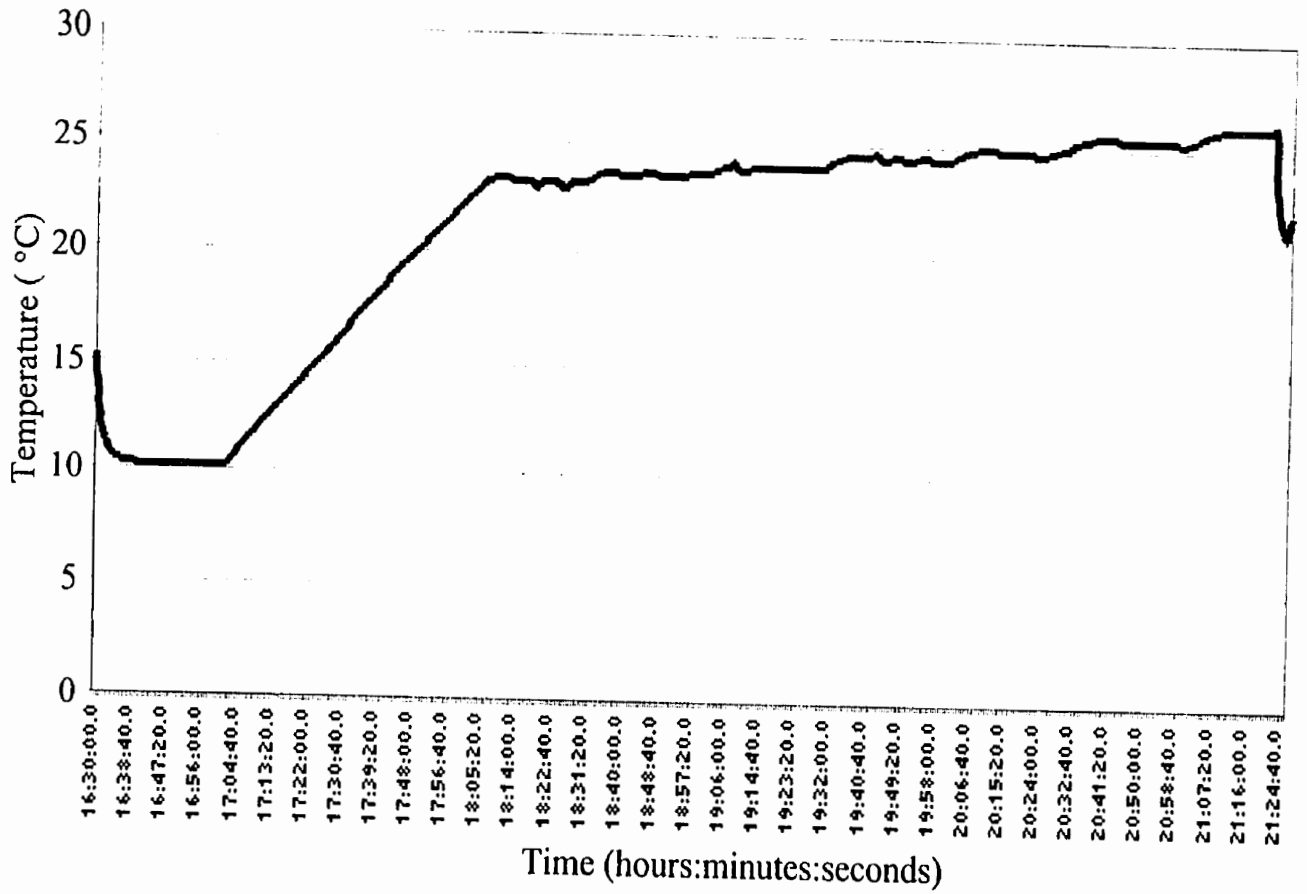
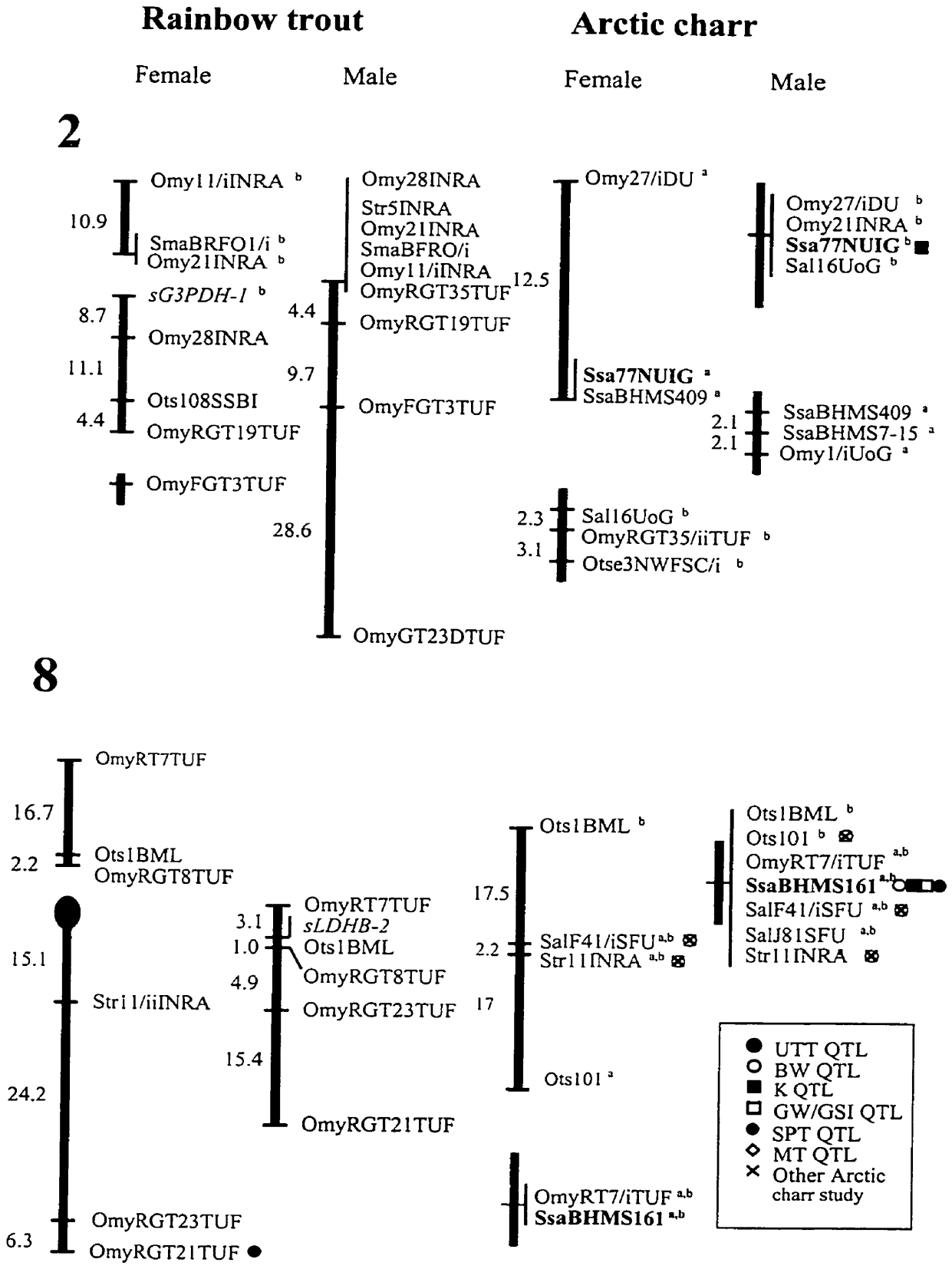
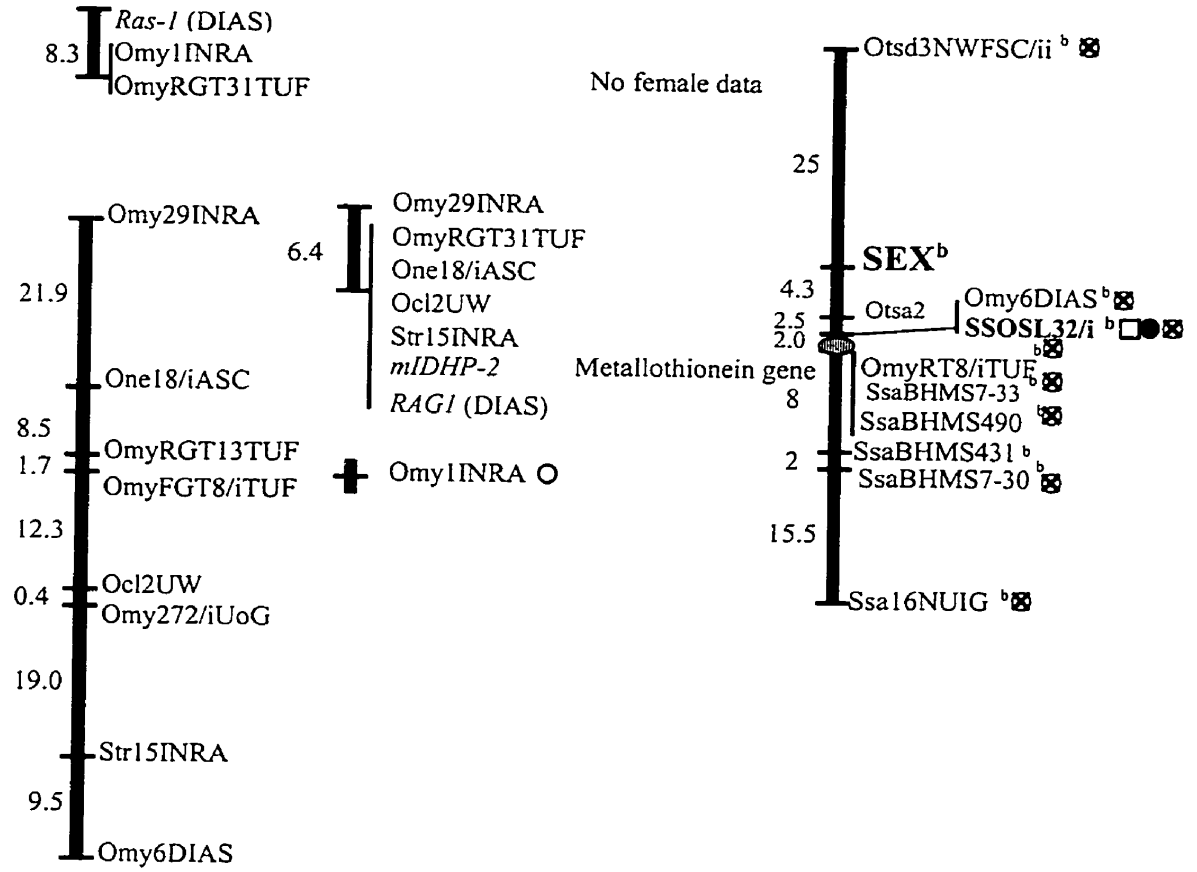

30
25
20
15
10
5
0
Temperature ( °C)
16:30:00.0
16:38:40.0
16:47:20.0
16:56:00.0
17:04:40.0
17:13:20.0
17:22:00.0
17:30:40.0
17:39:20.0
17:48:00.0
17:56:40.0
18:05:20.0
18:14:00.0
18:22:40.0
18:31:20.0
18:40:00.0
18:48:40.0
18:57:20.0
19:06:00.0
19:14:40.0
19:23:20.0
19:32:00.0
19:40:40.0
19:49:20.0
19:58:00.0
20:06:40.0
20:15:20.0
20:24:00.0
20:32:40.0
20:41:20.0
20:50:00.0
20:58:40.0
21:07:20.0
21:16:00.0
21:24:40.0
Time (hours:minutes:seconds)

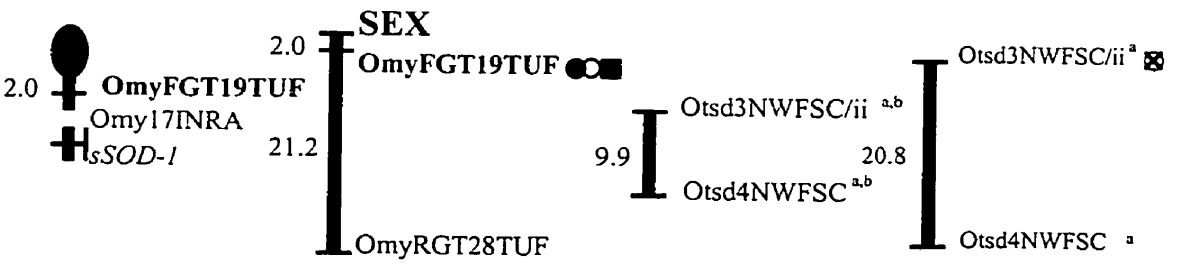
Figure 2-2. Comparative female (left) and male (right) linkage maps in rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*) produced primarily using microsatellite markers. Rainbow trout linkage groups are depicted on the left (taken from Sakamoto et al 2000), while homologous Arctic charr linkage groups are depicted on the right (R. Woram, pers. comm.) on the basis of conserved markers in both species. Numbered linkage groups were identified based on anchoring with allozyme markers, and correspond to Arabic numerals in May and Johnson (1990). Lowercase "i" and "ii" after the alphabetic linkage group designation indicate homeologous chromosomes (ancestrally duplicated). Similarly, duplicated loci are designated by /i or /ii after the locus name. Solid ovals represent centromeres localised in rainbow trout. Numbers to the left of linkage "stems" represent recombination-based distance measures (in centiMorgans) between markers. Abbreviations used in marker names are explained in Table 2-1. In Arctic charr, superscripts a and b indicate results from mapping in two separate backcross families. Markers in **bold** were amplified in this study. Putative quantitative trait loci (QTL) are also depicted beside the markers with which they are associated in each species (rainbow trout: Jackson et al 1998; Sakamoto et al 1999; Danzmann et al 1999; Martyniuk 2001; O'Malley 2001; Perry et al 2001; Arctic charr: R. Woram, pers. comm.; this study). Interactions between alleles derived from the sire and the dam are not shown. Legend: ■ = condition factor (K) QTL; ◘= gonad weight/GSI (GW/GSI) QTL; ◇ maturation time (MT) QTL; ◆ =spawning time (SPT) QTL; ●=upper temperature tolerance (UTT) QTL; ○ =body weight or fork length (BW/FL) QTL; ⊗=growth QTL identified in Arctic charr by R. Woram (body weight and specific growth rate). Unpublished marker-trait associations are not shown.

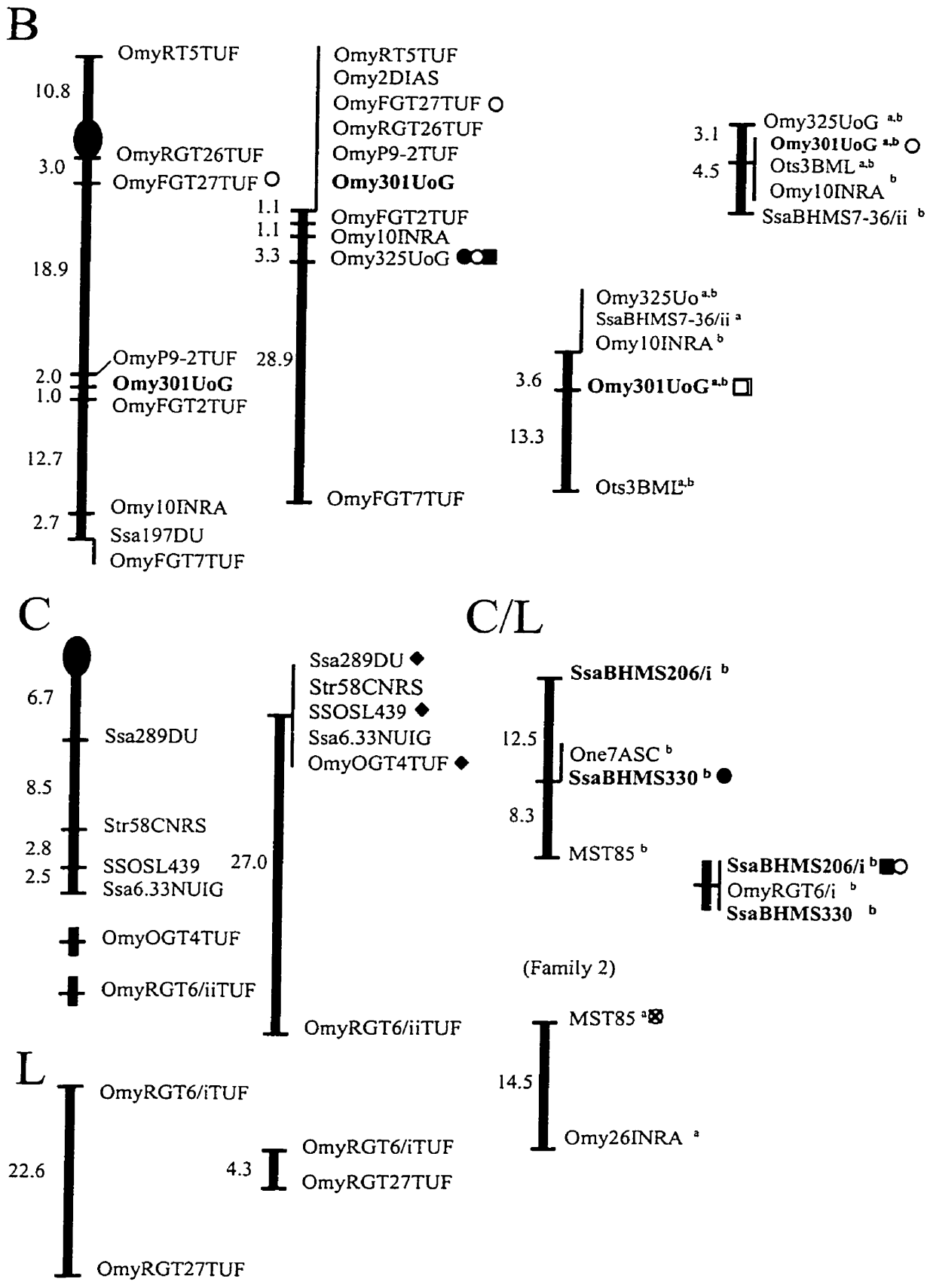

Rainbow trout
Arctic charr
Female
Male
Female
Male
2
Omy11/iINRA b
10.9
SmaBRFO1/i b
Omy21INRA b
sG3PDH-1 b
8.7
Omy28INRA
11.1
Ots108SSBI
4.4
OmyRGT19TUF
OmyFGT3TUF
Omy28INRA
Str5INRA
Omy21INRA
SmaBFRO/i
Omy11/iINRA
OmyRGT35TUF
4.4
OmyRGT19TUF
9.7
OmyFGT3TUF
28.6
OmyGT23DTUF
Omy27/iDU a
12.5
Ssa77NUIG a
SsaBHMS409 a
Omy27/iDU b
Omy21INRA b
Ssa77NUIG b
Sal16UoG b
SsaBHMS409 a
2.1
SsaBHMS7-15 a
2.1
Omy1/iUoG a
2.3
Sal16UoG b
OmyRGT35/iiTUF b
3.1
Otse3NWFSC/i b
8
OmyRT7TUF
16.7
2.2
Ots1BML
OmyRGT8TUF
15.1
Str11/iiINRA
24.2
6.3
OmyRGT23TUF
OmyRGT21TUF
OmyRT7TUF
3.1
sLDHB-2
1.0
Ots1BML
4.9
OmyRGT8TUF
OmyRGT23TUF
15.4
OmyRGT21TUF
Ots1BML b
17.5
SalF41/iSFU a,b
2.2
Str11INRA a,b
17
Ots101 a
OmyRT7/iTUF a,b
SsaBHMS161 a,b
Ots1BML b
Ots101 b
OmyRT7/iTUF a,b
SsaBHMS161 a,b
SalF41/iSFU a,b
SalJ81SFU a,b
Str11INRA
UTT QTL
BW QTL
K QTL
GW/GSI QTL
SPT QTL
MT QTL
Other Arctic charr study

# 15

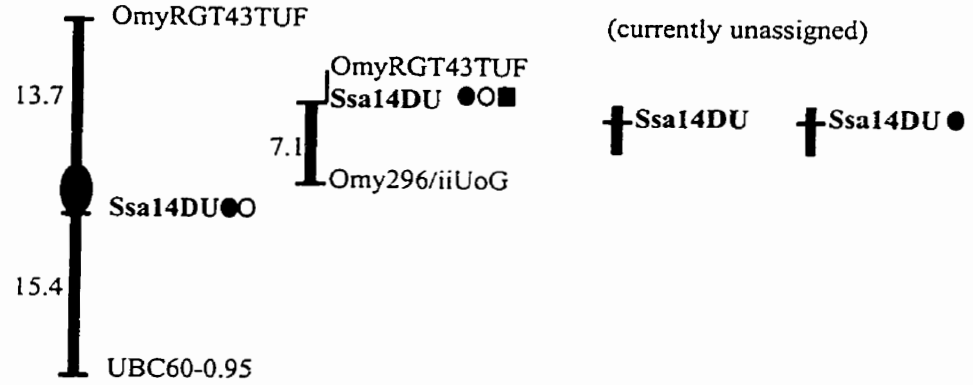

Ras-1 (DIAS)
8.3
Omy1INRA
OmyRGT31TUF
Omy29INRA
21.9
One18/iASC
8.5
OmyRGT13TUF
1.7
OmyFGT8/iTUF
12.3
Ocl2UW
0.4
Omy272/iUoG
19.0
Str15INRA
9.5
Omy6DIAS
Omy29INRA
6.4
OmyRGT31TUF
One18/iASC
Ocl2UW
Str15INRA
mIDHP-2
RAG1 (DIAS)
Omy1INRA
No female data
Otsd3NWFSC/ii b
25
SEX b
4.3
2.5
Otsa2
Omy6DIAS b
SSOSL32/i b
2.0
Metallothionein gene
OmyRT8/iTUF b
8
SsaBHMS7-33 b
SsaBHMS490 b
2
SsaBHMS431 b
SsaBHMS7-30 b
15.5
Ssa16NUIG b

# 18

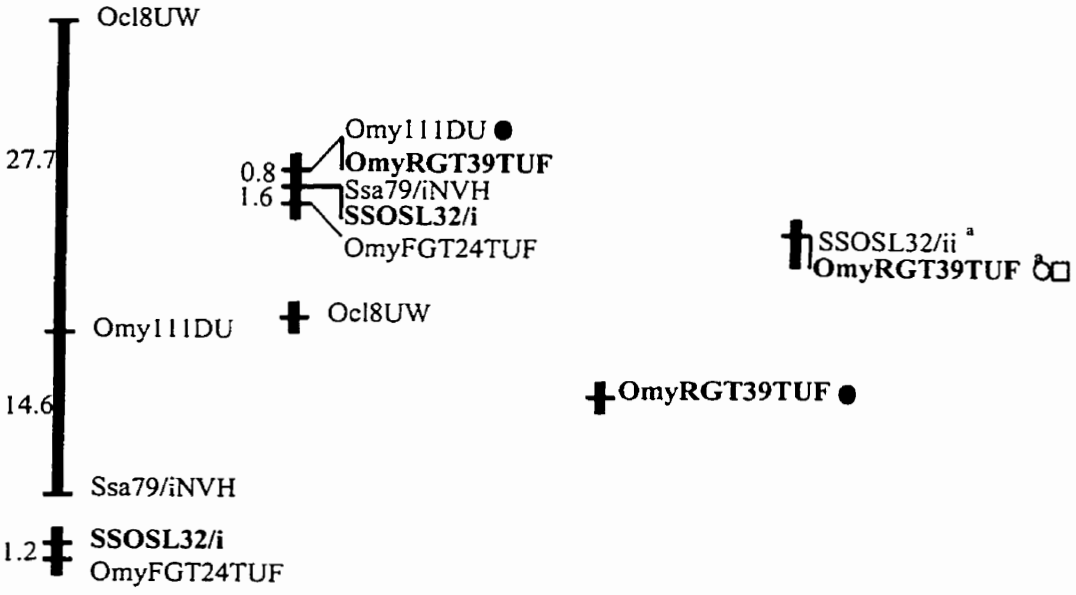

2.0
OmyFGT19TUF
Omy17INRA
sSOD-1
2.0
SEX
OmyFGT19TUF
21.2
OmyRGT28TUF
9.9
Otsd3NWFSC/ii a,b
Otsd4NWFSC a,b
20.8
Otsd3NWFSC/ii a
Otsd4NWFSC a

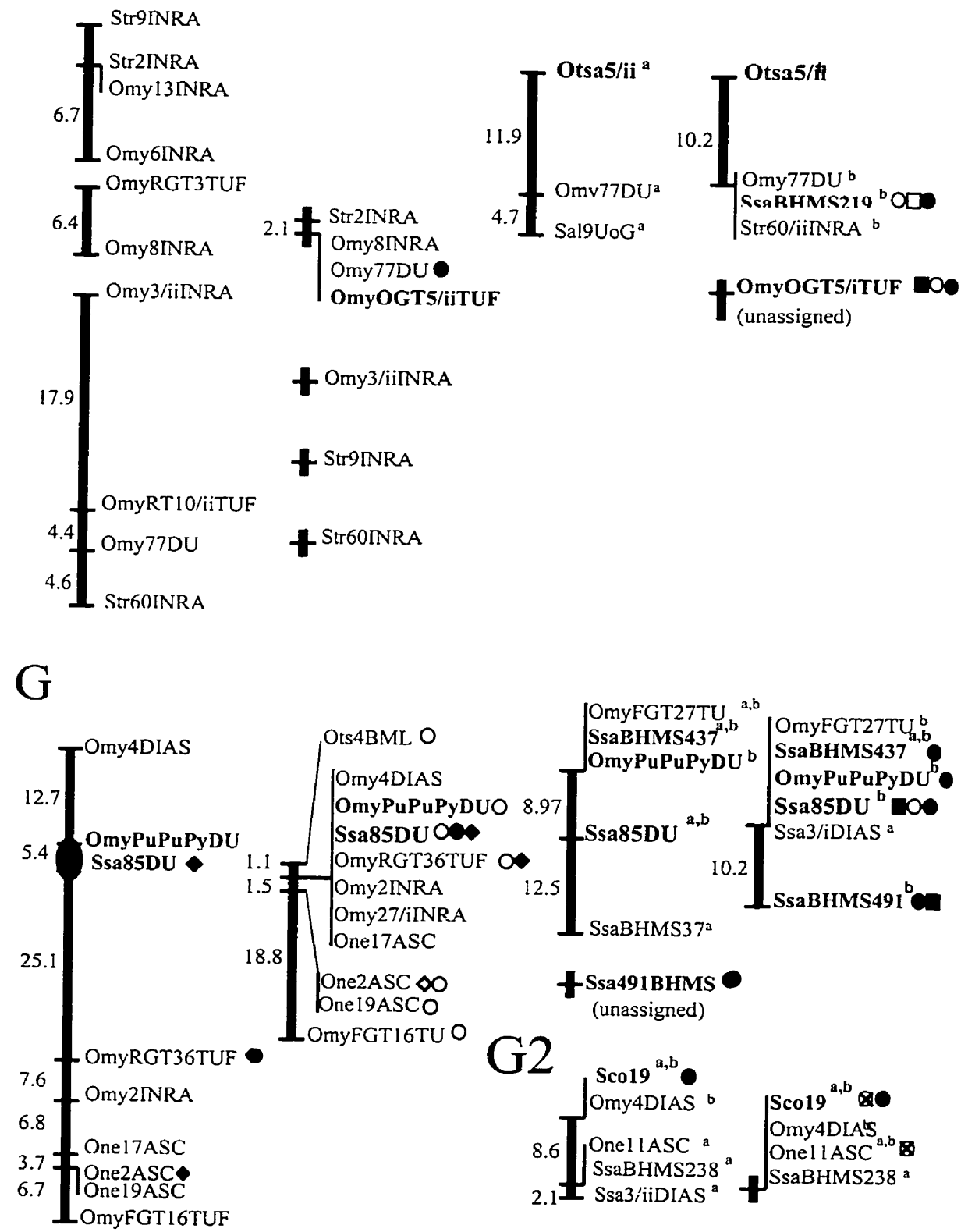

B
OmyRT5TUF
10.8
OmyRGT26TUF
3.0
OmyFGT27TUF
18.9
OmyP9-2TUF
2.0
Omy301UoG
1.0
OmyFGT2TUF
12.7
Omy10INRA
2.7
Ssa197DU
OmyFGT7TUF
OmyRT5TUF
Omy2DIAS
OmyFGT27TUF
OmyRGT26TUF
OmyP9-2TUF
Omy301UoG
1.1
OmyFGT2TUF
1.1
Omy10INRA
3.3
Omy325UoG
28.9
OmyFGT7TUF
3.1
Omy325UoG a,b
Omy301UoG a,b
4.5
Ots3BML a,b
Omy10INRA b
SsaBHMS7-36/ii b
Omy325Uo a,b
SsaBHMS7-36/ii a
Omy10INRA b
3.6
Omy301UoG a,b
13.3
Ots3BML a,b
C
6.7
Ssa289DU
8.5
Str58CNRS
2.8
SSOSL439
2.5
Ssa6.33NUIG
OmyOGT4TUF
OmyRGT6/iiTUF
Ssa289DU
Str58CNRS
SSOSL439
Ssa6.33NUIG
OmyOGT4TUF
27.0
OmyRGT6/iiTUF
C/L
SsaBHMS206/i b
12.5
One7ASC b
SsaBHMS330 b
8.3
MST85 b
SsaBHMS206/i b
OmyRGT6/i b
SsaBHMS330 b
(Family 2)
MST85 a
14.5
Omy26INRA a
L
OmyRGT6/iTUF
22.6
OmyRGT27TUF
OmyRGT6/iTUF
4.3
OmyRGT27TUF

D

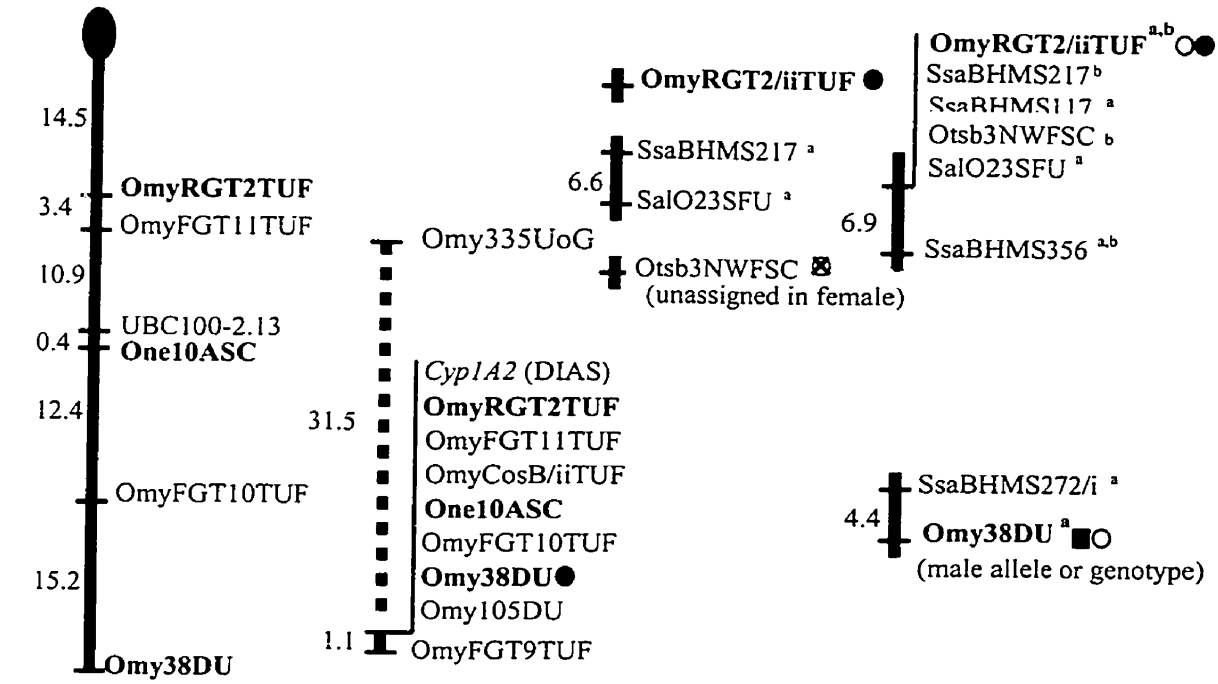

OmyRGT43TUF
13.7
Ssa14DU
15.4
UBC60-0.95
OmyRGT43TUF
Ssa14DU
7.1
Omy296/iiUoG
(currently unassigned)
Ssa14DU
Ssa14DU

E


Ocl8UW
27.7
Omy111DU
14.6
Ssa79/iNVH
1.2
SSOSL32/i
OmyFGT24TUF
Omy111DU
0.8
1.6
OmyRGT39TUF
Ssa79/iNVH
SSOSL32/i
OmyFGT24TUF
Ocl8UW
SSOSL32/ii [a]
OmyRGT39TUF [a]
OmyRGT39TUF


Fii
Str9INRA
Str2INRA
Omy13INRA
6.7
Omy6INRA
OmyRGT3TUF
6.4
Omy8INRA
Omy3/iiINRA
17.9
OmyRT10/iiTUF
4.4
Omy77DU
4.6
Str60INRA
2.1
Str2INRA
Omy8INRA
Omy77DU
OmyOGT5/iiTUF
Omy3/iiINRA
Str9INRA
Str60INRA
11.9
Otsa5/ii a
Omv77DU a
4.7
Sal9UoG a
10.2
Otsa5/ii
Omy77DU b
SsaBHMS219 b
Str60/iiINRA b
OmyOGT5/iTUF
(unassigned)
G
Omy4DIAS
12.7
OmyPuPuPyDU
5.4
Ssa85DU
25.1
OmyRGT36TUF
7.6
Omy2INRA
6.8
One17ASC
3.7
One2ASC
6.7
One19ASC
OmyFGT16TUF
Ots4BML
Omy4DIAS
OmyPuPuPyDU
Ssa85DU
1.1
OmyRGT36TUF
1.5
Omy2INRA
Omy27/iINRA
One17ASC
18.8
One2ASC
One19ASC
OmyFGT16TU
OmyFGT27TU a,b
SsaBHMS437 a,b
OmyPuPuPyDU b
8.97
Ssa85DU a,b
12.5
SsaBHMS37a
Ssa491BHMS
(unassigned)
OmyFGT27TU b
SsaBHMS437 a,b
OmyPuPuPyDU b
Ssa85DU b
Ssa3/iDIAS a
10.2
SsaBHMS491 b
G2
Sco19 a,b
Omy4DIAS b
8.6
One11ASC a
SsaBHMS238 a
2.1
Ssa3/iiDIAS a
Sco19 a,b
Omy4DIAS b
One11ASC a,b
SsaBHMS238 a

H


14.5
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3.4
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10.9
UBC100-2.13
0.4
One10ASC
12.4
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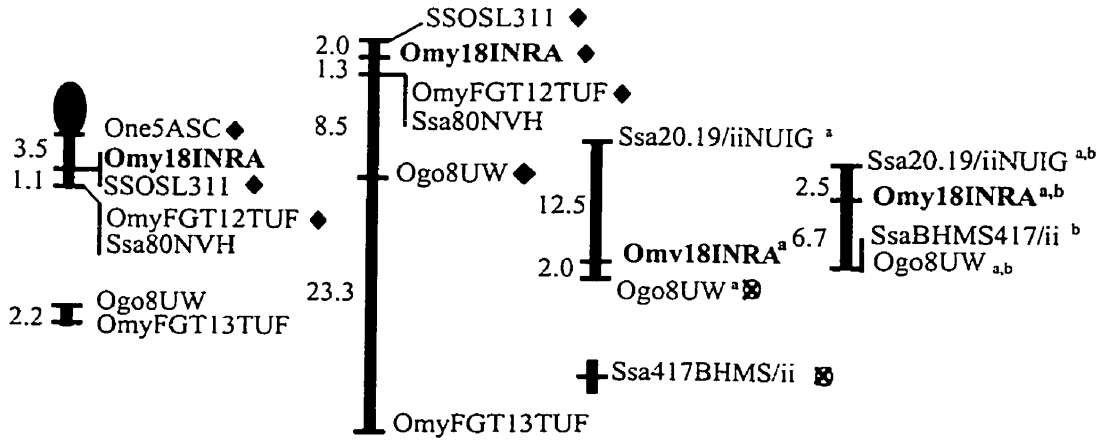
15.2
Omy38DU
OmyRGT2/iiTUF
6.6
SsaBHMS217 a
SalO23SFU a
Otsb3NWFSC
(unassigned in female)
OmyRGT2/iiTUF a,b
SsaBHMS217 b
Otsb3NWFSC b
SalO23SFU a
6.9
SsaBHMS356 a,b
Omy335UoG
31.5
Cyp1A2 (DIAS)
OmyRGT2TUF
OmyFGT11TUF
OmyCosB/iiTUF
One10ASC
OmyFGT10TUF
Omy38DU
Omy105DU
1.1
OmyFGT9TUF
SsaBHMS272/i a
4.4
Omy38DU a
(male allele or genotype)

J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1.3
OmyFGT12TUF
Ssa80NVH
8.5
Ogo8UW
23.3
OmyFGT13TUF
One5ASC
3.5
Omy18INRA
1.1
SSOSL311
OmyFGT12TUF
Ssa80NVH
2.2
Ogo8UW
OmyFGT13TUF
Ssa20.19/iiNUIG a
12.5
Omv18INRA a
2.0
Ogo8UW a
Ssa417BHMS/ii
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2.5
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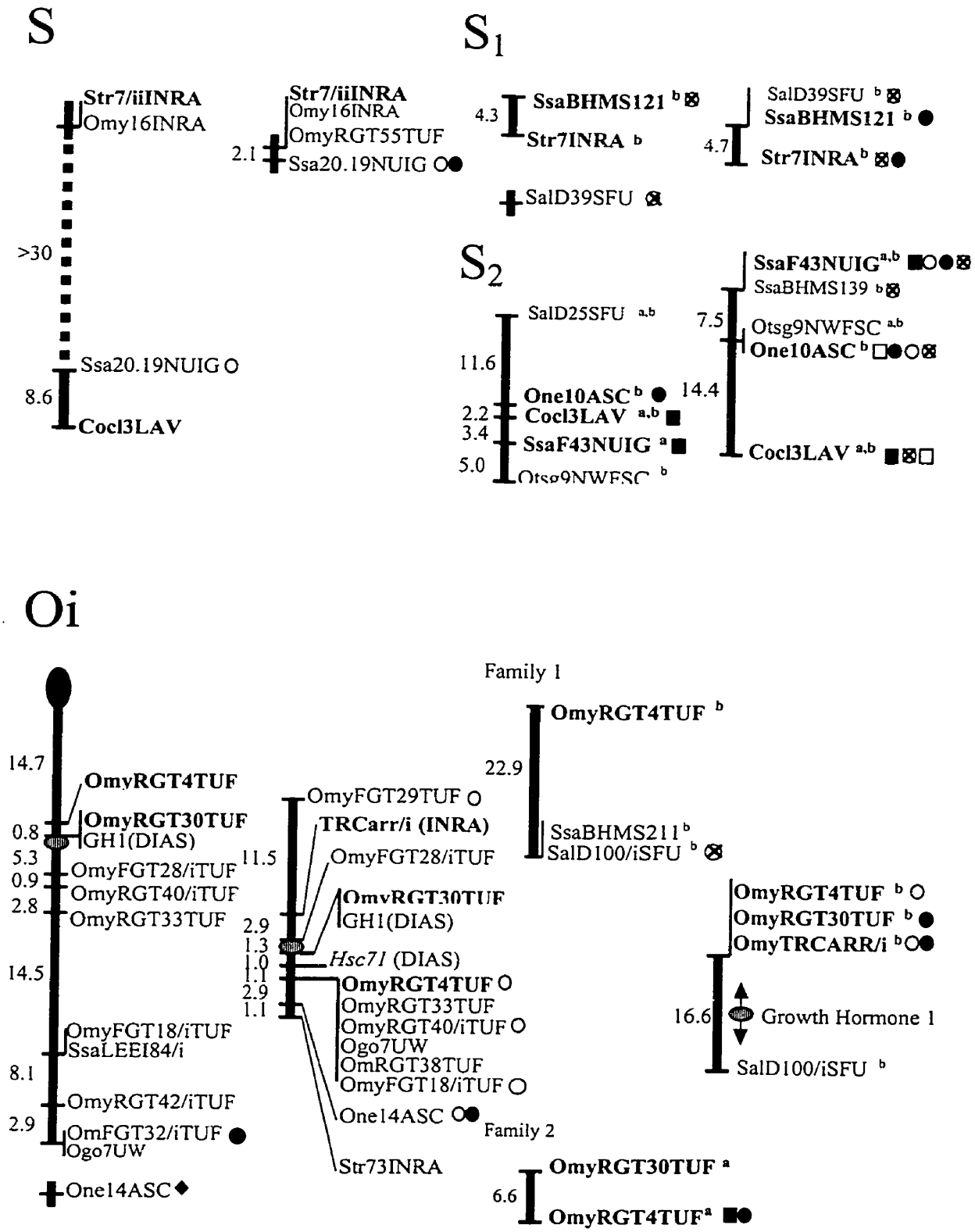
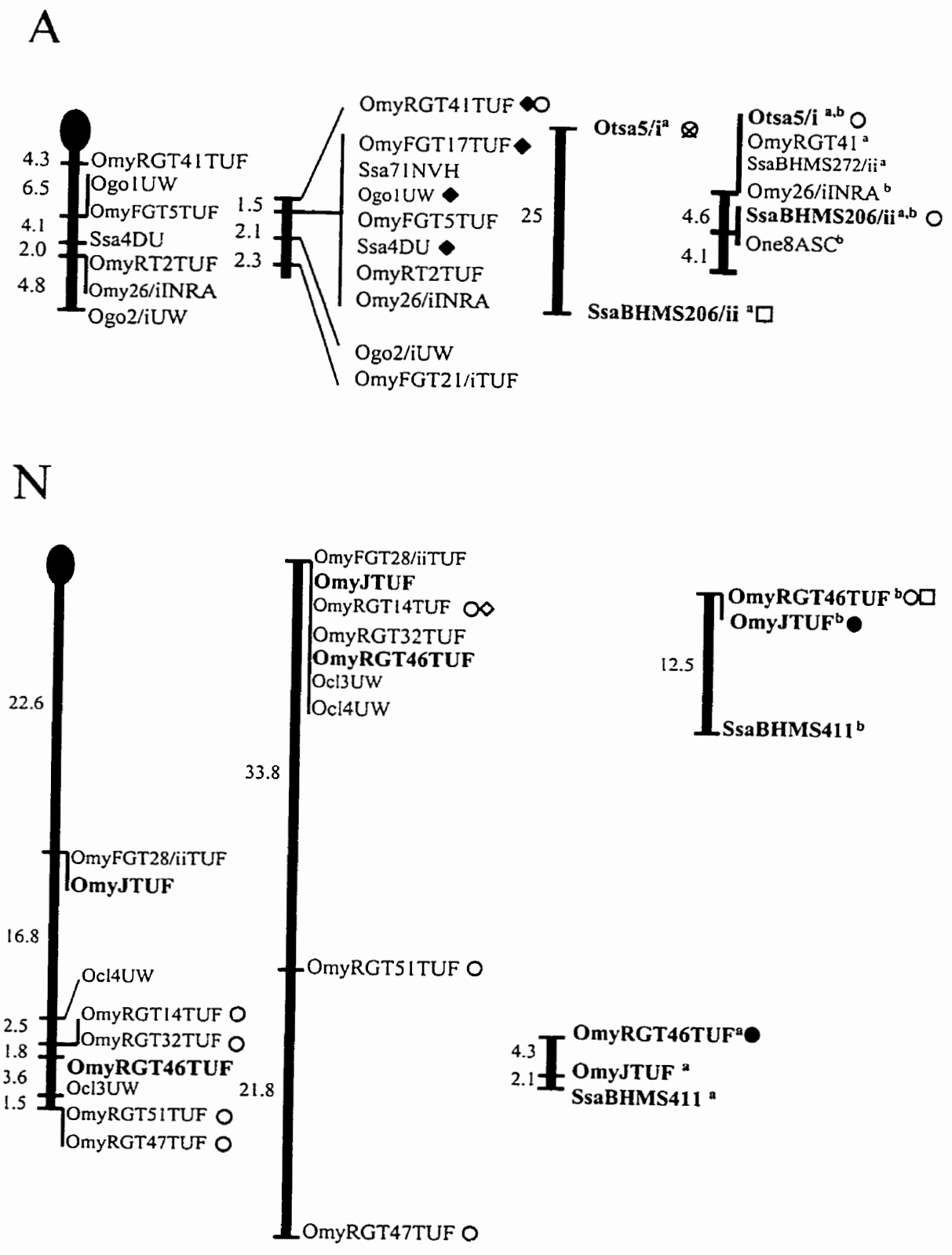
6.7
SsaBHMS417/ii b
Ogo8UW a,b


S
Str7/iiINRA
Omy16INRA
>30
Ssa20.19NUIG
8.6
Cocl3LAV
Str7/iiINRA
Omy16INRA
2.1
OmyRGT55TUF
Ssa20.19NUIG
S1
4.3
SsaBHMS121 b
Str7INRA b
SalD39SFU
SalD39SFU b
SsaBHMS121 b
4.7
Str7INRA b
S2
SalD25SFU a,b
11.6
One10ASC b
2.2
Cocl3LAV a,b
3.4
SsaF43NUIG a
5.0
SsaF43NUIG a,b
SsaBHMS139 b
7.5
Otsg9NWFSC a,b
One10ASC b
14.4
Cocl3LAV a,b
Oi
14.7
OmyRGT4TUF
0.8
OmyRGT30TUF
GH1(DIAS)
5.3
OmyFGT28/iTUF
0.9
OmyRGT40/iTUF
2.8
OmyRGT33TUF
14.5
OmyFGT18/iTUF
SsaLEEI84/i
8.1
OmyRGT42/iTUF
2.9
OmFGT32/iTUF
Ogo7UW
One14ASC
OmyFGT29TUF
TRCarr/i (INRA)
11.5
OmyFGT28/iTUF
OmyRGT30TUF
2.9
GH1(DIAS)
1.3
Hsc71 (DIAS)
1.0
1.1
OmyRGT4TUF
2.9
OmyRGT33TUF
1.1
OmyRGT40/iTUF
Ogo7UW
OmRGT38TUF
OmyFGT18/iTUF
One14ASC
Str73INRA
Family 1
OmyRGT4TUF b
22.9
SsaBHMS211 b
SalD100/iSFU b
OmyRGT4TUF b
OmyRGT30TUF b
OmyTRCARR/i b
16.6
Growth Hormone 1
SalD100/iSFU b
Family 2
OmyRGT30TUF a
6.6
OmyRGT4TUF a


A
4.3
6.5
4.1
2.0
4.8
OmyRGT41TUF
Ogo1UW
OmyFGT5TUF
Ssa4DU
OmyRT2TUF
Omy26/iINRA
Ogo2/iUW
1.5
2.1
2.3
OmyRGT41TUF
OmyFGT17TUF
Ssa71NVH
Ogo1UW
OmyFGT5TUF
Ssa4DU
OmyRT2TUF
Omy26/iINRA
Ogo2/iUW
OmyFGT21/iTUF
25
Otsa5/i[a]
SsaBHMS206/ii [a]
4.6
4.1
Otsa5/i [a,b]
OmyRGT41 [a]
SsaBHMS272/ii[a]
Omy26/iINRA [b]
SsaBHMS206/ii[a,b]
One8ASC[b]
N
22.6
16.8
2.5
1.8
3.6
1.5
OmyFGT28/iiTUF
OmyJTUF
Ocl4UW
OmyRGT14TUF
OmyRGT32TUF
OmyRGT46TUF
Ocl3UW
OmyRGT51TUF
OmyRGT47TUF
33.8
21.8
OmyFGT28/iiTUF
OmyJTUF
OmyRGT14TUF
OmyRGT32TUF
OmyRGT46TUF
Ocl3UW
Ocl4UW
OmyRGT51TUF
OmyRGT47TUF
12.5
OmyRGT46TUF [b]
OmyJTUF[b]
SsaBHMS411[b]
4.3
2.1
OmyRGT46TUF[a]
OmyJTUF [a]
SsaBHMS411 [a]

# CHAPTER 3:

# QTL FOR BODY SIZE, CONDITION FACTOR AND GONADOSOMATIC INDEX IN ARCTIC CHARR, *SALVELINUS ALPINUS:* EVIDENCE FOR CORRELATED EFFECTS AMONG FITNESS TRAITS

## INTRODUCTION

Salmonid fishes exhibit a variety of life history strategies. In particular, different species are characterised by varying levels of semelparity and iteroparity (frequency of post-spawning mortality), anadromy and residency (migratory behaviour), age at maturity, spawning time and growth parameters (references in Meerburg 1986; Fleming 1998; Metcalfe 1998; Unwin et al. 1999). These life history decisions often reflect local environmental conditions, resulting in the potential for spatial and temporal differences among populations within a species (Hendry and Quinn 1997). Moreover, the strategy adopted depends upon tradeoffs among fitness components (McCann and Shuter 1997; Roff 2000) and in particular, those associated with reproduction and growth. Most optimality models assume that a sensitive, threshold period in the early growth trajectory of salmonids controls whether smoltification or early maturation occurs. These decisions are based primarily upon available energy reserves and the costs incurred by the individual in response to the required physiological changes (Metcalfe 1998; Unwin et al. 1999). Thus, life history parameters may differ in males and females, whose lifetime fitnesses may be maximised by employing different strategies at different times (Fleming 1998).

Evidence for the existence of a common genetic architecture to life history characters in salmonids may explain the similarity of phenotypes observed across species. Studies with allozyme polymorphisms have revealed a genetic basis for aspects of performance and maturation in salmonids (Ferguson et al. 1987; Jordan et al. 1990; Pollard and Danzmann 1994). Similarly, genes for several hormones, such as prolactin, somatolactin and growth hormone have been shown to affect osmoregulation, reproduction and

metabolism in several species (reviewed in Rand-Weaver and Kawauchi 1993). In addition, coho salmon (*Oncorhynchus kisutch*) with a growth hormone transgene show increased appetite and ability to compete for food (Devlin et al. 1999). The growth hormone 1 gene has been mapped to linkage group Oi in rainbow trout (Holm and Brusgaard 1999, Sakamoto et al. 2000). In addition, quantitative trait loci (QTL = chromosomal regions that control a quantitative trait) for spawning time, maturation and growth in rainbow trout have also been identified using microsatellite linkage maps (Sakamoto et al. 1999; Martyniuk 2001; O'Malley 2001). The latter studies suggest that multiple genes, some of which may affect more than one character, control life history traits.

Likewise, the genetics of sex determination, which undoubtedly affects many life history parameters, has received much attention. Evidence that the expression of quantitative characters may be sex-dependent is apparent in multiple species, including salmonids. Sex by marker effects have been reported for bristle number in *Drosophila* (reviewed in Zwick et al. 2000), and sex strongly affects growth and maturation parameters in salmonids (Myers 1986). Comparative mapping suggests that the loci controlling sex determination in salmonid fishes are on different linkage groups (Phillips and Ihssen 1985; Reed and Phillips 1997; R. Woram, pers. comm.). In rainbow trout (*Oncorhynchus mykiss*), markers on linkage group 18 are linked to the sex determining locus (May and Johnson 1990; Sakamoto et al. 2000), while in species in the *Salvelinus* genus, at least two different linkage groups are involved (Phillips et al. 1989; Reed and Phillips 1997; R. Woram pers. comm.). A sex-specific repeat sequence has been reported in chinook salmon, *Oncorhynchus tshawytscha* (Devlin et al. 1998). In addition, males of

*O. masou* and *O. rhodurus* can be differentiated based on the presence or absence of a growth hormone pseudogene on the putative Y chromosome (Nakayama et al. 1999). These differences may reflect genome rearrangements in salmonid species since their divergence from a common tetraploid ancestor, as well as more recent evolution of the sex-determining mechanism.

Correlations between traits may reflect either a common underlying biological cause, resulting from a similar genetic basis, the interaction of gene complexes, or a common environmental cue, which produces correlated responses among sets of traits. Correlations among fitness characters are complex and may reveal energy-dependent tradeoffs. Roff (2000) suggests that the phenotypic correlation between growth and other life history traits represents minimal genetic correlations, but that the correlations between fitness traits are difficult to predict. Growth related traits and maturation may have high phenotypic and genetic correlations in some strains of Arctic charr (*Salvelinus alpinus*), although the magnitude and direction of these effects appear to be environmentally-dependent (Nilsson 1992). Studies in other salmonid species further suggest that the relationship among these traits is complex and difficult to predict (Silverstein and Hershberger 1992; Heath et al. 1994).

Temperature can either negatively or positively affect poikilotherm biology, depending on whether or not it acts as a stressor. Salmonids exhibit enhanced growth at the high end of their optimal thermal range, and temperature may control spawning, smolting and maturation time. However, in the critical temperature range between optimal growth and death, stress results in decreased reproductive and growth performance due to increased metabolic or maintenance costs (Elliott 1981; Pankhurst et

al. 1996). This association may translate into a genetic correlation between stress resistance (temperature or otherwise) and life history characters due to common genes affecting their physiological responses. In brook charr (*Salvelinus fontinalis*), fish weight has a significant effect on thermotolerance, which is lost when sex is taken into account according to Benfey et al. (1997), who propose that lower thermal tolerance in males (larger) may be due to reproductive investment. Thus, it may be informative to examine the relationship among various fitness traits (stress tolerance, growth and reproductive traits) and the genetic basis for these traits in closely related species.

Among salmonids, Arctic charr represents perhaps the most polymorphic species, in terms of life history adaptations, morphology and behaviour. High intraspecific variation in body size and life history strategy has been documented (McCart 1980; Jensen 1994; Langeland and L'Abee-Lund 1998). The morphotypes of Thingvallavatn, Iceland, with their varied ecological and morphological specialisations, provide one of the best arguments for proponents of sympatric speciation (Skúlason et al. 1996; Gíslason et al. 1999). Similar forms have been identified worldwide, and differ in growth rate and spawning site and season (e.g. Klemetsen et al. 1997; Adams et al. 1998). Some of this variation may be the result of phenotypic plasticity, wherein slight modifications of the environment alter the expression of the phenotype (Sheiner 1993; Via et al. 1995; Skúlason et al. 1996; de Moed et al. 1997; Pétavy et al. 1997). Thus, if life history traits are under similar genetic control in different salmonid species (under controlled conditions), it is possible that Arctic charr phenotypes are more environmentally labile.

The molecular architecture of life history traits, and in particular genetic effects and mode of action, are less well understood in Arctic charr than other salmonid species. The

relatively high heritability for growth, condition factor and sexual maturity in Arctic charr (Nilsson 1992; Nilsson 1994), as well as the effects of growth hormone (Krasnov et al. 1999; Pitkänen et al. 1999) are conserved in salmonids in general. However, unlike other salmonids, Arctic charr exhibit great variation in body size and weight even under optimal aquaculture conditions and after repeated size-sorting and selection (Jobling and Reinsnes 1986; Baardvik and Jobling 1995; de March 1997). This trend is also apparent within individual families reared under controlled laboratory conditions, which suggests complex polygenic as well as environmental (social and physical) control of body size.

The purpose of this chapter is twofold. First, this study aims to locate QTL for body size, condition factor and relative gonad size in five Arctic charr families, previously used to detect QTL for upper temperature tolerance (UTT, Chapter 2). Based on marker assignment in rainbow trout and Arctic charr (Sakamoto et al. 2000; R. Woram, pers. comm.), loci on at least sixteen linkage groups were surveyed (Table 2-1). Second, markers associated with more than one trait were used to examine possible genetic correlations among growth-related traits (fork length/body weight and condition factor) and reproductive parameters (gonad weight and gonadosomatic index).

## Materials and Methods

Five families derived from the Nauyuk Lake and Fraser River strains of Arctic charr were used in this study. The Nauyuk Lake and Fraser River populations differ in various life history characters (Dempson and Green 1984; Gyselman 1994) the Arctic and Labrador mitochondrial DNA lineages, respectively (Wilson et al. 1996). The families (designated by fish identification number, female x male) consisted of different types of

inter-strain hybrids. Specifically, family 12-111 was a Fraser backcross (Fraser female x F1 male), families 12-114 and 21-114 were Fraser female x Nauyuk male F1 hybrids, and families 27-139 and 30-136 were Nauyuk female x Fraser male F1 hybrids. The five families had been subjected to temperature tolerance trials in December of 1998, when the fish were approximately 13 months post-fertilisation (Chapter 1, Table 1-1). I used genotypic data to confirm the family identity of individual fish using PROBMAX (Danzmann 1997) because the families had been reared together without tagging.

Body weight in grams (BW, nearest 0.1) and fork length in cm (FL, nearest mm) were recorded subsequent to the thermal trials. Fulton's condition factor, a measure of fish's girth, was calculated as $K= 100\ BW\ X\ FL^{-3}$ (Rikardsen and Elliott 2000). This particular measure of K was employed due to its widespread use in population studies, and because regression of log $FL^3$ onto log BW was approximately linear ($r=0.98$, $F(1, 209)=5736.5$, $p<0.000$).

In addition, gonad weight in grams (GW, nearest 0.001) was recorded for families 12-111, 30-136 and 27-139. No data were collected for families 12-114 or 21-114 because individuals were selectively genotyped in order to detect upper temperature tolerance (UTT) QTL (see Chapter 2). Gonadosomatic index (GSI) was calculated using the formula $GSI= 100\ GW/BW$, and is a rough estimate of investment into gonad production. Since GW and GSI are highly and positively correlated (Table 3-2), data are reported for GSI only.

QTL detection

Microsatellite markers used to search for body size, condition factor and relative gonad size QTL are listed in Table 2-1. These markers were previously used to detect

QTL for UTT (Chapter 2). The DNA extraction and PCR protocols used here have been described previously in Chapter 2. The distribution of trait values was tested for conformation to normality by the Kolmogorov-Smirov test for small sample sizes. FL, BW and K did not deviate from normality in any of the families (Table 2-2), or GW in 12-111. In contrast, GSI was not normally distributed in any family ($p<0.01$).

For normally distributed traits, linear regression was used to estimate QTL location. The general linear model has the form

$$\gamma_i = \mu + \alpha + e_i,$$

where $\gamma_i$ is the phenotypic value for offspring *i*, $\mu$ is the trait mean, $\alpha$ is the effect of the parental allele, and $e_i$ is a random error term. When both parents were heterozygous for the same alleles, progeny were assigned by genotypic class: homozygotes for small or large alleles were scored as 11 or 22, and heterozygotes as 12. Initially all genotypic classes were regressed onto phenotype, and subsequently all pairwise comparisons were made. Influential points greater than $\pm 2\ \delta$ (outliers) were removed if they increased the coefficient of determination ($R^2$). The $R^2$ value provides an estimate of the amount of variance in the trait contributed by the model (Lynch and Walsh 1998), and thus the QTL effect. This was appropriate because the phenotypic data were regressed onto the locus term only (i.e. no additional variables). Therefore, this represents the reduced model, and the regression sums of squares equals the locus sums of squares.

Associations between markers and GSI were initially examined using the non-parametric Mann Whitney U rank test. This allows comparison of the phenotypic means between individuals receiving alleles from either parent (sire or dam component). Subsequently, all loci exhibiting an association with GSI were tested using the same

regression model as presented above. This was to ensure consistency across traits and to permit the estimation of QTL effects. Outliers were removed (>>2±σ) if they improved the fit of the model.

Probability values were adjusted using Bonferonni correction for multiple tests on sixteen linkage groups (Rice 1989). Associations that failed to pass Bonferonni correction but for which $p<0.05$ during the initial tests were considered marginal.

Correlations between QTL for fitness traits

In order to determine whether or not a single chromosomal region is associated with multiple traits, markers linked to QTL for body size and weight, condition factor, and gonadosomic index (GSI) were compared. Correlations among fitness traits were calculated using the Pearson Product-Moment Correlation for normally distributed traits (BW, FL, K). The distributions of GW and GSI were not appreciably improved by transformation. Therefore, the nonparametric Kendal Tau was calculated to test for a relationship between GW with GSI, and those measures with all other traits (Table 3-2). Statistical outliers were removed (>>±2σ) and all analyses were performed using STATISTICA software (1995).

## RESULTS

Phenotypic correlations among traits

Families differed in mean phenotypic values and standard deviations (Table 2-2). In addition, correlations between values of fitness traits varied both in magnitude and pattern across families (Table 3-2). Fork length and body weight were highly correlated in all families ($r > 0.95$). Further, FL and BW showed identical results for the QTL

analysis. Thus, only the data for FL are presented below. Similarly, GW and GSI were highly correlated and yielded highly congruent results in the QTL analysis. As a result, only results from QTL analyses for GSI are provided below. In contrast, K and GSI were not correlated ($p<0.05$) in the three families for which GSI was recorded.

QTL analysis

Allelic variation at OmyRGT4TUF (linkage group Oi) from the sire was significantly associated with FL (Figure 2-2, Table 3-1). A marginal association was detected for a second marker (TRCarr) on linkage group Oi. Additional significant associations were detected with SsaBHMS161 (linkage group 8), SsaBHMS206/ii, (A), Omy38DU (H) and SsaF43NUIG (S2). Loci demonstrating significant associations with FL contributed between approximately 19% and 33% of the variation in the trait (Table 3-1).

The existence of marginal associations with FL at four markers on each of linkage groups C/L, E, F and G also provide suggestive evidence for QTL in those locations. These were detected in alleles derived from the sire. The variance for loci exhibiting marginal associations with FL ranged from 12-32%.

A single unassigned marker (Ssa3NUIG, sire effect) was associated with variation in K (Table 3.1). In addition, a significant effect was detected in the dam at SsaF43NUIG (linkage group S2). Ssa3NUIG and SsaF43NUIG contributed ~29% and 26% of the variation in K, respectively.

Marginal associations for Cocl3LAV on linkage group S2 further support the presence of a QTL for K in that chromosomal region. Other marginal associations were detected with loci on four linkage groups (2, F, G, and Oi). Loci that marginally affected

K were inconsistent in their effects and produced between 5-24% of the variance in the locus.

Allelic variation at loci on linkage groups 15 (SSOSL32/i), E (OmyRGT39TUF) and N (OmyRGT46TUF) was significantly associated with GSI (Table 3-1). The associations with linkage groups 15 and E were each detected in two families. The putative QTL on N explains 24% of the variation in GSI. The effects detected on linkage group 15 for variation in GSI ranged from 56-85%. The removal of additional outliers in family 30-136 would increase the explained variance to 93%, but resulted in small sample size. Similarly, locus OmyRGT39TUF on linkage group E accounted for 16-25% of the variance in GSI. However, when comparing genotypic classes 11 and 22 alone (30-136) the model explains 92% of the variance.

Marginal associations with GSI were detected for linkage groups 8, A, B, and Fii. The locus SSOSL456, which has not yet been assigned to any linkage group, also exhibited a marginal association with GSI. Marginal associations contribute between 8% and 17% of the variation in the trait (Table 3-1).

## DISCUSSION

Significant associations between fork length and marker alleles on five linkage groups (8, A, H, Oi and S2) were detected in Arctic charr (Table 3-1). Additional QTL may occur on linkage groups C/L, E, F and G based on marginal associations with fork length. Likewise, two putative QTL for condition factor were detected on linkage group S2 and with an unassigned marker (Ssa3NUIG). In addition, a QTL for condition factor may be located on linkage group 2. Ssa77NUIG was weakly associated with condition

factor in the male parent of three families, implying either a weak QTL effect in that location, or a great distance of the marker from the actual QTL. Marginal associations between variation in condition factor and markers on linkage groups F, G and Oi further suggest that QTL may exist in these locations. Thus, at least seven chromosomal regions may affect growth in Arctic charr based on condition factor and fork length data.

Inclusion of results from two other backcross families of Arctic charr, also derived from Nauyuk Lake and Fraser River strain fish, further supports the data found here (figure 2-2; R. Woram, pers. comm.). Not only are QTL for growth parameters substantiated on linkage groups A, Oi, H and S2, but so are the weaker associations detected on 8, C/L and G or G2. Mapping in the sire (e.g. C/L) may result in the detection of weaker associations over longer map distances due to the reduced amounts of recombination in male salmonids (Sakamoto et al. 2000; O'Malley 2001). In contrast, QTL detected in the dam may be more representative of the true QTL location because recombination rates are more representative of map distance in female salmonids compared to males. Thus, specific growth weight or body size QTL localised in the dam to linkage group C/L, using different markers and families than those used here (R. Woram, pers. comm.) support the existence of a body size QTL on this linkage group. However, the QTL is probably some distance away from the locus tested in this study.

The results found here further corroborate the association of markers on linkage groups F and S2 with body size in Fraser strain fish of unknown family structure. Progeny from twenty-one families were pooled and tested for significant differences in genotype frequencies at microsatellite loci between large and small fish (Johansen 1999). Johansen (1999) showed that variation at Omy77DU (Fii) and SsaF43NUIG (S2) was

associated with body weight in single-year classes. The broodstock used to produce these families is descended from a collection of wild Arctic charr from the Fraser River, and is therefore related to the broodstock from which my families are derived (deMarch 1997). In contrast, I did not detect the association between alleles at SSOSL456 and body size that was reported in the previous analysis (Johansen 1999).

Putative QTL for GSI occur on linkage groups E, 15 and N. Marginal associations on linkage groups 8, A, B, F and with SSOSL456 (currently unassigned to a linkage group) suggest the presence of QTL near these locations. The detection of associations between loci on linkage group 15 and GSI in the two F1 hybrid families analyzed (27-139 and 30-136) may be explained by linkage with the sex locus. In Arctic charr, loci on linkage group 15 are linked to the sex-determining factor, which is closely linked to SSOSL32/i (Woram, pers. comm. and figure 2-2). In all families used here, the female parent is homozygous for the 84 allele at this locus, while the sires are heterozygous 84/94. Preliminary data indicate complete linkage disequilibrium between the genotype at the sex-determining locus (SSOL32/i) and phenotypic sex in adult Nauyuk and F1 charr (Sormorjai unpublished). Thus, based on the dominant Y sex-determination system in salmonids (Allendorf and Thorgaard 1984), progeny inheriting the 84 allele from the sire will generally be female, while those inheriting the 94 allele will be male. Differences in GSI between progeny with the 94 or the 84 allele in 27-139 and 30-136 may therefore reflect differential gonad size between the sexes. Unfortunately, it was not possible to sex the progeny with confidence in order to confirm this.

The absence of a similar effect in the backcross 12-111 may be caused by either family differences in gonadal differentiation or incomplete disequilibrium between alleles

at SSOSL32/i and sex. The backcross was produced by a mating between an F1 male and a Fraser strain female; linkage disequilibrium between alleles at SSOSL32/i and sex is lower in the Fraser strain (Somorjai unpublished). Thus, there may be a varied association between a particular allele at SSOSL32/i and the sex-determining locus in this strain (i.e. Y allele associated with both 84 and 94 alleles). In contrast with the Nauyuk Lake strain (Papst and Hopky 1984), the Fraser River strain was derived from multiple collections (de March 1992). It would therefore not be surprising if the Fraser strain were composed of individuals with alleles in opposite phase at the sex locus.

This study revealed that many significant associations between traits and a locus occurred in the F1 hybrid families. For example, QTL for GSI on linkage groups E and N were present in 30-136 and 27-139, while the majority of marginal associations were detected in the backcross family (12-111). The detection of QTL in F1 hybrid families indicates that the original strains are not fixed for alternate QTL alleles at autosomal loci. If that had been the case, QTL detection would only have been possible in the backcross because fixation within strains would have yielded 100% heterozygotes in F1 hybrids.

Examination of the coefficient of determination ($R^2$) for each locus indicates that multiple large effect QTL have been identified for all traits examined in this study (Table 3-1). Several loci account for as much as 30% of the variation in fork length and condition factor, and SSOSL32/i alone accounts for 85% of the variation in GSI in interstrain hybrid family 27-139. The variance in GSI explained by this locus is not surprising given the association of several markers with sex on linkage group 15 in Arctic charr (R. Woram, pers. comm.). Similarly, OmyRGT39TUF is associated with GSI and explains 92% of the variation in this trait. Some of these effects may be inflated due to

the small sample sizes available for the families used here, the regression of phenotype onto genotype when parents were heterozygous, as well as the removal of outliers when fitting the linear model. In addition, full sib data incorporate dominance effects, which may further inflate the variance beyond that accounted for in additive models (e.g. half sibs, Lynch and Walsh 1998).

Evidence for the autotetraploid ancestry of salmonids includes gene duplication, a DNA content per cell double that of diploid relatives, and residual tetrasomy at telomeric isoloci (Wright et al. 1983; Allendorf and Thorgaard 1984; Johnson et al. 1987). The general observation that duplicated loci are not classically linked suggests that homeologous segments tend not to fuse following a fission and translocation event. These models suggest that greater affinity (homology) between homeologues results in their pairing during meiosis I in males. Thus, homeology can be inferred by the presence of duplicated loci on different linkage groups (Sakamoto et al. 2000). The possibility of a single linkage group being homeologous to two different linkage groups arises because of the fusion of two acrocentric chromosomes to form a large metacentric (Wright et al. 1983). In Arctic charr, homeologous chromosome arms might include A and C/L (SsaBHMS206), A and Fii (Otsa5NWFSC), E and 15 (SSOSL32) and several others (see figure 2-2). The fate of disomically inherited duplicated loci is either loss of function of the redundant locus, partial or complete expression of both loci either temporally or physically, or evolution of novel functions in one or both copies (Cronn et al. 1999; Lynch and Force 2000a; Wagner 2000). Thus, the identification of QTL in potentially homeologous regions can provide insight into the evolution of salmonid genetic function since the tetraploid event.

The strong association of OmyRGT39TUF and SSOSL32/i with GSI on linkage groups E and 15, respectively, suggests that functional conservation has occurred on homeologues in Arctic charr (Table 3-1). Although SSOSL32/ii was not amplified in this study, it is linked to OmyRGT39TUF on linkage group E in Arctic charr (figure 2-2; Woram, pers. comm.). Suggestive evidence for conservation of body size QTL on linkage groups A and C/L is also supported by this study. Alleles derived from hybrid male 111 at SsaBHMS206/i (marginal) and SsaBHMS206/ii are both associated with fork length (figure 2-2). Conservation of homeologous effects for body weight and spawning time is supported by QTL studies in rainbow trout (O'Malley 2001).

There is also some evidence for lack of functional conservation between ancestrally duplicated segments in Arctic charr. Linkage groups A and Fii may be homeologues based on localisation of Otsa5NWFSC/i and /ii on these chromosomes. Although not itself associated with any trait in my families, Otsa5NWFSC/ii is within 11 cM of SsaBHMS219 on Fii in Arctic charr (male map). Poompuang and Hallerman (1997) report that QTL can be detected reliably within 20cM. Furthermore, considerable variation in recombination rates occurs across families (Sakamoto et al. 2000; O'Malley 2001). Therefore, the marginal association with GSI at SsaBHMS219 may provide evidence for a QTL on Fii. In contrast, variation at Otsa5NWFSC/i on linkage group A is associated with FL. These results might suggest that genes in these regions have diverged in expression. Allendorf and Thorgaard (1984) report spatio-temporal patterns of expression for duplicated allozymes. It is difficult to infer loss or divergence in function of a chromosomal region using QTL mapping studies. Even when examining simple marker-trait associations, the power of the test depends heavily upon marker density

(Tanksley 1993; Poompuang and Hallerman 1997). Since large genomic tracts may be in linkage disequilibrium in hybrids (reviewed in Peterson et al. 1999; Kohn et al. 2000), the actual gene may be a considerable distance from the marker associated with the trait. This is a particularly important consideration in male salmonids, for which recombination "cold-spots" can be extensive (Sakamoto et al. 2000; O'Malley 2001, K. Gharbi pers. comm.). Of greater importance is that homeologous chromosome arms consist of a multitude of genes whose functions may cover a wide range of integrated developmental and regulatory processes. However, since this is a concern in all QTL studies, further testing will be required in order to confirm QTL location even on non-homeologues.

This study illustrates how a point approach to QTL detection may benefit greatly from a comparative mapping framework, when regions associated with QTL in one related species have already been mapped. For instance, of the six linkage groups (8, A, H, N, Oi, S2) significantly associated with body size or condition factor in Arctic charr in this study alone, three (H, N, Oi) correspond to those previously linked to growth in rainbow trout. Marginal associations (here $p<0.05$) may also be evident in both species on B, C/L, F and G (Martyniuk 2001; O'Malley 2001). However, since the data are derived from the sire in Arctic charr and dam alleles in rainbow trout, it is difficult to infer the actual QTL location on linkage group H. The marginal association with Ssa85DU on G (sire) is paralleled by moderate effects for the same locus in the sire in rainbow trout; a QTL for body size is likely present in this region (O'Malley 2001). Furthermore, the marginal association of linkage group B with fork length in a single family of charr is supported by recent data in rainbow trout (Martyniuk 2001; O'Malley 2001). The weakness of this effect in Arctic charr is probably the result of distance

between locus Omy301UoG and the QTL (figure 2-2). In particular, the strongest effect in rainbow trout was observed at OmyFGT27TUF (sire and dam), which is over 25 cM from Omy301UoG on the female map (O'Malley 2001). QTL resolution is most accurate within 5-20cM (Cheverud and Routman 1993; Poompuang and Hallerman 1997), and fine mapping requires even greater marker density than currently exists in salmonids.

The localisation of a major GSI QTL on N (sire effect) deserves further attention. Martyniuk (2001) identified a putative QTL for male precocious maturation in the same region on linkage group N in rainbow trout. In salmonids, the phenomenon of precocious maturation is fairly common among males, and strain, family-dependent, and maternal effects have been observed (Glebe and Saunders 1986). Few data are available for females, but it is reported that age of first maturity in Atlantic salmon is associated with ovarian development in smolts (Chadwick et al. 1986). Thus, precocious males may arise in the population at least one year before the earliest maturing females. Although the fish used here were still too young to mature, several small individuals exhibited the characteristic early maturation phenotype seen in spermiating males, including the retention of parr marks and bright orange fins and ventrum (R. Woram, pers. comm.). Although it is possible that the QTL for GSI identified at OmyRGT46TUF on linkage group N is also tracking sex (e.g. like at SSOSL32/i), this result may also be due to a correlation between GSI and early maturation. Research on underyearling masu salmon (*Oncorhynchus masou*) indicates that future precocious males can be differentiated from immatures based on GSI (0.203% vs. 0.055%), and that GSI correlates with levels of gonadotropin-releasing hormone mRNA in the brain (Amano et al. 1997). Further, females can be separated into individuals adopting a resident or an anadromous strategy

based upon gonad weight as early as two years of age (Kiso and Kosaka 1992). However, Jobling and Baardvick (1991) report distinct size differences between Arctic charr of different maturational status. Further, growth rates of immature males and females are similar, while maturing males tend to be smaller than immature males (Jobling et al. 1993). This complicates the differentiation between sex–specific growth and variation resulting from maturational status when using young fish. It is clear that there exists a complex association between fitness parameters, and it is likely that males and females adopt different strategies in order to maximize their lifetime fitness. The future identification of QTL for maturation and gonad differentiation in both males and females is therefore warranted.

Based on the results of this study, QTL for growth-related traits and GSI do not generally map to the same regions in Arctic charr, since I could not demonstrate significant associations for the same marker across both sets of traits. A possible exception to this is the association of OmyRGT39TUF (linkage group E) with both GSI and FL ($p<0.004$) in 30-136 F1 hybrids (Table 3-1). Thus, although growth rate or condition factor may affect reproductive investment (associated with sex or maturation), other sets of genes must also independently control (or additively contribute to) the early stages of gonad development. In contrast, several chromosomal regions controlling spawning time or maturation correspond to those for body weight in rainbow trout (Figure 2-2; Sakamoto et al. 1999; Martyniuk 2001; O'Malley 2001). Strong correlations between traits would be expected if the expression of both traits were affected by similar genes. Martyniuk (2001) found a correlation between early maturation (2 years of age) and body size in the Rainbow Springs strain of rainbow trout. Precocial individuals were

significantly larger than individuals that did not mature at two years. However, no correlation between these traits was observed in the Spring Valley strain. Interestingly, putative QTL for precocious maturation were only detected in the Rainbow Springs strain, suggesting genetic covariation between growth and precocity. As a result, one might predict a similarly strong (positive or negative) relationship between body size and GSI in Arctic charr families in which QTL were identified. Unexpectedly, the major QTL for GSI were identified in 27-139 and 30-136 (linkage groups N and E, in addition to 15), for which the relationship between body size and GSI was weak. In contrast, weaker QTL effects for GSI were detected in 12-111, for which the strongest correlation between fork length and GSI was detected ($p<0.036$, Table 3-2). However, several large effect QTL for growth traits were identified. These data illustrate how complex genetic and physiological control may result in diverse patterns of phenotypic response, and explain inter-population variation in traits related to life history.

QTL for upper temperature tolerance and body size or GSI mapped to the same region in several cases. In particular, a significant effect for both UTT and FL was detected for alleles at SsaF43NUIG (linkage group S2) in backcross 12-111. Additional marginal associations for thermal tolerance and growth-related traits occur within families on linkage groups 15, F and H, and possibly G and Oi based on data from closely linked loci (Table 3-1; figure 2-2). Across families, the importance of these chromosomal regions to multiple traits is confirmed, in addition to the effect of loci on linkage group E to both GSI and UTT. This, in combination with the negative phenotypic correlations between thermotolerance (UTT) and growth or reproductive traits in Arctic

charr (depending upon the family, Table 3-2) may suggest the existence of negative genetic correlations (Roff 2000).

The correspondance between chromosomal regions influencing both thermal tolerance and growth-related parameters (Tables 2-4, 3-2) illustrates the difficulty inherent in QTL studies of complex traits. In particular, spurious results can occur if the marker is linked to a QTL for body size, and body size is significantly correlated with UTT. For example, in backcross 12-111, locus Ssa206BHMS/ii (linkage group A) may be a growth QTL exclusively. This locus is not associated with UTT using survival analysis. However, its strong association with UTT using regression analysis with body weight as a covariate would suggest otherwise. Thus it may be informative to address the effect of a chromosomal region on multiple traits by examining the correlations among traits, as well as by locating QTL for both traits using the same genetic markers and multiple statistical methodologies.

Although conclusions based on multi-trait comparisons must be interpreted with caution due to lack of knowledge on the underlying genes, these results emphasize the importance of the relative localization of genes in these regions. Co-localization of QTL for fitness traits may be due to tight linkage between loci. Since tight linkage results in strong linkage disequilibrium between genes, selection on one locus may result in hitchhiking of a second locus, and hence an effect on two traits. Alternatively, a single gene may control the expression of multiple phenotypes via pleiotropy.

Pleiotropy at QTL has been found for developmentally integrated complexes of mandibular morphology in mice (Cheverud et al. 1997), and is likely to be prevalent among physiological traits. The strongest evidence for antagonistic pleiotropy is

supported by associations at SsaF43NUIG in family 27-139, for which the 110 allele confers resistance to thermal stress, but also results in small fork length. Although small body size per se may not be detrimental, it is generally associated with low growth rates and subordinate rank within social hierarchies in Arctic charr (Jobling and Reinsnes 1986). However, growth responses are particularly complicated in Arctic charr, and appear to result from a mixture of social, genetic and environmental factors (Adams and Huntingford 1996; de March 1997; Seppä et al. 1999).

The observation that families in which body weight significantly affected temperature tolerance exhibited a greater variance in body weight and fork length (Tables 2-1, 3-5) may be related to metabolic rate. Cutts et al. (1998) showed that individual variation in standard metabolic rates in *Salmo salar* might effect differences in aggression, thereby explaining reduced size variance in a small-size-sorted group. Dominance status is correlated with aggression, and large individuals tend to exhibit greater competitive ability. In contrast, high stress as inferred from seratonin levels may cause depressed growth rates in subordinate individuals, and therefore small size (Alanärä et al. 1998). Increased growth results from improved protein retention and body-weight corrected metabolism in growth hormone-transgenic salmon (Krasnov et al. 1999). Since temperature increases metabolic rates (Lin and Regier 1995), and oxygen consumption and protein turnover are higher per unit tissue weight in young ectotherms (Hawkins 1995), the metabolic cost incurred by large fish may be highest during a thermal challenge.

Besides the effects of *Pgm1-t* on developmental rate and agonistic behaviour in rainbow trout (Ferguson et al. 1987), few loci have been identified that contribute to our

understanding of the genetic basis of resource allocation (body size, condition factor and GSI) in salmonids. However, recent QTL studies are shedding light on potential regions of interest for candidate gene studies (Martyniuk 2001; O'Malley 2001; this study). In particular, OmyRGT4TUF is associated with body size in Arctic charr, and is located on linkage group Oi. This region harbours a putative QTL for growth in rainbow trout (Martyniuk 2001) and is closely associated with both growth hormone and *hsc71*, a heat shock cognate with many housekeeping functions. Trout red cell arrestin (TRCarr) is also located on Oi, and may function in beta-adrenergic activation and desensitisation of the membrane transport system (Jahns et al. 1996). Further, the metallothionein gene in Arctic charr is located on linkage group 15 (figure 2-2), and may explain the GSI effects at SSOSL32/i. This gene is important in embryonic and sexual development in fish, and may act as a zinc donor during early ovarian development (Olsson 1993). In addition, the strong body size effect seen at OmyRGT2/iiTUF could result from its proximity to the cytochrome P450 gene (Cyp450), which is also mapped to linkage group H in rainbow trout (Sakamoto et al. 2000). Cyp450 mediates the metabolism of both xenobiotic and steroid compounds, and its induction appears to be altered by temperature acclimation and estradiol (Stegeman 1993).

In addition to growth hormone, candidate genes affecting both aspects of growth and sexual development might include estrogen receptors and gonadotropin, which may modify the choice of fitness strategy adopted via temperature and photoperiod sensitive modifications in expression (GH effects reviewed in Björnsson 1998). Gonadal steroids, with their concomitant effects on growth hormone secretion, are associated with gonad development and therefore maturation rate in salmonids (Holloway and Leatherland

1997); this in turn may affect smoltification and the decision to migrate to sea or remain resident after the first year. In Arctic charr, maturation inhibits seawater tolerance, possibly via the involvement of sex steroids in Na+/K+ ATPase activity (Staurnes et al. 1994). In addition, dose effects of sex steroids and growth hormone may result in differences in metabolism (Pytkäinen et al. 1999); in particular, they influence the rate of protein breakdown (catabolism) and lipid synthesis, including PUFAs (polyunsaturated fatty acids), triglycerides and phospholipids (Krasnov et al. 1999). PUFAs affect growth and lipid metabolism (Yang and Dick 1994), and phospholipid arrangement and composition of membranes are key to the homeoviscous adaptation that occurs during temperature acclimation (reviewed in Logue et al. 1995). Clearly, many genetic factors must control the direction and magnitude of trait correlations, potentially reflecting a fitness tradeoff between the metabolic requirements of the physiological alternatives.

Several major QTL were detected for growth and reproductive investment in Arctic charr, some of which may have reflected unknown sex differences. Low correlations among growth and reproduction related traits, and high negative correlations between these and upper temperature tolerance suggest a tradeoff between fitness traits. QTL associated with both temperature tolerance and growth or reproductive investment might be related to maturation, and emphasize the importance of candidate gene detection in these regions. Comparative mapping is therefore a useful tool in evaluating the effects, if any, of genome reorganization on gene expression and evolutionary processes it might affect.

Table 3-1. Putative QTL for growth (FL), condition factor (K) and gonadosomatic index (GSI) in five families of Arctic charr (*Salvelinus alpinus*). Superscripts 'a' and 'b' indicate that reports of linkage groups are based on comparative linkage mapping in Arctic charr and rainbow trout (*Oncorhynchus mykiss*), respectively. A '-' shows that the locus has not been assigned to a linkage group in one or both species. 'Sex' indicates the QTL was detected in alleles derived from the sire (M) or the dam (F), while 'Allele' denotes the particular allele producing increased effects for the trait. '*': Comparison of genotypic classes rather than alleles for identically heterozygous parents. $R^2$ is the variance in % contributed by the model, and represents the QTL effect. FL = fork length in cm, K = Fulton's condition factor and GSI = gonadosomatic index, and represents relative gonad size (see text). Values in **bold** indicate significant effects at $p<0.05$. Loci passing the Bonferroni experimentwise threshold are indicated by **. #: Locus for which the removal of additional outliers would improve model fit, but would also reduce sample size significantly.

| *AC*[a] | *RT*[b] | Locus | Family | Sex | Allele | FL ($R^2$ %) | K ($R^2$ %) | GSI ($R^2$ %) |
|---|---|---|---|---|---|---|---|---|
| 2 | - | Ssa77NUIG | 12-111 | M | 139 | 0.059 (22.2) | 0.073 (19.6) | n.s. |
| 2 | - | Ssa77NUIG | 27-139 | M | 141 | n.s. | 0.099 (3.3) | n.s. |
| 2 | - | Ssa77NUIG | 30-136 | M | 139 | n.s. | **0.024** (14.0) | n.s. |
| 8 | - | SsaBHMS161 | 12-111 | M | 199 | **0.000****(31.5) | 0.077 (5.8) | n.s. |
| 8 | - | SsaBHMS161 | 30-136 | M | 191 | n.s. | n.s. | **0.048** (8.6) |
| - | - | Ssa3NUIG | 12-111 | M | 246 | n.s. | **0.027** (12.8) | n.s. |
| - | - | Ssa3NUIG | 12-114 | M | 246 | n.s. | **0.001****(29.1) | - |
| - | - | SSOSL456 | 12-111 | M | 180 | n.s. | n.s. | **0.017** (11.1) |
| A | - | Otsa5/iNWFSC | 12-111 | M | 172 | **0.011** (14.2) | n.s. | n.s. |
| A | - | SsaBHMS206/ii | 12-111 | M | 190 | **0.000****(32.4) | n.s. | n.s. |
| A | - | SsaBHMS206/ii | 12-111 | F | 180 | n.s. | n.s. | **0.020**(12.1) |
| B | B | Omy301UoG | 12-111 | M | 136 | 0.055 (5.5) | n.s. | n.s. |
| B | B | Omy301UoG | 27-139 | F | 108 | n.s. | n.s. | **0.010** (13.6) |
| C/L | - | Ssa206iBHMS | 12-111 | M | 86 | **0.015** (13.4) | n.s. | n.s. |

Table 2-1, continued

| *AC*[a] | *RT*[b] | Locus | Family | Sex | Allele | FL ($R^2$ %) | K ($R^2$ %) | GSI ($R^2$ %) |
|---|---|---|---|---|---|---|---|---|
| E | E | OmyRGT39TUF | 27-139 | F | 109 | n.s. | n.s. | **0.003****(16.6) |
| E | E | OmyRGT39TUF, all | 30-136 | N/A | 2 | **0.004** (20.8) | n.s. | **0.001****(25.0) |
| E | E | OmyRGT39TUF* | 30-136 | N/A | 2 | **0.014** (31.5) | n.s. | **0.000****(92.0) |
| 15 | E | SSOSL32i | 27-139 | M | 84 | n.s. | n.s. | **0.000****(85.0) |
| 15 | E | SSOSL32i | 30-136 | M | 84 | n.s. | n.s. | **0.000****(56.3#) |
| Fii | - | SsaBHMS219 | 12-111 | F | 148 | n.s. | n.s. | **0.008** (15.5) |
| Fii | - | SsaBHMS219* | 21-114 | N/A | 1 | 0.086 (12.9) | n.s. | - |
| Fii | - | SsaBHSM219* | 12-114 | N/A | 1 | 0.078 (6.0) | n.s. | - |
| - | F | OmyOGT5TUF, all | 27-139 | N/A | 1 | **0.004** (12.8) | n.s. | n.s. |
| - | F | OmyOGT5TUF* | 27-139 | N/A | 1 | 0.074 (8.4) | **0.026** (16.5) | n.s. |
| G | G | SsaBHMS491 | 27-139 | M | 125 | n.s. | 0.086 (3.6) | n.s. |
| G | G | Ssa85DU | 12-114 | M | 170 | **0.006** (21.7) | **0.022** (14.4) | - |
| H | H | Omy38DU | 30-136 | M | 173 | **0.005** (19.9) | n.s. | n.s. |
| H | H | Omy38DU, all | 12-111 | N/A | 1 | **0.001****(26.2) | n.s. | n.s. |
| H | H | Omy38DU* | 12-111 | N/A | 1 | **0.011** (31.9) | 0.087 (12.8) | n.s. |
| H | H | OmyRGT2iiTUF | 27-139 | M | 360 | **0.000****(25.6) | n.s. | n.s. |
| N | N | OmyRGT46TUF | 27-139 | M | 165 | 0.064 (4.5) | n.s. | **0.000****(24.2) |
| Oi | Oi | OmyRGT4TUF | 27-139 | F | 139 | n.s. | **0.040** (5.8) | n.s. |
| Oi | Oi | OmyRGT4TUF | 30-136 | M | 137 | **0.003****(20.5) | n.s. | n.s. |
| Oi | Oi | OmyTRCarr | 30-136 | M | 126 | **0.008** (17.0) | n.s. | n.s. |
| S2 | S | Cocl3Lav | 12-111 | M | 246 | n.s. | n.s. | 0.064 (7.1) |

Table 3-1, continued 2

| *AC*[a] | *RT*[b] | Locus | Family | Sex | Allele | FL ($R^2$ %) | K ($R^2$ %) | GSI ($R^2$ %) |
|---|---|---|---|---|---|---|---|---|
| S2 | S | Cocl3Lav | 27-139 | F | 228 | n.s. | **0.008** (23.1) | n.s. |
| S2 | S | Cocl3Lav | 27-139 | M | 246 | n.s. | **0.008** (12.9) | n.s. |
| S2 | H | One10ASC | 30-136 | M | 160 | n.s. | n.s. | 0.078 (6.2) |
| S2 | H | One10ASC | 12-111 | M | 166 | **0.006** (16.7) | n.s. | n.s. |
| S2 | - | SsaF43NUIG | 12-111 | M | 110 | **0.002****(19.7) | n.s. | n.s. |
| S2 | - | SsaF43NUIG | 12-114 | M | 110 | 0.061(7.0) | **0.023** (11.5) | - |
| S2 | - | SsaF43NUIG | 21-114 | F | 100 | n.s. | **0.029** (12.0) | - |
| S2 | - | SsaF43NUIG | 27-139 | F | 114 | n.s. | **0.000****(25.7) | n.s. |

Table 3-2. Correlations among fitness characters in five families of Arctic charr (*Salvelinus alpinus*) calculated using the Pearson Product Moment (BW, K, FL) or the Kendall Tau (GW, RI) tests. Values in **bold** are considered significant at $p<0.05$. Abbreviations are BW= body weight (g), FL= fork length (cm), K= condition factor, GW= gonad weight (g), GSI= gonadosomatic index, and Time= time until death (min) during thermal trials. A dash ('-') above the diagonal indicates there were no data available for this trait (i.e. GW or GSI in 12-114 and 21-114, which were selectively genotyped for thermal tolerance). Please see text for details.

| Family | Trait | BW | K | GW | GSI | Time |
|---|---|---|---|---|---|---|
| 12-111 | FL | **0.96** | **0.22** | -0.02 | **-0.26** | **-0.54** |
| | BW | - | **0.54** | 0.10 | **-0.30** | **-0.61** |
| | K | - | - | 0.18 | 0.10 | **-0.34** |
| | GW | - | - | - | **0.78** | -0.08 |
| | GSI | - | - | - | - | 0.05 |
| 12-114 | FL | **0.96** | 0.24 | - | - | -0.07 |
| | BW | - | **0.48** | - | - | -0.18 |
| | K | - | - | - | - | **-0.33** |
| 21-114 | FL | **0.96** | 0.31 | - | - | **-0.38** |
| | BW | - | **0.46** | - | - | **-0.45** |
| | K | - | - | - | - | **-0.45** |
| 30-136 | FL | **0.95** | -0.02 | -0.05 | -0.12 | 0.21 |
| | BW | - | 0.08 | -0.05 | -0.12 | 0.10 |
| | K | - | - | 0.10 | 0.15 | -0.09 |
| | GW | - | - | - | **0.91** | -0.20 |
| | GSI | - | - | - | - | -0.22 |
| 27-139 | FL | **0.95** | -0.01 | -0.01 | -0.09 | -0.11 |
| | BW | - | 0.15 | 0.00 | -0.08 | -0.17 |
| | K | - | - | -0.01 | -0.00 | -0.09 |
| | GW | - | - | - | **0.91** | **-0.19** |
| | GSI | - | - | - | - | **-0.21** |

# CHAPTER 4:

# EVOLUTIONARY IMPLICATIONS AND FUTURE PROSPECTS

Speciation is a consequence of the accumulation of unique adaptations (via selection) in an ancestral population, which, in combination with drift, result in the divergence of reproductively isolated gene pools. The mechanisms by which this process originates are diverse; in effect, many models have been proposed to explain the evolution of phenotypic novelties contributing to species formation (reviewed in Futuyma 1998; Lynch and Walsh 1998; Schlichting and Pigliucci 1998). Recently, there has been a reawakening of scientific interest into the role of polyploidy, and gene duplication more generally, as a creative evolutionary force (Soltis and Soltis 1999; Otto and Whitton 2000). The ubiquity of polyploid plant species derived by hybridisation (Cronn et al.. 1999; Otto and Whitton 2000), as well as evidence for at least two duplication events early in vertebrate evolution (e.g. *Hox* gene clusters; Sidow 1996; Holland 1998; Meyer and Malaga-Trillo 1999) supports the involvement of genome duplication in many successful adaptive radiations.

Members of the family Salmonidae represent important vertebrate taxa for the study of evolutionary diversification following polyploidy. The persistence of species that are still undergoing diploidisation allows for comparative studies of gene function (QTL), the evolution of homeologues (ancestrally duplicated segments) and chromosome rearrangement. Further, although there is considerable uncertainty about the phylogeny of salmonid species, *Salvelinus, Salmo* and *Oncorhynchus* diverged as little as 10-16 million years ago (Grewe et al.. 1990; Phillips et al.. 1992; Pleyte et al.. 1992; Andersson et al.. 1995; Oakley and Phillips 1999), and flourish despite having adopted many different evolutionary strategies. Thus, they provide a unique opportunity for gaining an understanding of the speciation process. This study suggests that gene location and

general function have been conserved in rainbow trout and Arctic charr over a significant period of time, despite extensive alteration in chromosome structure. The mechanisms by which phenotypic differences among taxa arise remain to be elucidated, but may result from more complex gene interactions (epistasis) or changes in expression. Recent work in *Tribolium* beetles suggests that epistasis may accelerate adaptive divergence among populations due to variable genetic responses to selection in different demes (Wade 2000). Furthermore, founder-flush episodes may be critical to the release of additive genetic variance from epistatic variance (Cheverud et al.. 1999), thereby altering genetic correlations among fitness traits, alleviating antagonistic pleiotropy and potentially allowing the evolution of divergent phenotypes (Bradshaw and Holzapfel 2000). Lukens and Doebley (1999) argue that selection on a gene complex was important in the evolution of maize from a teosinte-like ancestor, and that phenotypic differentiation was accomplished via epistasis and regulatory differences between alleles at two QTL affecting organ growth. These studies indicate the importance of interactions among genes in the speciation process, and highlight the need for comparative functional genetic analyses at conserved QTL loci in salmonids.

The fate of loci after a duplication event is still a matter of debate. Accumulation of mutations in one gene copy can result in pseudogenisation (silencing) of the gene, with general function maintained by the duplicate. Alternatively, subfunctionalisation is suggested as a mechanism by which duplicate genes are preserved in most genomes. Degenerative mutations at each locus result in the complementary expression of both genes in order to maintain ancestral gene function (Lynch and Force 2000a). Given a sufficient period of time for selection to operate, small enough population sizes, and

limited pleiotropy (Lynch and Force 2000a; Wagner 2000), duplicated genes may also acquire novel functions. Preliminary data in rainbow trout (O'Malley 2001) and Arctic charr (this study) suggest functional conservation of fitness QTL on homeologues within species. Subfunctionalisation may be revealed as a common mode of duplicate gene persistence in salmonids. Rescan et al.. (1999) show that duplicate MyoD genes are differentially expressed in the developing somite in rainbow trout, which may be suggestive evidence for this model.

The genomic flexibility inherent in chromosomal rearrangement may also provide an arena for the evolution of innovation. Reduction in chromosome number via Roberstsonian fusions is generally considered to be a derived state, and has been documented in multiple animal groups including marsupials (Svartman and Vianna-Morgante 1998) and many fishes (reviewed in Galetti et al.. 2000). Karyotype variation in salmonid species is extensive (Phillips and Ihssen 1985; Hartley 1991), suggesting a role for chromosome evolution in speciation following tetraploidisation. Fusion of acrocentrics to form metacentrics has resulted in a reduced number of chromosomes in *Oncorhynchus* (2n=58-64; 12-24 acrocentrics) relative to the inferred ancestral state (2n~50; all acrocentrics), while maintaining a relatively constant number of chromosome arms (NF~100; Allendorf and Thorgaard 1984; Lockwood and Derr 1992). In contrast, species of *Salvelinus* are characterised by larger numbers of chromosomes, consisting mainly of acrocentrics (2n=78-80, 58-60 acrocentrics; Phillips and Ihssen 1985; Hartley 1991; Frolov 1995;). Segregation distortion of certain metacentrics over acrocentric homologues during male meiosis in the common shrew (*Sorex araneus*) suggests that structural or functional constraints favour genome compaction (Wyttenbach et al.. 1999).

However, environmental heterogeneity may promote the maintenance of chromosome polymorphism (Wójcik et al. 1996). Unfortunately, it is difficult to determine the selective advantage, if any, of karyotype evolution. Nonetheless, recent evidence suggests that chromosomal context may affect gene expression and activity (Graham et al. 1998; Festenstein 1999). Moreover, Weiler and Wakimoto (1998) have shown that chromosomal rearrangements juxtaposing hetero- and euchromatic blocks altered the expression of viability genes. Although it is currently impossible to differentiate between genic and structural differences in the adaptive potential of loci in salmonids, the results from these studies suggest that it may be informative to examine the chromosomal context of genes on homologous acrocentric and metacentric chromosomes in various species.

The high intraspecific genomic diversity characteristic of salmonids species may suggest that they are still experiencing a period of genetic transilience. Transilience has been proposed as a mechanism for chromosomal speciation in rodents (Ortells and Barrantes 1994), whereby karyotypic variation among populations leads to reproductive isolation. Such a mechanism may have contributed to salmonid speciation, since polyploidy would allow for a relaxation of genetic constraints (Soltis and Soltis 1999), and fragmented ancestral populations may have persisted in refugia during the last glaciation. Arctic charr in particular are highly polymorphic in terms of chromosome banding, as well as in the number, size and position of nucleolar organiser regions (NOR) (Phillips and Ihssen 1985; Hartley 1991; Reed and Phillips 1997). Variability in NOR length and chromosome arm number have been documented in *Salmo trutta lacustris*, but are generally confined to a single chromosome (Castro et al. 1997; Woznicki et al. 1997).

Phillips and Ihssen (1985) have proposed that the greater number of NOR in *Salvelinus* species is due to chromosomal dispersion of NOR-bearing chromosomes beyond the two that would have resulted from duplication. However, Frolov (1995) suggests that the presence of multiple NOR represents a basal character derived from salmonids' tetraploid ancestry. The latter is supported by an increased number of NOR in the polyploid-derived sygnathid *Nerophis ophidion* compared to related diploid taxa (Vitturi et al. 1998). Thus, chromosomal polymorphisms may be typical of polyploid-derived organisms, and suggest genomic flexibility.

The observation that increased numbers of NOR correlate with greater cell volume (Phillips and Ihssen 1985), and that the intensity of NOR staining is proportional to rDNA expression (Zurita et al. 1999) may point to an adaptive role for such chromosome polymorphisms. Increased cell volume may be advantageous in cold or harsh environments due to its effects on metabolic processes, and has been suggested as an explanation for the success of polyploids at high altitudes (Otto and Whitton 2000). This environmental effect has been demonstrated in plants, for which genome size is proportional to the length of the chromosome complement. Caceres et al. (1998) further suggest that intraspecific variation in genome size can explain phenotypic plasticity in morphological traits. Intraspecific variation is also reported to be greater than interspecific variation in genome size among salmonids, the basal coregonines possessing the greatest nuclear DNA content (Lockwood and Derr 1992). Interestingly, Arctic charr show similarly large genome sizes (Hartley 1990), as well as large variability between sympatric morphs (Hartley et al. 1992). Thus, the adaptive functional and ecological basis of genome size variation deserve further attention.

The observation that the maximum genome size is related to maternal genome size in plants (Caceres et al. 1998) provides a tantalising link to the relationship between thermal habit, metabolic rate and mtDNA genome size in ectothermic vertebrates (reviewed by Rand 1994). Arctic charr are the exclusive mitochondrial donor in hybridisations with *Salvelinus fontinalis* (Glémet et al. 1998) and *Salvelinus namaycush* (Wilson and Bernatchez 1998) in Canada, which has been suggested to result from a selective advantage of "cold" Arctic charr haplotypes in more southerly distributed salmonid species. Given that Nauyuk Lake and Fraser River strain charr are phenotypically (Gyselman 1994 and others), mitochondrially (Brunner et al. 2001) and genomically differentiated (e.g. NOR number and position; Phillips et al. 1988), it was unexpected to find that families with Nauyuk mothers were the most temperature tolerant. Although the effects of founder events cannot be ruled out, it is tempting to speculate that there was an adaptive reason for the fixation of the different mitochondrial lineages in the Arctic and Labrador strain charr (see Ballard and Kreitman 1995 for selection on mtDNA).

Speciation by hybridisation, although commonly associated with allotetraploidy in plants, is a contentious topic where animals are concerned. Lynch and Force (2000b) provide a model for hybrid inviability due to the accumulation of deleterious mutations at duplicated loci, which may be of particular importance to salmonids. Although certain types of interspecific crosses are sterile due to preferential chromosome elimination (Fujiwara et al. 1997), and segregation distortion has been documented even in viable intraspecific hybrids (Poteaux et al. 2000), hybridisation may have had an important "diversifying" role in salmonids post-duplication. This may explain why molecular and morphological variation in *Salvelinus* confounds attempts to resolve taxonomic

relationships among "species" of the complex, in particular *S. malma* and *S. alpinus* in North America (Frolov 1995; Hamada et al. 1998 and many others). Thus, hybrids, by virtue of possessing two genomes, represent a unique opportunity for characterising species-isolating factors. However, the usefulness of hybrids will depend on the degree of both phenotypic and genomic divergence between the parental stocks. The detection of QTL in the F1 and backcross interstrain hybrids used in this study suggests that genes with effects on fitness segregate both within and between populations of Arctic charr. However, fitness loci of adaptive interest (i.e. major genes) would be most difficult to detect due to the potential loss of truly deleterious genes (ie no viability of that particular genotype) in hybrids. For instance, Rieseberg et al. (1999) identified chromosomal blocks with genes or rearrangements (including translocation breakpoints and inversion polymorphisms) that reduced fertility in a natural sunflower hybrid zone. Although these regions may represent reproductive and genetic isolating factors between parental species, the observation that certain linkage group segments were underrepresented in laboratory crosses relative to wild hybrids suggests that measures of selection may be underestimated under experimental conditions. It would be of great interest to assess rates of introgression using a similar method in natural hybrids between the Labrador and Arctic lineages of Arctic charr.

The usefulness of QTL mapping to our understanding of genetic differentiation is exemplified by the results obtained in this study for Arctic charr. Quantitative variation for traits such as body size parameters, stress tolerance, sex ratio or maturational status in natural populations is the raw material upon which selection may act to promote diversification. In Arctic charr, individuals within populations are known to segregate

based on the life history strategy they have adopted, and may differ in spawning time and site, age of seaward migration and growth rate (Noakes et al. 1989; Strand and Heggberget 1994; Klemetsen et al. 1997; Adams et al. 1998; Gíslason et al. 1999). For instance, changes in temporal or spatial expression of QTL for body size or condition factor may lead to the formation of dwarf and normal morphs via environmentally-dependent cues on growth rates. If GSI reflects maturation status, then segregation and reproductive isolation between stocks within a single body of water could ensue. Similarly, local adaptation of thermotolerant populations may occur given rapid response to environmental stress through individual variation at metabolic genes. Such high variability within populations may lead to significant genetic and phenotypic differentiation between isolated populations. Orr (1998) suggests that adaptive differences between taxa can be estimated using a sign test, when the number of QTL that have been identified and the phenotypic difference between taxa are known. This has proven to be useful in QTL studies examining male secondary sexual traits in *Drosophila* species, for which directional sexual selection leads to conspicuous interspecific differences in male genital morphology (Macdonald and Goldstein 1999). Future studies examining genetic and phenotypic differentiation in Arctic charr should apply a similar methodology. However, this study shows that genetic correlations among quantitative traits due to pleiotropy or linkage may produce complicated patterns of intraspecific variation. Thus, in combination with candidate gene analysis, it may be most informative to investigate environmentally induced patterns of gene expression at loci controlling traits of evolutionary interest.

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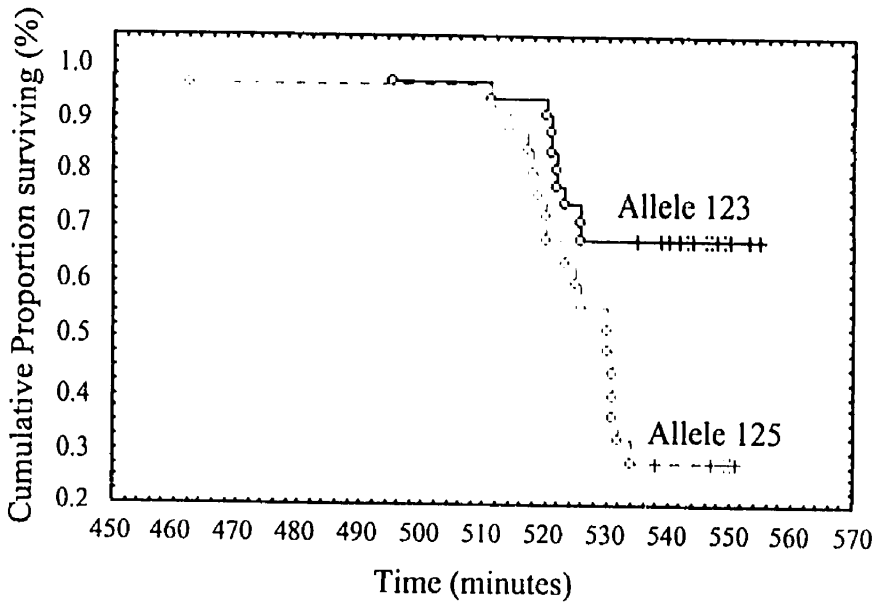
Appendix. Table 1. Data used to calculate the survival function for SsaBHMS491 in groups 1 (allele 123) and 2 (allele 125) in F1 hybrid family 27-139. The dashed line indicates when censoring occurred: all individuals (dead + censored) are used to calculate the survival function prior to censoring, where censoring is defined as the midway point (when 50% of all fish have lost equilibrium) in the experiment within each family. Individuals that survive past the midpoint are termed censored, and given a value of 100% survival below the dashed line because they are all alive at the censoring point. This forces the cumulative survival function to become fixed at the % survival attained at censoring for the group (i.e. 67.7% of individuals are still alive midway through the experiment for group 1). # entered = number of individuals in each group minus the number dead or censored at that time. # censored = number of individuals alive after the experiment's halfway point, # dead = number that die in the given interval in each group prior to the censoring point; % survival = percentage of individuals that are alive in the interval; cumulative % = cumulative survivorship through time.

| Lower limit of failure interval | # entered group 1 | # entered group 2 | # censored group 1 | # censored group 2 | # dead group 1 | # dead group 2 | % survival group 1 | % survival group 2 | cumulative % survival group 1 | Cumulative % survival group 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| 462.0 | 31 | 25 | 0 | 0 | 0 | 1 | 100.0 | 96.0 | 100.0 | 100.0 |
| 472.3 | 31 | 24 | 0 | 0 | 0 | 0 | 100.0 | 100.0 | 100.0 | 96.0 |
| 482.7 | 31 | 24 | 0 | 0 | 0 | 0 | 100.0 | 100.0 | 100.0 | 96.0 |
| 493.0 | 31 | 24 | 0 | 0 | 1 | 0 | 96.8 | 100.0 | 100.0 | 96.0 |
| 503.3 | 30 | 24 | 0 | 0 | 1 | 1 | 96.7 | 95.8 | 96.8 | 96.0 |
| 513.7 | 29 | 23 | 0 | 0 | 6 | 7 | 79.3 | 69.6 | 93.5 | 92.0 |
| 524.0 | 23 | 16 | 0 | 0 | 2 | 9 | 91.3 | 43.8 | 74.2 | 64.0 |
| 534.3 | 21 | 7 | 7 | 1 | 0 | 0 | 100.0 | 100.0 | 67.7 | 28.0 |
| 544.7 | 14 | 6 | 13 | 6 | 0 | 0 | 100.0 | 100.0 | 67.7 | 28.0 |
| 555.0 | 1 | 0 | 1 | 0 | 0 | 0 | 100.0 | 100.0 | 67.7 | 28.0 |

Appendix. Table 2. Estimate of survival function for SsaBHMS491 in F1 hybrid family 27-139. Dinstinct ordered failure times (time of death ordered from earliest to latest), number of individuals who have survived at least until distinct failure time (risk set =R(I)), number of individuals who fail at each distinct failure time (M(I)), and the ratio of the two (M/R) are shown. KAP/MEIR is the survival function; -- indicate that the censoring point (midway point during the experiment within a family) has been reached.

| Distinct failure times | R(I) | M(I) | M(R) | KAP/MEIR |
|---|---|---|---|---|
| 462 | 56 | 1 | .018 | 1 |
| 495 | 55 | 1 | .018 | 0.98 |
| 511 | 54 | 2 | .037 | 0.96 |
| 514 | 52 | 1 | .019 | 0.93 |
| 517 | 51 | 1 | .020 | 0.91 |
| 518 | 50 | 1 | .020 | 0.89 |
| 519 | 49 | 1 | .020 | 0.88 |
| 520 | 48 | 3 | .063 | 0.86 |
| 521 | 45 | 2 | .044 | 0.80 |
| 522 | 43 | 2 | .047 | 0.77 |
| 523 | 41 | 2 | .049 | 0.73 |
| 525 | 39 | 1 | .026 | 0.70 |
| 526 | 38 | 3 | .079 | 0.68 |
| 530 | 35 | 2 | .057 | 0.63 |
| 531 | 33 | 3 | .091 | 0.59 |
| 532 | 30 | 1 | .033 | 0.54 |
| 534 | 29 | 1 | .034 | 0.52 |
|  | -- | -- | -- | 0.50 |

Appendix. Figure 1. Cumulative proportion of individuals surviving in two allele classes at SsaBHMS491 (female data) in F1 hybrid family 27-139 using the Kaplan Meier Product limit estimator. Group 1 refers to progeny inheriting the 123 allele, while group 2 refers to progeny inheriting the 125 allele. Complete data indicate that individuals "died" (lost equilibrium) prior to censoring, while censored data indicate that individuals survived past the censoring point (defined as the midway point of the experiment in each family). + indicates censored individuals, while o indicates complete observations (individuals that have died).


Cumulative Proportion surviving (%)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
Allele 123
Allele 125
450 460 470 480 490 500 510 520 530 540 550 560 570
Time (minutes)