QUANTITATIVE TRAIT LOCI FOR SPAWNING TIME AND BODY WEIGHT IN RAINBOW TROUT: TESTING FOR CONSERVED EFFECTS ACROSS ANCESTRAL HOMEOLOGUES

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ABSTRACT

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I incorporated 53 microsatellite markers into an existing data set of 132 markers to construct an updated genetic linkage map of rainbow trout (*Oncorhynchus mykiss*) spanning 26 linkage groups. Synteny of duplicated markers was used to confirm eight homeologous chromosome pairs. I detected eleven spawning time QTL and nine body weight QTL using segregating maternal and paternal alleles at 185 microsatellite loci. Spawning time QTL were conserved across two homeologous pairs with strong indication for three additional pairs. Body weight QTL were conserved across a single homeologous pair with compelling evidence for two additional pairs. Conservation was not universal as three spawning time and two body weight QTL were detected on a single homeologue. Three spawning time and four body weight QTL were detected on linkage groups with unknown homeologous relationships. This suggests that some duplicated genes controlling spawning time and body weight have been preserved in rainbow trout.

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CHAPTER 1:

GENERAL INTRODUCTION

Gene mapping is the fundamental approach towards reconstructing and deciphering the genetic blueprint of an organism. Localizing and defining individual genes will enhance our understanding of genome structure and organization. Moreover, detailed genetic maps can be used for comparative analysis to assess the extent of conserved synteny (the linkage or chromosomal association of two or more gene homologues in maps of compared species) thus providing new insights into genome evolution (O'Brien et al. 1999).

The idea of gene mapping was first stimulated by Thomas Hunt Morgan's observation that the recombination fraction between two loci increases with the distance between them. Based on this premise, the first genetic map covering six loci was constructed in *Drosophila* by Alfred Sturtevant, a 19-year-old college student working in Morgan's laboratory (Sturtevant 1913). The statistical foundations of gene mapping were subsequently established by Haldane, Hogben, Fisher, Penrose, Smith, and Morton (Morton 1955). The lack of genetic markers, however, hampered linkage analysis for more than a half century. The recent advent of new genetic markers has greatly accelerated mapping efforts in a wide range of taxa.

Advances in molecular biology techniques have led to the development of a variety of marker types. Nuclear markers such as allozymes, randomly amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs) and variable number of tandem repeat loci (VNTRs) have all been used to develop linkage maps (May and Johnson 1990; Postlethwait et al. 1994; Young et al. 1998; Shimoda et al. 1999). Each marker system, however, varies in applicability and success. Allozymes are relatively inexpensive but inadequate because of their lower levels of polymorphism.

RAPDs are of limited value since they show dominance and demonstrate poor repeatability (Ferguson and Danzmann 1998). AFLPs can be used to produce a genetic map within a relatively short period of time yet they appear to cluster around the centromere in some organisms thus limiting their usefulness (Ferguson and Danzmann 1998). Currently, genetic mapping efforts are focused on VNTR loci (Rohrer et al. 1996; Kappes et al. 1997; De Gortari et al. 1998; Shimoda et al. 1999). VNTR loci, which include minisatellites and microsatellites, consist of short, tandemly repeated DNA sequences distributed randomly throughout the genome (O'Connell and Wright 1997). Microsatellites, or simple sequence repeats (SSRs), are powerful single locus markers for a variety of genetic studies (Queller et al. 1993). Microsatellites are thought to occur approximately once every 10kbp (Wright 1993), which indicates their value as a tool in genome mapping studies. They are co-dominantly expressed (allowing the differentiation of heterozygotes and homozygotes) and easily amplified using the polymerase chain reaction (PCR). Also, microsatellite markers are hypervariable, resulting in the detection of all four segregating alleles (including null alleles) in the progeny.

Microsatellite genetic maps are constructed through the detection of linkage disequilibrium between DNA marker loci. In pairwise determination, linkage is indicated by an excess of progeny expressing either parental genotype at the two loci of interest. The frequency of non-parental types provides an estimate of recombination and therefore the distance between two loci on a chromosome (Poompuang and Hallerman 1997).

Genetic linkage mapping is not only the fundamental strategy to understanding the evolution of the genome but also in identifying critical regions affecting traits of

phenotypic and economic importance. Contrary to simple Mendelian inheritance, most fitness traits are controlled by many loci. Identifying and localizing positions on the chromosome affecting polygenic traits has been a primary objective of several mapping projects (Barendse et al. 1994; Crawford et al. 1995; Rohrer et al. 1996; Kocher et al. 1998). The first detection of quantitative trait loci (QTL) was for bunt and rust resistance in wheat (Sax 1923). Since that initial detection, QTL have been mapped in a variety of species including plants (Mohan et al. 1997), pigs (Andersson et al. 1994), cattle (Arranz et al. 1998), chickens (Vallejo et al. 1998) and fish (Jackson et al. 1998).

Essentially, there are two methods of QTL mapping – candidate locus and genome scan (Cheverud and Routman 1993; Tanksley 1993). The candidate locus approach investigates loci that have a known biochemical or physiological relationship with the trait and has proven successful in cattle, sheep, swine and poultry (Haley 1995). This method, however, is limited by the number of loci available and genome coverage. In contrast, genome scans, also known as the marker locus approach, incorporate a large number of loci thus saturating the genome to provide high resolution. The marker locus approach is the optimal method for surveying the entire genome in search of unknown QTL in phenotypically divergent crosses (Cheverud and Routman 1993).

Through single marker analysis, QTL effects can be estimated as the difference between phenotypic means of offspring inheriting alternative marker alleles from a heterozygous parent. If the QTL is not closely linked with the marker, it is difficult to link a QTL location to its effect (Lander and Botstein 1989). Least Squares (LS) interval mapping alleviates this problem by incorporating two flanking markers to locate QTL.

This method is ideal when a high-resolution linkage map is available (Cheverud and Routman 1993; Tanksley 1993).

The construction of a dense linkage map is fundamental to the accurate detection of QTL. According to Soller and Brody (1976), a segregating QTL allele can be mapped accurately to a marker locus occurring within approximately 20 cM along the chromosome. However, characterization of loci with small phenotypic effects requires even higher resolution mapping (1-2 cM average interval).

Ultimately, QTL mapping studies provide evidence to examine the two models proposed to explain the genetic architecture of polygenic characters. Fisher's infinitesimal model states that quantitative traits are controlled by a very large number of loci, each with small phenotypic effect (Fisher 1930). In contrast, the oligogenic model describes phenotypic variation as the result of a few loci with very large effects (reviewed by Tanksley 1993). Estimating the number of loci segregating for a character and the distribution of allelic effects will elucidate the nature of quantitative variation. In particular, one can examine the role of epistasis in evolutionary change, the genetic response to phenotypic selection and the effect of pleiotropy on fitness within populations (Cheverud and Routman 1993).

Polyploidy has proven to be a common and successful evolutionary transition in many plant and some animal lineages yet the creative role of polyploidy in evolution remains a mystery (Otto and Whitton 2000). Some argue that "polyploidy has contributed little to progressive evolution" (Stebbins 1971), while others claim that polyploidization events have been an important evolutionary force in the origin of vertebrates (Ohno 1970; Schultz 1980). Evidence indicates that polyploidization can

produce genetic shifts in systems potentially increasing evolutionary diversification. However, conclusive results that polyploidy has altered rates of diversification remain insubstantial (Otto and Whitton 2000).

The general conclusion that at least two rounds of genome duplication probably coincided with the appearance of vertebrates is well supported by the observation that the number of paralogous (arising from duplication in an ancestor, followed by speciation) gene clusters in mammals is generally four (Aparicio 2000). For instance, mammals possess four Hox complexes in comparison to the one Hox complex found in *Amphioxus* (Bailey et al. 1997). The increase in genetic complexity following a polyploidization event is thus believed to promote organismal complexity and diversification (Ohno et al. 1967; Ohno 1970; Iwabe et al. 1996). Furthermore, evidence of an extra set of Hox complexes in two bony fishes suggests that a more recent genome duplication event occurred after the divergence of teleosts from the tetrapod lineage (Aparicio et al. 1997; Prince et al. 1998).

The evolutionary fate of duplicate gene pairs arising subsequent to a polyploidization event has been widely discussed. Haldane (1933) first suggested that one member of a duplicate pair would eventually be silenced by mutation, while the other locus continued to perform the original function. On rare occasions, a beneficial mutation would cause divergence in function between duplicate genes thus preserving both copies. However, estimates indicate that about 99% of duplicate genes would devolve into pseudogenes by this process (Walsh 1995).

Data from a variety of ancient polyploids suggest, however, that a much larger proportion of duplicate gene copies are preserved than predicted by the classical model.

The fraction of genes retained in duplicate has been estimated as ~8% in yeast over ~100 MY (Seoighe and Wolfe 1999), ~72% in maize over ~11 MY (Ahn and Tanksley 1993), ~77% in *Xenopus* over ~30 MY (Hughes and Hughes 1993), ~70% in salmonids over 25-100 MY (Bailey et al. 1978), ~47% in catostomids over ~50 MY (Ferris and Whitt 1979), and ~33% in vertebrates over ~500 MY (Nadeau and Sankoff 1997).

The persistence and ubiquity of genomic redundancy has lead evolutionary biologists to reexamine the selective forces acting on duplicate genes (Otto and Whitton 2000). Force et al. (1999) suggest that degenerative mutations may increase rather than decrease the probability of duplicate gene conservation. The duplication-degeneration-complementation (DDC) model predicts that the common mechanism of preservation is the partitioning of ancestral functions rather than the evolution of novel functions. Alternatively, instances may exist in which there is positive selection for the maintenance of multiple copies of genes (Clark 1994; Nowak et al. 1997). Ultimately, the reason why genes with overlapping functions have been retained, in some cases for hundreds of millions of years, remains elusive.

Fish in the family Salmonidae are believed to be descended from a single taxon, which underwent chromosome replication without cell division (autopolyploidy), about 25-100 MY (Allendorf and Thorgaard 1984). Four features of the salmonid genome provide convincing evidence for an ancestral tetraploid event. Salmonids have approximately twice the amount of DNA per cell as closely related fish and about 80% as much DNA as mammals. The genome consists of about 100 chromosome arms (58-65 chromosomes), which is doubled that of closely related species. Multivalents have been

observed during meiosis while numerous duplicated enzyme loci have been detected throughout the genome (Ohno 1970).

Immediately following genome duplication, an autotetraploid lineage is expected to demonstrate tetrasomic chromosome segregation. Over time, however, diploidization of the genome occurs and disomic segregation becomes prevalent (i.e. four homologues begin to pair as two pairs of chromosomes) (Allendorf and Danzmann 1997). Disomy permits the structural and regulatory divergence of the newly duplicated gene loci. The general trend since the tetraploid event has been a reduction in chromosome number by centric fusion, while conserving the chromosome arm number at about 100 (Allendorf and Thorgaard 1984).

Many homeologous chromosome arms still exchange chromatid segments as a result of multivalent formations (Lee and Wright 1981; Wright et al. 1983; Allendorf and Thorgaard 1984). Interestingly, this meiotic event appears to be almost exclusive to male salmonids (Allendorf and Thorgaard 1984; Allendorf and Danzmann 1997). Differential crossovers between homeologous chromosomes result in pseudolinkage (an excess of nonparental types at duplicated loci) and thus may regulate the duplication of some genes and diploidization of others (Sakamoto et al. 2000). These forms of residual tetrasomy suggest that diploidization is still in progress.

Genetic linkage mapping in salmonids began over a decade ago with the first comprehensive map constructed using 54 allozyme loci from several species and their hybrids (May and Johnson 1990). Although this map has provided valuable information, it is of limited utility for some applications because only a few markers were characterized for any one species. Recent progress has lead to the construction of more

detailed linkage maps in rainbow trout, *Oncorhynchus mykiss* (Young et al. 1998; Sakamoto et al. 2000). For instance, a genetic linkage map based on 190+ microsatellites, 3 RAPD, 7 ESMP and 7 allozyme markers has been constructed using three rainbow trout backcross families (Sakamoto et al. 2000). In comparison to other linkage maps, however, this map is still in the initial phase. Shimoda et al. (1999) have produced a zebrafish, *Danio rerio*, genetic map consisting of 2000 microsatellite markers. The average resolution is 1.2 cM (intermarker distance of 3.0 cM), sufficient to initiate positional cloning for most mutant genes.

The rainbow trout linkage map constructed by Sakamoto et al. (2000) is characterized by the largest sex-specific recombination differences for any known vertebrate. Similar patterns have previously been documented in human, mouse, and pig, where the female map distances are usually greater than the male (Dib et al. 1996; Dietrich et al. 1996; Barendse et al. 1994) although the recombination ratios are not as great as those observed in rainbow trout. Due to the sex-specific alignment of chromosomes during meiosis in salmonids (Lee and Wright 1981), female recombination rates appear much higher around the centromere while male recombination rates appear to be higher in telomeric regions. Furthermore, large intrachromosomal differences in recombination rates were evident between mapping families of rainbow trout (Sakamoto et al. 2000).

The microsatellite linkage map can be utilized to identify QTL of both evolutionary and economic significance in rainbow trout such as spawning time and body weight. Rainbow trout are seasonal spawners and each genetic group (stock) has a characteristic season of spawning (Bromage et al. 1992). Spawning season is a critical

component of rainbow trout production since many aquaculture facilities are based on year-round production for the market. Therefore, restricted seasonal spawning can be the limiting factor of production efficiency (Siitonen and Gall 1989). While spawning season has been successfully manipulated (i.e. modified photoperiods), this technique is costly and requires special housing for the broodstock (Billard 1985).

An alternative method to develop broodstocks that spawn at different times of the year is to alter spawning season through genetic selection. Genetic variation has been detected in strains of rainbow trout spawning in different seasons. An analysis of mitochondrial DNA (mtDNA) variation revealed that fish spawning in the fall, winter, and spring were genetically divergent (Ferguson et al. 1993). These results indicate a strong genetic component exists for the determination of this trait. Selection programs designed to advance spawning season in rainbow trout broodstocks have been successful reporting relatively high heritability estimates of 0.5. It is noted that these figures may be biased upwards due to influences of management and selection involving other traits and should therefore be considered as the upper limit (Siitonen and Gall 1989).

Body weight is another important heritable fitness-trait in the life history of salmonid fishes. In general, body weight of rainbow trout has a large phenotypic variation, providing a high potential for genetic improvement by selective breeding (Su et al. 1996). Body weight heritability tends to increase with the age of the fish with pre-yearling estimates more variable than those for post-yearling body weight (Su et al. 1996). The covariation between spawning time and body weight has been examined in several salmonid species. Crandell and Gall (1993a) found that genetic and phenotypic correlations between spawning time and body weight tend to increase with age in

rainbow trout. Low genetic correlations between spawning time and body weight prior to maturity were detected whereas genetic correlations between time of spawning and body weight at maturity were fairly high. Hendry et al. (1999) found a negative correlation between body size and breeding date in a natural population of sockeye salmon, *Oncorhynchus nerka*, where late spawning females were smaller relative to early breeding females.

The purpose of my project was to expand the current rainbow trout linkage map utilizing microsatellite markers and to locate QTL for spawning time and body weight. QTL for upper temperature tolerance (Jackson et al. 1998; Perry et al. in press) and spawning time (Sakamoto et al. 1999) have previously been reported in rainbow trout. My primary objective was to identify additional QTL regions and test for conservation of effects across inferred homeologous chromosome pairs.

CHAPTER 2:

A MICROSATELLITE LINKAGE MAP OF RAINBOW TROUT (*Oncorhynchus mykiss*)

Introduction

Genome mapping is the initial step towards resolving and interpreting patterns of genome evolution (O'Brien et al. 1999). The development of a dense genetic map will facilitate the location, definition and utilization of genes affecting traits of economic and evolutionary significance. Furthermore, comparative genetic analysis expands the utility of these maps in the study of genome organization. Identifying parallels in genome assemblages will provide new evolutionary insight into species and individual variation (O'Brien et al. 1999).

Genetic linkage maps have become prominent research tools in many organisms (Dietrich et al. 1996; Kocher et al. 1998; Gates et al. 1999). A few potential applications of a dense linkage map include quantitative trait locus (QTL) analysis (Lander and Botstein 1989), comparative analysis and localization of genes (Gates et al. 1999), and marker-based selection (Montgomery and Kinghorn 1997).

Genetic linkage is determined by the proportion of recombinants (crossover events) between markers defined as polymorphic coding or non-coding DNA sequences (loci). If alleles of two loci tend to co-segregate, the loci are likely to be linked and thus located on the same chromosome. The more recombination events observed, the larger the distance between the two loci. Efficient linkage analysis depends on the existence of a dense genetic marker map. Therefore, the essential feature of any DNA marker is that it must be highly polymorphic and evenly distributed throughout the genome. Recent technological advances have led to the development of a wide variety of genetic markers. Genetic marker systems commonly used in genome mapping include amplified fragment

length polymorphisms (AFLPs; Young et al. 1998), restriction fragment length polymorphisms (RFLPs; Botstein et al. 1980), and microsatellites (Shimoda et al. 1999).

Microsatellites are ubiquitous in eukaryotes (Mohan et al. 1997) and have proven to be powerful single locus markers for a variety of genetic studies (Queller et al. 1993). Microsatellites consist of short (1-6 base pair) tandem arrays (Tautz and Renz 1984). Microsatellite markers are codominantly expressed, evenly distributed throughout the genome, and surveyed rapidly in many individuals using PCR techniques (Lee and Kocher 1996; Knapik et al. 1998). Also, microsatellite markers are hypervariable, which often results in the detection of all four segregating chromosome regions (including null alleles) in the progeny.

Rainbow trout, *Oncorhynchus mykiss*, is one of the most intensively studied fish species because of its economic importance (Stickney 1991) and as a model for genome evolution following tetraploidization (Ohno 1970). Initial mapping efforts relied mainly on allozyme loci for several salmonid species and their hybrids (May and Johnson 1990). The development of molecular techniques that identify nucleotide-level DNA sequence polymorphisms between individuals has created an unlimited source of genetic markers (Botstein et al. 1980; Vos et al. 1995). Subsequently, more detailed linkage maps have recently been constructed in rainbow trout (Young et al. 1998; Sakamoto et al. 2000).

As a member of the family Salmonidae, rainbow trout have evolved by tetraploidization from a diploid ancestor (Allendorf and Thorgaard 1984). Evidence of tetrasomic inheritance suggests that the process of diploidization is not yet complete. Many homeologous arms still exchange chromatid segments during meiosis. Interestingly, this event appears to be almost exclusive to males (Allendorf and

Danzmann 1997). The formation of multivalents likely obstructs crossovers proximal to the centromere thus facilitating the diploidization of loci located in this region (Sakamoto et al. 2000). Alternatively, crossovers between homeologous chromosomes in the telomeric region preserve duplicated gene regions. Differential chromosomal pairing has been postulated to account for the unusually large differences in recombination rates between the sexes.

A rainbow trout linkage map has previously been constructed incorporating segregation data from three experimental backcross families (Sakamoto et al. 2000). In salmonids, microsatellite markers are often conserved among closely related species (Morris et al. 1996; Sakamoto et al. 1999). Thus, microsatellite markers from Artic charr (*Salvelinus alpinus),* Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), chinook salmon (*Oncorhynchus tshawytscha*), cutthroat trout (*Oncorhynchus clarkii*), pink salmon (*Oncorhynchus gorbusha*), and sockeye salmon (*Oncorhynchus nerka*) are located within the rainbow trout map. In this study, segregation data from the backcross family selected for spawning time was used to create a male and female-specific microsatellite linkage map. I integrated information from 53 microsatellite markers into the existing data (Sakamoto et al. 2000) to assemble a genetic linkage map consisting of 26 linkage groups. Evidence for differences in recombination rates between the sexes is provided.

Materials and methods

Source of the fish

The spring and fall spawning strains used in this study were reared by the Ontario Ministry of Natural Resources (OMNR), at Maple, Ontario, Canada. In 1990, a spring spawning male (Ganaraska River strain) was crossed to a fall spawning female (Blue

Springs commercial strain) to produce a hybrid family. In 1992, an F_1 hybrid male was backcrossed to a fall spawning female to produce the backcross family (Lot 44) used to construct the microsatellite linkage map. Neither the spring spawning nor the fall spawning strain was an inbred line so the genotypes of the backcross parents were not expected to be completely homozygous. Similarly, the F_1 (S x F) parent was not completely heterozygous for the marker loci examined.

Microsatellite analysis

Genomic DNAs were extracted from liver tissue from 90 backcross progeny and the sire by the method of Bardakci and Skibinski (1994). Genotypes at 53 microsatellite markers were scored in this study. PCR was performed in an 11 ul reaction volume containing 5 pmol of unlabeled primer, plus 0.375 mM of each dNTP, 4 mM labeled TAMRA dCTP, 20 mM Tris-HCL (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 $\mu g \cdot ml^{-1}$ BSA, 0.25 unit *Taq* DNA polymerase and 30 ng template DNA. The PCR program consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles consisting of 30 s at 95 °C, 1 min at the annealing temperature, 1 min at 72 °C, 30 s at 95 °C, and a final extension of 10 min at 72 °C. PCR products were separated in a 6% polyacrylamide-7-M urea gel and the resulting DNA fragments were visualized with a Hitachi FMBIOII fluorescence imaging system. Allele base pair size was determined using 350-Tamra lane standard (Figure 2.1).

Genetic nomenclature

The naming of microsatellite markers follows the standard proposed by Jackson et al. (1998) and implemented in Sakamoto et al. (2000). The label begins with a three-letter acronym usually specifying the species (i.e. Omy = *O. mykiss*) followed by a lab-

specific term and a suffix acronym indicating the lab of origin (Table 2.1). When a primer pair has been published, the adopted format is that designated by the respective lab.

Linkage groups labeled with Arabic numbers represent those previously described by May and Johnson (1990) using allozyme markers. Linkage groups described after May and Johnson (1990) are designated alphabetically. Potentially homeologous linkage groups are designated with a lowercase i or ii following the assigned letter. Similarly, duplicated microsatellite markers detected with a single pair of primers are indicated by a forward slash and a lowercase i or ii to distinguish each separate locus.

Linkage analysis

A Visual Basic program, LINKMFEX (Danzmann www.uoguelph.ca/~rdanzman), was used to perform linkage analysis through a series of pairwise comparisons between loci. The analysis was executed using segregation data at 185 microsatellite markers from 90 progeny (Appendix 1). The program considers the segregation of each parental complement of alleles separately to permit the construction of sex-specific linkage arrangements. The program generates two-point recombination estimates among markers. To overcome the complicating factor of pseudolinkage in measuring classical linkage, the program was written to assume that the least abundant pairs of genotypes are the recombinants. Log of Odds ratio (LOD) scores were calculated to determine linkage. A LOD score of 3 or higher is accepted as demonstrating linkage between markers (Botstein et al. 1980). The LOD threshold value of 3 roughly corresponds to the 5% level of significance used in conventional statistical tests. The program, MAPORD (Danzmann www.uoguelph.ca/~rdanzman), was used to determine linear assignments of markers within a

linkage group. MAPORD uses two methods in attempting map construction. Method 1 compares nearest neighbor orientations (NNO) when constructing the linear order of markers in a linkage group. The program designates a marker position as a "source" marker and attempts to build a linear NNO marker order with the principle that the shortest recombination distance to a "test" marker represents the NNO from the source marker. Markers identified as NN are considered the source marker in the next search step, while the source marker used in the previous search is considered the "proceeding marker" position. Method 2 involves arraying all map distances among markers in a matrix and assigning a linear rank order of additive distances from each source marker in turn. The relative position of the source marker in the marker vector is determined by a multipoint assessment of recombination distances among the markers from the source.

Recombination rates

Sex-specific linkage maps were generated as large differences in recombination rates between the sexes has previously been reported in rainbow trout (Johnson et al. 1987; Sakamoto et al. 2000). Estimates of the differences in sex-specific recombination rates along chromosome intervals were calculated using the program RECOMDIF (Danzmann www.uoguelph.ca/~rdanzman). RECOMDIF allows one to compare recombination differences between both parents for markers distributed along a given linkage group. The program gives the results of a contingency G-test (Sokal & Rohlf 1981) testing differences in the actual counts of recombinants and non-recombinants for the pair of markers indicated. Values exceeding 3.84 for 1 df are deemed as showing significant ($p < 0.05$) differences in recombination values between the parents for the marker region being tested.

Results

Segregation data at 53 microsatellite markers was integrated into the existing data of 132 markers (Sakamoto et al. 2000) to construct a genetic linkage map for rainbow trout. Twenty-six linkage arrangements with 170 microsatellite markers were identified using segregation information from the Lot 44 backcross family. The female map spans ~ 992 cM compared to the male map of ~ 545 cM. Six informative markers have been assigned to the male map yet remain unlinked in the female map (Figure 2.2). In addition, there are 15 markers that remain unassigned at a LOD threshold of 3.0 in both sexes.

Several of the microsatellite markers had four alleles instead of two in each parent. It was possible to score these duplicated loci as disomically segregating (Allendorf and Danzmann 1997). Thirty duplicated loci have been mapped to fourteen linkage groups (Figure 2.2). All duplicated loci mapped in female linkage groups with a centromere were located at least 20 cM from the centromere (Oi, 8, R, N, Fi, and 5) (Figure 2.2). The distribution of duplicated markers along the chromosomes arms varied between linkage groups. In three linkage groups, duplicated markers were located in the intercalary region (N [22 cM]; Fi [33 cM]; 5 [20 cM]) whereas duplicated markers mapped in linkage groups R (31 – 67 cM) and 8 (47 cM) appear to be located in the telomeric region. In linkage group Oi, duplicated markers were not located proximal to the centromere (28 – 54 cM).

Conservation of duplicated microsatellite markers had been used to identify nine homeologous relationships between linkage groups mostly in a different mapping family from that used here (Sakamoto et al. 2000). I was able to confirm all homeologous

relationships in this mapping family except for three. For instance, I was able to map Omy27/iiINRA to linkage group Q but was unable to map the duplicate Omy27/iINRA to linkage group G because the locus did not amplify well enough to score the alleles. Similarly, OmyFGT21/iiTUF has been mapped in linkage group K but the duplicate OmyFGT21/iTUF could not be analyzed since it was not variable in the family used here. Also, linkage group T has yet to be identified with microsatellite markers in this mapping family whereas a homeologous relationship between linkage group T and D has previously been identified in the other reference family (Sakamoto et al. 2000). However, homeology can be inferred based on segregating marker data from the other backcross families (Sakamoto et al. 2000). Homeologous relationships have yet to be identified in the remaining ten linkage groups (Figure 2.2).

Differences in sex-specific recombination rates were examined in all linkage groups except U, L, P, 2, D, 18, Fi and Fii. The ratio of female:male recombination rates among all adjacent markers is 3.33:1. Female recombination rates around the centromere were consistently higher than those of males (< 30 cM) (H, Oi, A, R, G, C, D,) (Figure 2.2; Table 2.2). The recombination ratio in regions proximal to the centromere is estimated to be ~ 4.30:1 (female:male). Conversely, male recombination rates appeared to be higher in telomeric regions (> 30 cM) (8, B, N, and J) (Figure 1.2; Table 2.2). For example, male map distances between terminal marker pairs in these four linkage groups are substantially larger than those of female (OmyRGT23TUF and OmyRGT21TUF; OmyFGT2TUF and OmyFGT7TUF; OmyRGT51TUF and OmyRGT47TUF; Ogo8UW and OmyFGT13TUF)(Table 2.2). Male recombination rates

in the telomeric region are not necessarily higher across all linkage groups as the estimated recombination ratio in telomeric regions is ~ 1.3:1 (female:male).

Discussion

I incorporated 53 microsatellite markers into the existing rainbow trout linkage map (Sakamoto et al. 2000) to construct an updated genetic linkage map of rainbow trout. I focused on one of the three backcross families since it was also selected for spawning time and would thus facilitate the future detection of QTL for this trait. The map consists of 26 linkage groups with 19 identified centromeric regions. Fifteen markers remain unassigned at a LOD threshold of 3.0 in both sexes. There are six pairs of chromosomes which show homeology to one another in this mapping family (Sakamoto et al. 2000).

Molecular deciphering of the genome has advanced in a number of species through genetic mapping (Dib et al. 1996; Dietrich et al. 1996; Shimoda et al. 1999). Whether focusing on homologous gene sequences, gene segments, chromosomes, or entire genomes, these blueprints provide new insight into the individual components governing genetic variation. Improved technologies have expanded the study of genome organization beyond the human and mouse to include organisms of distant evolutionary lineages (O'Brien 1999). The rainbow trout linkage map will serve as a template for locating genetic determinants of heritable traits, such as spawning time and body weight (Chapter 3), in addition to examining the patterns of genome evolution following a polyploidization event.

Duplicated loci mapped in the female were not located proximal to the centromere (> 20 cM). The distribution of duplicated loci varied among linkage groups. For instance, duplicated loci appeared to be clustered in the intercalary region of several

linkage groups (N, Fi, and 5) but mapped primarily to the distal region others (8 and R). There were six cases where the relative position of duplicated loci could not be determined as female linkage groups lack gene-centromere mapping information (Oii, U, K, Q, L, and 15). It has been postulated that differential crossovers between homeologous chromosomes may affect the diploidization of some genes and ensure the duplication of others. Crossovers of homeologous chromosome arms in distal regions (secondary tetrasomic inheritance) likely preserve duplicated gene regions (Wright et al.1983; Allendorf and Danzmann 1997). In contrast, multivalent formation limits the number of crossover events proximal to the centromere thus increasing the rate of diploidization among loci (Sakamoto et al. 2000). The observation that duplicated loci were not located proximal to the centromere (< 20 cM) suggests that crossover events may regulate their rate of diploidization. However, duplicated loci were not restricted to the telomeric regions of all chromosomes and in fact are evenly distributed along some chromosome arms.

Conservation of duplicated markers was used to confirm six homeologous pairs of linkage groups (U and H; Oi and Oii; 8 and R; C and L; 5 and 15; Fi and Fii). Three additional homeologous pairs have been identified in a different rainbow trout backcross family (A and K; G and Q; D and T) (Sakamoto et al. 2000). Immediately following genome duplication, a tetraploid lineage is expected to demonstrate tetrasomic chromosome segregation. Over time, however, diploidization of the genome occurs and disomic segregation becomes prevalent (Allendorf and Danzmann 1997). The four ancestral homologues begin to pair as two pairs of chromosomes. Following a tetraploid event there has been a reduction in chromosome number by centric fusion, while the

chromosome arm number has been conserved at about 100 (Allendorf and Thorgaard 1984). Multivalent formation increases the probability of pseudolinkage arrangements (i.e. chromosome arms showing homeology to one another during meiosis in males) which are characterized by the aberrant joint segregation of duplicated loci. These forms of residual tetrasomy suggest that diploidization of rainbow trout loci may still be in progress. Synteny of duplicated loci can thus be used to infer ancestral homeologous relationships among chromosomes pairs and permit the study of genome evolution following a polyploidization event.

Differences in recombination rate were observed between the sexes as female map distances were generally larger than those in the male. However, male recombination rates were higher in the telomeric region of several linkage groups (> 30 cM). Johnson et al. (1987) first provided evidence of large female:male recombination differences among salmonid species using allozymes. More recently, Sakamoto et al. (2000) reported largest sex-specific differences in recombination rates reported for any known vertebrate. Also, large intrachromosomal differences in recombination rates were observed between rainbow trout mapping families (Sakamoto et al. 2000). Differences in recombination rates are thought to arise from the differential sex-specific alignment of chromosomes during meiosis (Lee and Wright 1981). Multivalent formation appears almost exclusively in males (Wright et al. 1983; Allendorf and Thorgaard 1984). The structural constraints imposed by quadrivalent formation affect crossover events thus influencing the recombination levels in male salmonids. If chiasmata are localized to telomeric regions (Wright et al. 1983; Allendorf and Danzmann 1997), then regions proximal to the centromere may experience no crossing over while an exchange of

genetic material between homeologous regions may occur in the telomeric region. Consequently, male recombination levels would be higher in the telomeric region compared to females (Sakamoto et al. 2000).

The work described here is an interim step in the development of a high density genetic map based on microsatellite markers. The map is a valuable resource for locating quantitative trait loci (QTL) affecting traits of economic and evolutionary significance (Chapter 3). Furthermore, identifying homeologous relationships among chromosome pairs will provide new insights into genome evolution following a polyploidization event.

Table 2.1 Sources of the microsatellite primers used in this study

Species Abbreviation	Common name	Scientific name
Coc	Lake Whitefish	(*Coregonus clupeaformis*)
Ocl	Cutthroat trout	(*Oncorhynchus clarki*)
Ogo	Pink salmon	(*Oncorhynchus gorbuscha*)
Omy	Rainbow trout	(*Oncorhynchus mykiss*)
One	Sockeye salmon	(*Oncorhynchus nerka*)
Ots	Chinook salmon	(*Oncorhynchus tshawytscha*)
Ssa	Atlantic salmon	(*Salmo salar*)
Str	Brown trout	(*Salmo trutta*)
Sal	Artic charr	(*Salvelinus alpinus*)

Laboratory abbreviation	Official name and corresponding author or citation reference for primer sequences
ASC	Alaska Science Center (USA), Scribner et al. (1996)
BML	Bodega Marine Laboratory, University of California (Davis), Banks et al. (1999)
CNRS	Centre National de Recherche Scientifique (France), Chantal Poteaux
DIAS	Danish Institute of Agricultural Science, Tjele (Denmark), L-E. Holm, Holm and Brusgaard (1999)
DU	Dalhousie University, Halifax, Nova Scotia (Canada), J.M. Wright; Morris et al. (1996)
INRA	Institut National de la Recherche Agronomique (Jouy-en-Josas, France), K. Gharbi and R. Guyomard
NVH	Norwegian College of Veterinary Medicine (Oslo, Norway), B. Hoyheim
NUIG	National University of Ireland (Galway), R. Powell
NWFSC	Northwest Fisheries Science Center (USA), K. Naish
SSBI	SeaStar Biotech Incorporated (Victoria, British Columbia, Canada), Nelson and Beacham (1999)
TUF	Tokyo University of Fisheries (Japan), T. Sakamoto and N. Okamoto; Sakamoto et al. (1996)
UoG	University of Guelph (Canada), R. G. Danzmann and M. M. Ferguson; Jackson et al. (1998)
UW	University of Washington (USA), Olsen et al. (1998); Condrey and Bentzen (1998)

The acronym prefix for the species of origin with respect to primer design is indicated in the first part of the table.

Lab abbreviation specifies the source of the primer design. Primers are identified according to their lab of origin by the acronym that appears as a suffix or with the primer name given throughout the text.

Table 2.2. Differences in recombination rates between male and female rainbow trout along several chromosome arms possessing an identified centromere.

Linkage group	Marker 1	Marker 2	Ratio F:M	MapDis	Significance
H	BHMS117B	BHMS356	UnDef	5.2	NS
	BHMS356	OmyRGT2TUF	4.54	10.9	P < 0.05
	OmyRGT2TUF	OmyFGT11TUF	UnDef	3.3	NS
	OmyFGT11TUF	One10ASC	UnDef	11.2	P < 0.01
Oi	OmyRGT4TUF	OmyRGT30TUF	UnDef	1.8	0
	OmyRGT30TUF	BHMS184	UnDef	11.8	P < 0.05
	BHMS184	OmyRGT40/iTUF	UnDef	4.4	P < 0.05
	OmyRGT40/iTUF	OmyRGT33TUF	UnDef	2.2	NS
	OmyRGT33TUF	OmyFGT18/iTUF	UnDef	14.5	P < 0.001
	OmyFGT18/iTUF	OmyFGT29TUF	2.07	20.7	NS
A	OmyRGT41TUF	Ogo1UW	UnDef	5.5	NS
	Ogo1UW	Ssa4DU	UnDef	3.3	NS
8	OmyRGT23TUF	OmyRGT21TUF	0.58	8.3	NS
R	Omy3DIAS	OmyRGT17TUF	2.04	4.7	NS
	OmyRGT17TUF	OmyFGT26TUF	5.18	5.7	NS
	OmyFGT26TUF	Omy7INRA	12.82	10.4	P < 0.001
	Omy7INRA	One1/iASC	6.37	25.7	P < 0.001
G	OmyPuPuPy	Ssa85DU	UnDef	6.8	NS
	Ssa85DU	BHMS377	3.00	3.6	NS
	BHMS377	OmyRGT36TUF	20.00	24.0	P < 0.001
	OmyRGT36TUF	One2ASC	UnDef	15.5	P < 0.001
	One2ASC	OmyFGT16TUF	0.60	13.4	NS
C	Ssa289DU	Str58CNRS	1.00	6.2	NS
	Str58CNRS	SSOSL439	1.80	11.2	NS
	SSOSL439	Ssa6.33NUIG	UnDef	2.9	NS
B	OmyFGT27TUF	OmyRGT26TUF	UnDef	3.3	NS
	OmyFGT27TUF	Sal12UOG	3.06	8.1	P < 0.05
	Sal12UOG	OmyP9-2TUF	3.34	11.7	P < 0.05
	OmyP9-2TUF	Omy301UOG	UnDef	5.6	NS
	Omy301UOG	OmyFGT2TUF	3.00	3.3	NS
	OmyFGT2TUF	OmyFGT7TUF	0.50	15.5	P < 0.05
I	OmyFGT34TUF	One3ASC	5.65	21.1	P < 0.001
J	BHMS423	SSOSL311	0.33	2.4	NS
	SSOSL311	OmyFGT12TUF	0.33	1.1	NS
N	OmyFGT28/iiTUF	OmyJTUF	5.00	5.5	NS
	OmyJTUF	Ocl4UW	UnDef	15.6	P < 0.001
	Ocl4UW	OmyRGT14TUF	UnDef	2.4	NS

	OmyRGT14TUF	OmyRGT32TUF	1.00	0	NS
	OmyRGT32TUF	OmyRGT51TUF	0.21	6.6	P < 0.001
	OmyRGT51TUF	OmyRGT47TUF	0	0	NS
M	BHMS418	OmyRGT24TUF	0.50	2.3	NS
	OmyRGT24TUF	OmyRGT34TUF	UnDef	1.1	NS
E	BHMS7.5	SSOSL32	1.00	2.5	NS
	SSOSL32	OmyFGT24TUF	1.00	1.2	NS
5	BHMS254	OmyRGT1TUF	2.02	12.1	NS
	OmyRGT1TUF	OmyFGT8/iiTUF	6.18	6.8	P < 0.05
	OmyFGT8/iiTUF	One18/iiASC	1.00	1.1	NS

Ratio F:M indicates recombination difference for the pair of markers indicated in each respective linkage group. UnDef indicates 0 recombination in the male for the specific interval.

Sig refers to the results from a contingency G-test (1 d.f.) comparing the frequency of parental vs. recombination genotypes between the sexes.

MapDis refers to the distance (cM) between the two markers in the female map (the fraction of observed recombinant genotypes).

Figure 2.1 Allelic variation at microsatellite loci as seen in a 6% polyacrylamide-7-M urea gel with a Hitachi FMBIOII fluorescence imaging system. Allele base pair size was determined using 350-Tamra lane standard and the corresponding sizes for each gel image are listed down the right side. Each vertical lane shows the genotypic information for a single individual. Progeny will inherit one allele from each parent. (A) At Omy3DIAS progeny inherit either the 108 or 115 bp allele from the sire and either the 108 or 131 bp allele from the dam (B) At Str7INRA progeny inherit the either 263 bp allele or the 276 bp allele from the sire and either the 263 bp allele or the 273 bp allele from the dam.

A


115, 131
160 bp
150 bp
139 bp
108, 115
Sire 108, 115
100 bp

B

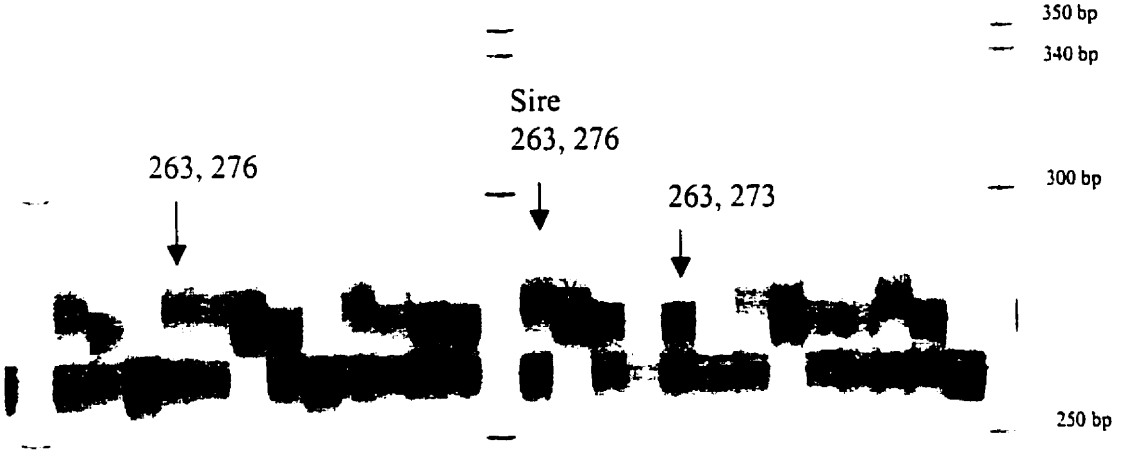
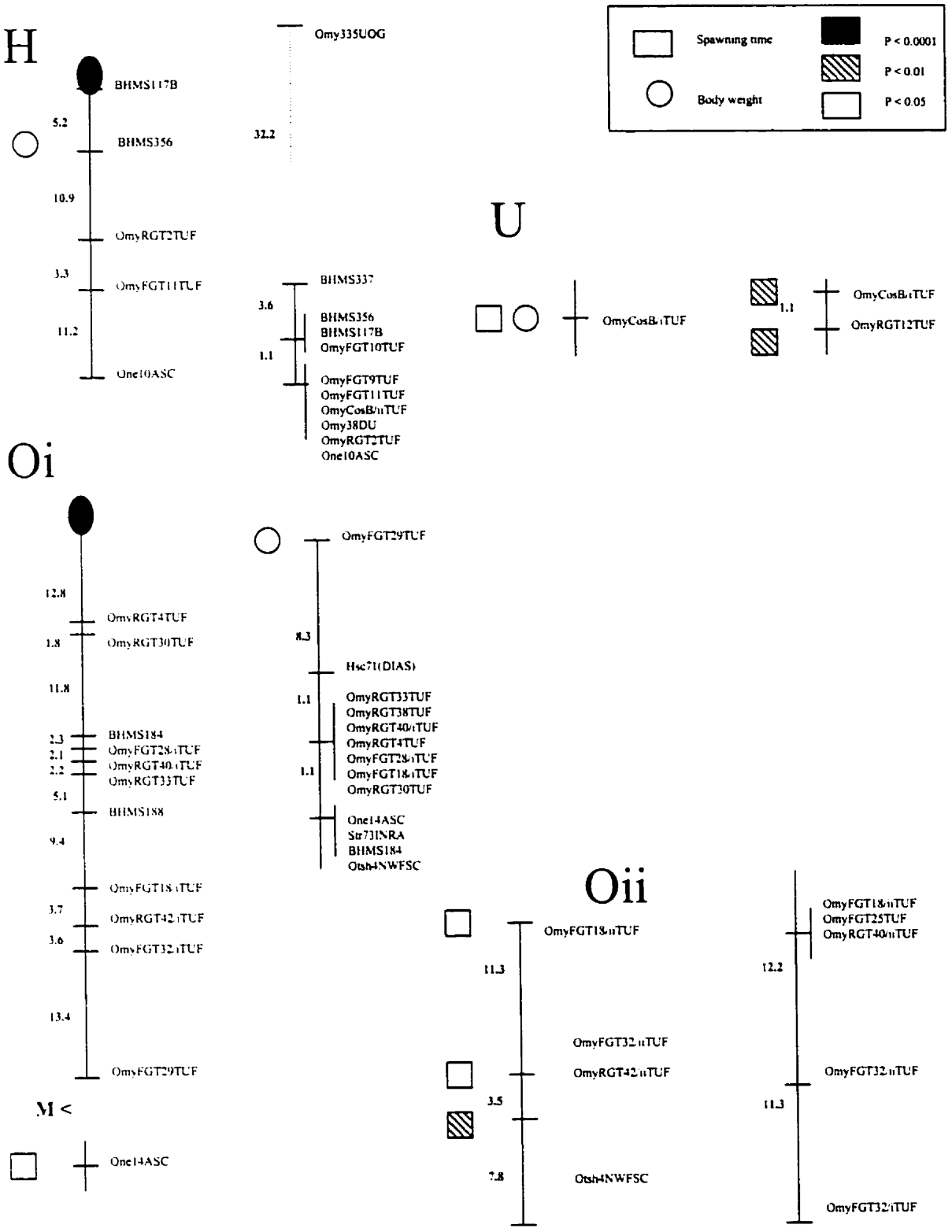

350 bp
340 bp
Sire
263, 276
263, 276
263, 273
300 bp
250 bp

Figure 2.2 Twenty-six comparative female (left) and male (right) linkage groups mapped in rainbow trout using microsatellite markers (Sakamoto et al. 2000; Chapter 2). Numbered linkage groups correspond to those of May and Johnson (1990) based upon syntenic linkages with an identified allozyme marker. Linkage groups identified as showing some homeology to one another because of the presence of duplicated markers are: chromosomes H and U (OmyCosB/iTUF and OmyCosB/iiTUF), chromosomes Oi and Oii (OmyFGT18/iTUF and OmyFGT18/iiTUF; OmyFGT32/iTUF and OmyFGT32/iiTUF; OmyRGT40/iTUF and OmyRGT40/iiTUF; OmyRGT42/iTUF and OmyRGT42/iiTUF), chromosomes 8 and R (OmyRGT15/iTUF and OmyRGT15/iiTUF), chromosomes C and L (OmyRGT6/iTUF and OmyRGT6/iiTUF), chromosomes 5 and 15 (Omy272/iiUOG and Omy272/iUOG; OmyFGT8/iiTUF and OmyFGT8/iTUF; One18/iiASC and One18/iASC) chromosomes Fi and Fii (OmyOGT5/iTUF and OmyOGT5/iiTUF). Markers showing significant associations with values for spawning time and body weight are indicated. The map distance (measured in centiMorgan) between adjacent markers is shown.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5.2
BHMS356
10.9
OmyRGT2TUF
3.3
OmyFGT11TUF
11.2
One10ASC
Omy335UOG
32.2
BHMS337
3.6
BHMS356
BHMS117B
OmyFGT10TUF
1.1
OmyFGT9TUF
OmyFGT11TUF
OmyCosB/uTUF
Omy38DU
OmyRGT2TUF
One10ASC
Spawning time
Body weight
P < 0.0001
P < 0.01
P < 0.05
U
OmyCosB/uTUF
1.1
OmyCosB/uTUF
OmyRGT12TUF
Oi
12.8
OmyRGT4TUF
1.8
OmyRGT30TUF
11.8
2.3
BHMS184
2.1
OmyFGT28/uTUF
2.2
OmyRGT40/uTUF
OmyRGT33TUF
5.1
BHMS188
9.4
OmyFGT18/uTUF
3.7
OmyRGT42/uTUF
3.6
OmyFGT32/uTUF
13.4
OmyFGT29TUF
M <
One14ASC
OmyFGT29TUF
8.3
Hsc71(DIAS)
1.1
OmyRGT33TUF
OmyRGT38TUF
OmyRGT40/uTUF
OmyRGT4TUF
1.1
OmyFGT28/uTUF
OmyFGT18/uTUF
OmyRGT30TUF
One14ASC
Str73INRA
BHMS184
Otsh4NWFSC
Oii
OmyFGT18/uTUF
11.3
OmyFGT32/uTUF
OmyRGT42/uTUF
3.5
7.8
Otsh4NWFSC
OmyFGT18/uTUF
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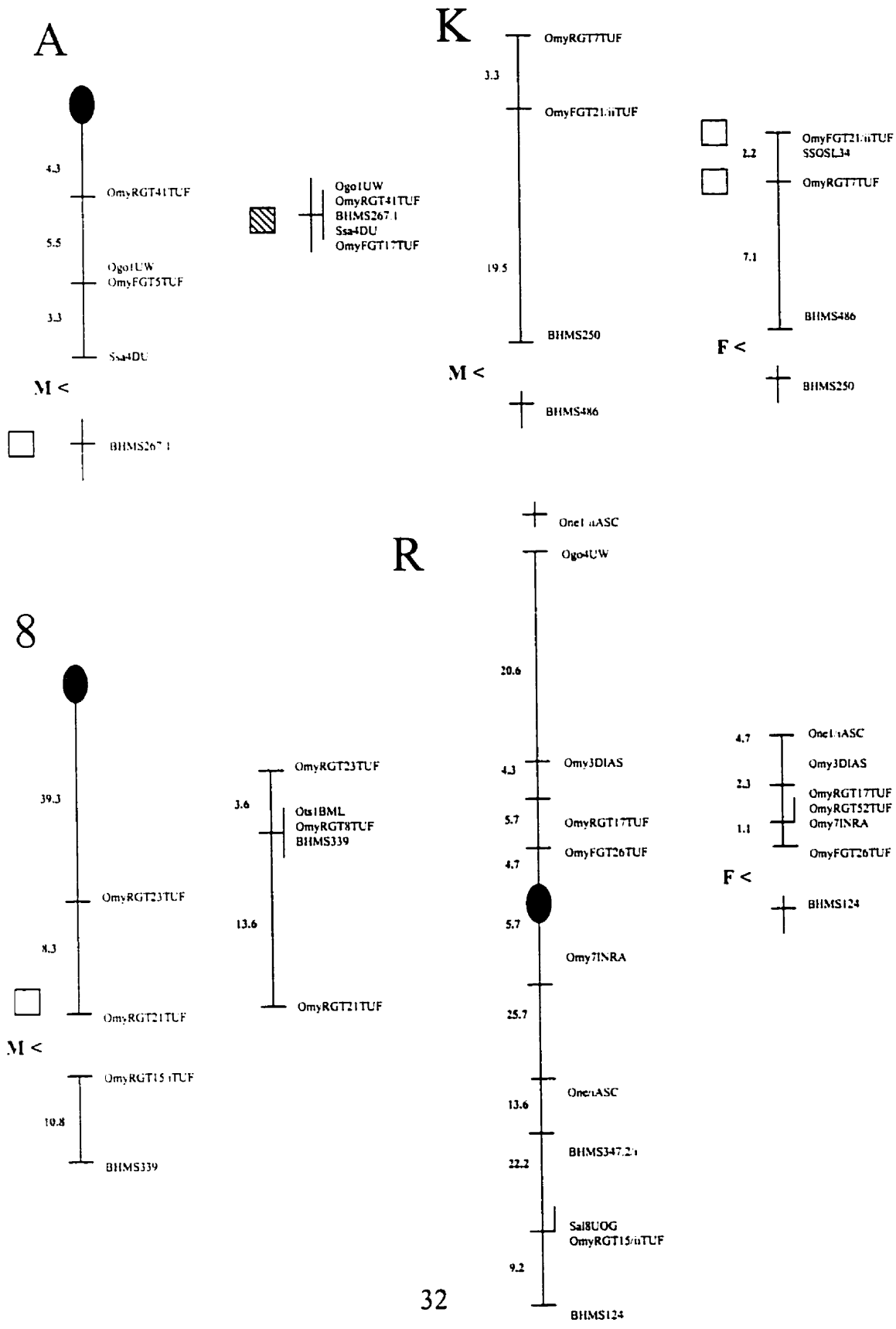
OmyRGT40/uTUF
12.2
OmyFGT32/uTUF
11.3
OmyFGT32/uTUF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OmyFGT5TUF
3.3
Ssa4DU
M <
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Ogo1UW
OmyRGT41TUF
BHMS267.1
Ssa4DU
OmyFGT17TUF
K
OmyRGT7TUF
3.3
OmyFGT21.6TUF
19.5
BHMS250
M <
BHMS486
OmyFGT21.6TUF
SSOSL34
2.2
OmyRGT7TUF
7.1
BHMS486
F <
BHMS250
8
39.3
OmyRGT23TUF
8.3
OmyRGT21TUF
M <
OmyRGT15.1TUF
10.8
BHMS339
OmyRGT23TUF
3.6
Ots1BML
OmyRGT8TUF
BHMS339
13.6
OmyRGT21TUF
R
One1.1ASC
Ogo4UW
20.6
Omy3DIAS
4.3
5.7
OmyRGT17TUF
4.7
OmyFGT26TUF
5.7
Omy7INRA
25.7
One1ASC
13.6
BHMS347.2.1
22.2
Sal8UOG
OmyRGT15.1TUF
9.2
BHMS124
4.7
One1.1ASC
Omy3DIAS
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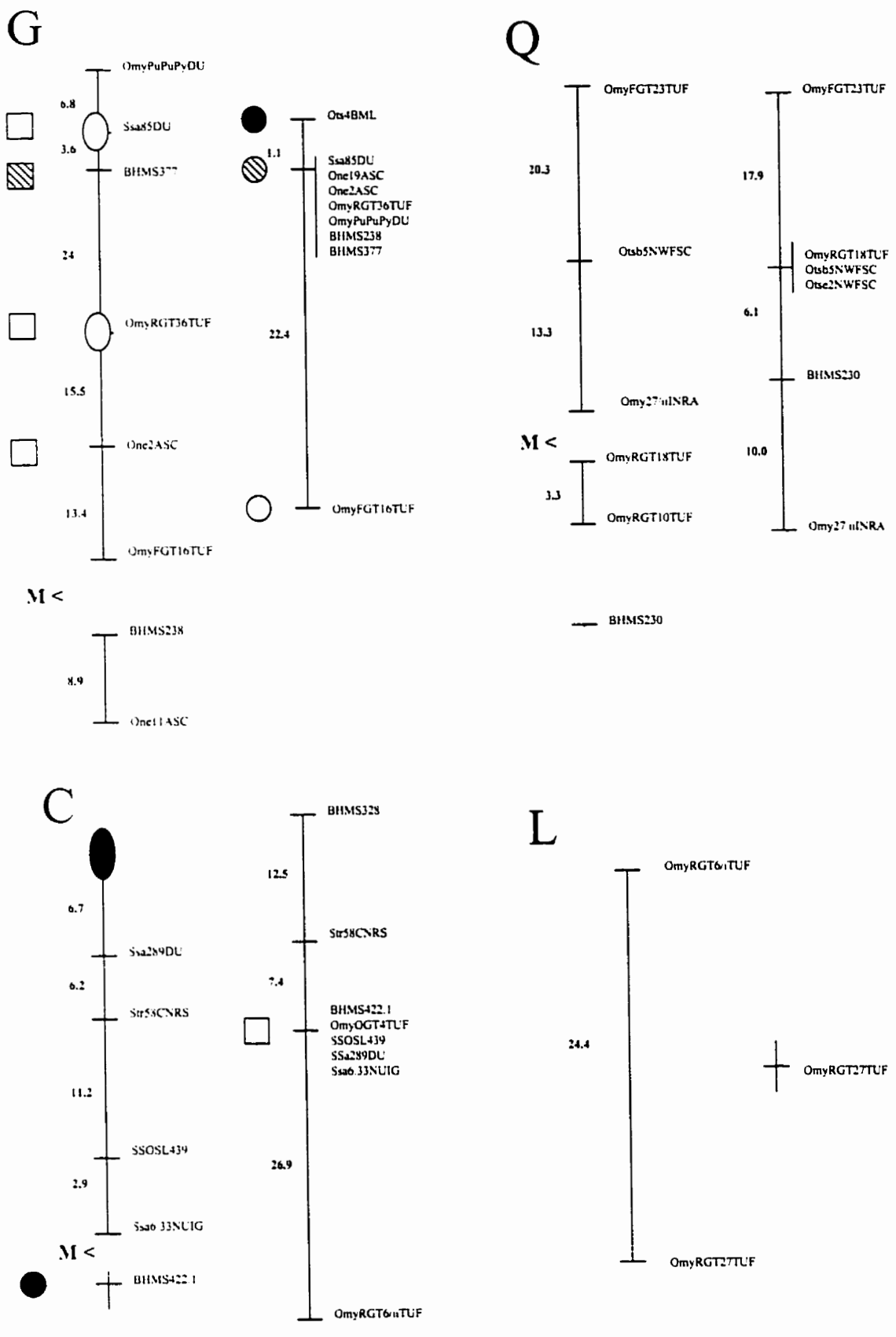
OmyRGT52TUF
1.1
Omy7INRA
OmyFGT26TUF
F <
BHMS124


G
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3.6
BHMS377
24
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15.5
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13.4
OmyFGT16TUF
Ots4BML
1.1
Ssa85DU
One19ASC
One2ASC
OmyRGT36TUF
OmyPuPuPyDU
BHMS238
BHMS377
22.4
OmyFGT16TUF
M <
BHMS238
8.9
One11ASC
Q
OmyFGT23TUF
20.3
Otsb5NWFSC
13.3
Omy27uINRA
M <
OmyRGT18TUF
3.3
OmyRGT10TUF
BHMS230
OmyFGT23TUF
17.9
OmyRGT18TUF
Otsb5NWFSC
Otse2NWFSC
6.1
BHMS230
10.0
Omy27 uINRA
C
6.7
Ssa289DU
6.2
Str58CNRS
11.2
SSOSL439
2.9
Ssa6 33NU1G
M <
BHMS422.1
BHMS328
12.5
Str58CNRS
7.4
BHMS422.1
OmyOGT4TUF
SSOSL439
SSa289DU
Ssa6.33NU1G
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L
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24.4
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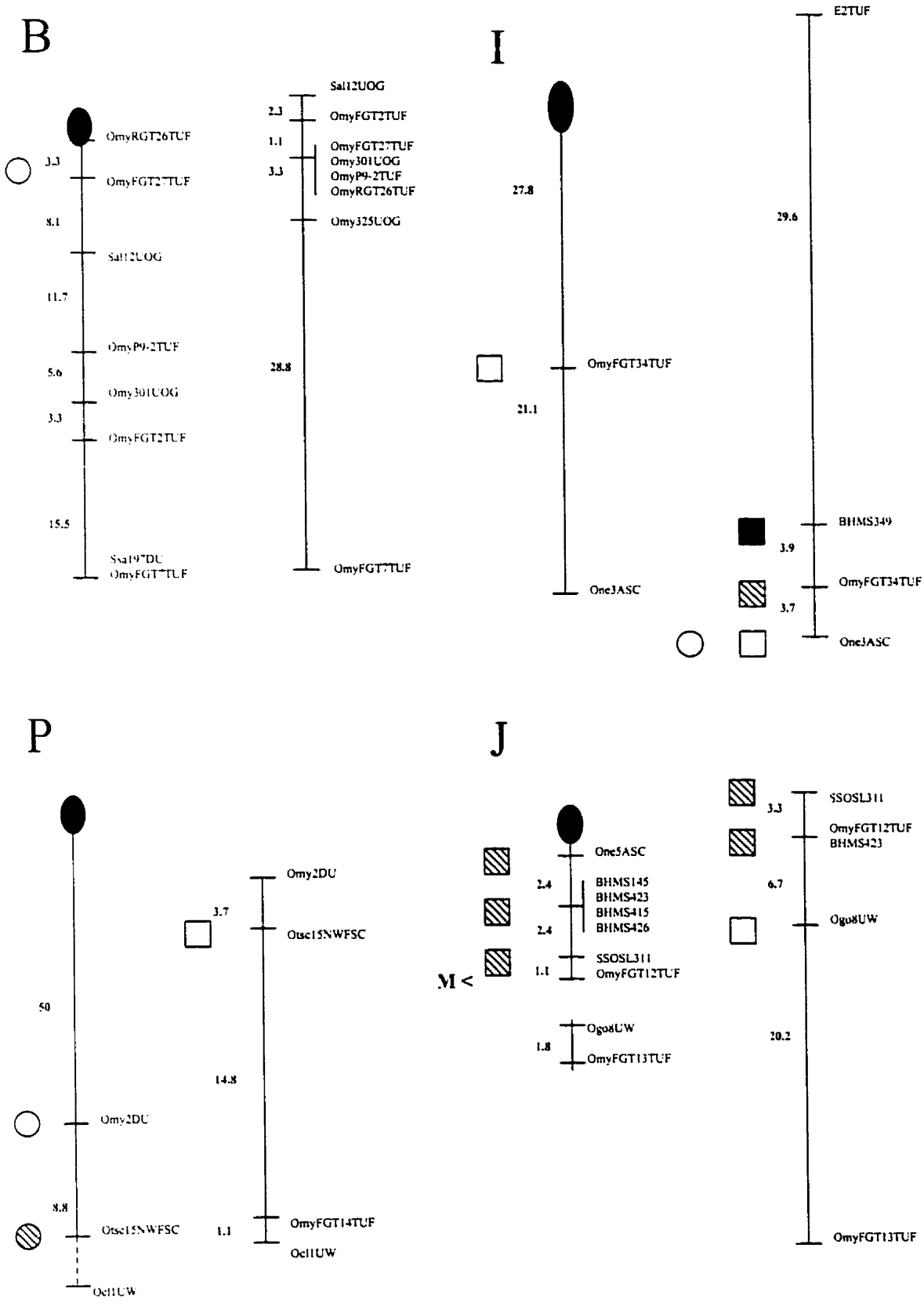
OmyRGT27TUF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Omy301UOG
3.3
OmyFGT2TUF
15.5
Ssa197DU
OmyFGT7TUF
Sal12UOG
2.3
OmyFGT2TUF
1.1
OmyFGT27TUF
Omy301UOG
3.3
OmyP9-2TUF
OmyRGT26TUF
Omy325UOG
28.8
OmyFGT7TUF
I
27.8
OmyFGT34TUF
21.1
One3ASC
E2TUF
29.6
BHMS349
3.9
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One3ASC
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Omy2DU
8.8
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3.7
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Otsc15NWFSC
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1.1
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J
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BHMS423
BHMS415
2.4
BHMS426
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Ogo8UW
1.8
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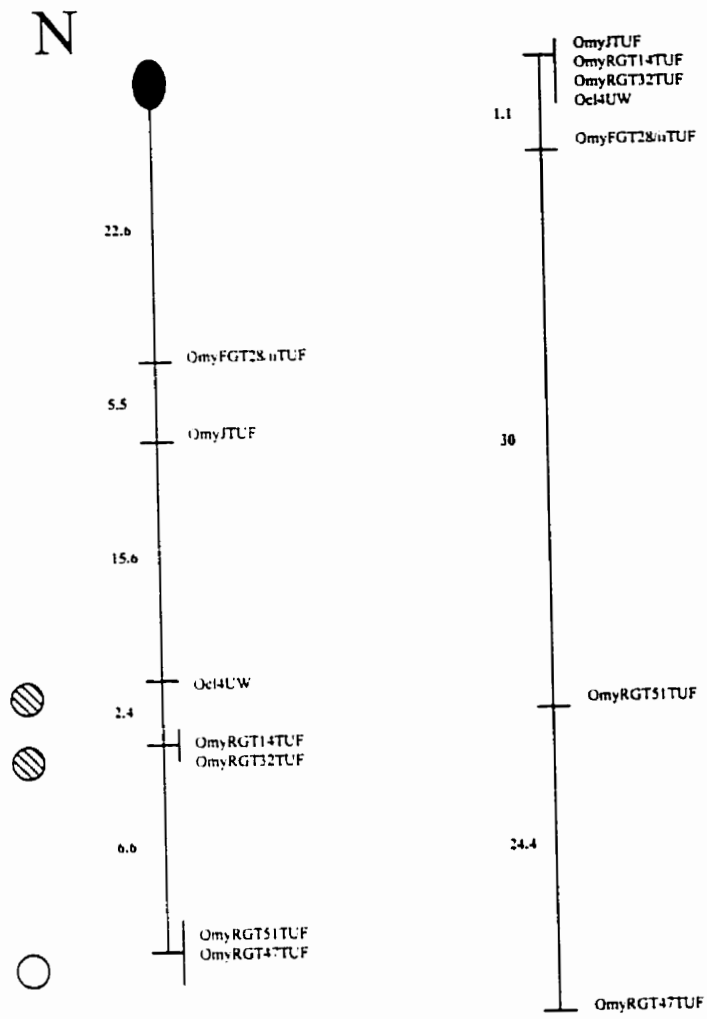
Ogo8UW
20.2
OmyFGT13TUF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5.5
OmyJTUF
15.6
Oci4UW
2.4
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OmyRGT32TUF
6.6
OmyRGT51TUF
OmyRGT47TUF
OmyJTUF
OmyRGT14TUF
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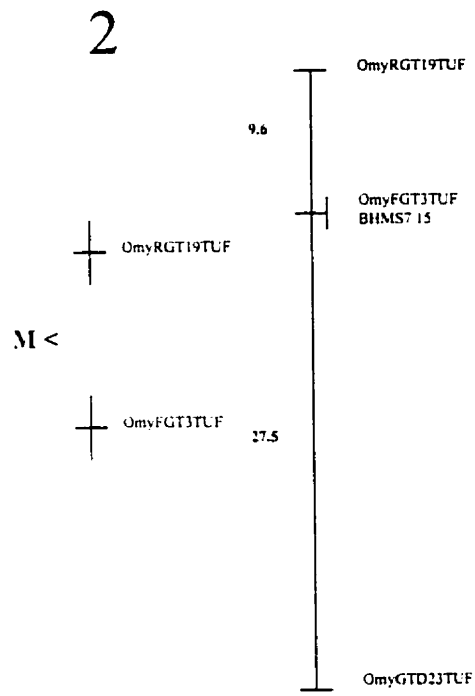
30
OmyRGT51TUF
24.4
OmyRGT47TUF


2
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BHMS7 15
OmyRGT19TUF
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OmyFGT3TUF
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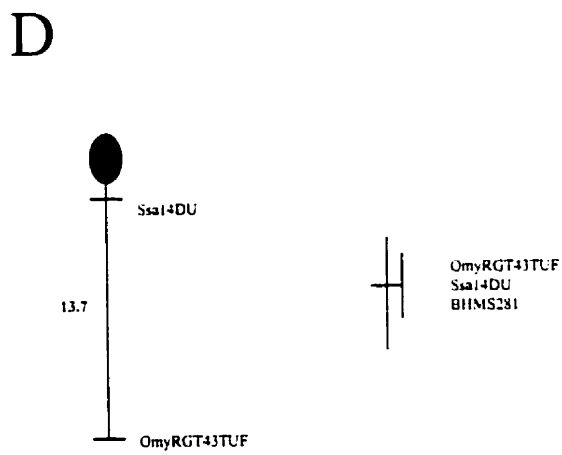
OmyGTD23TUF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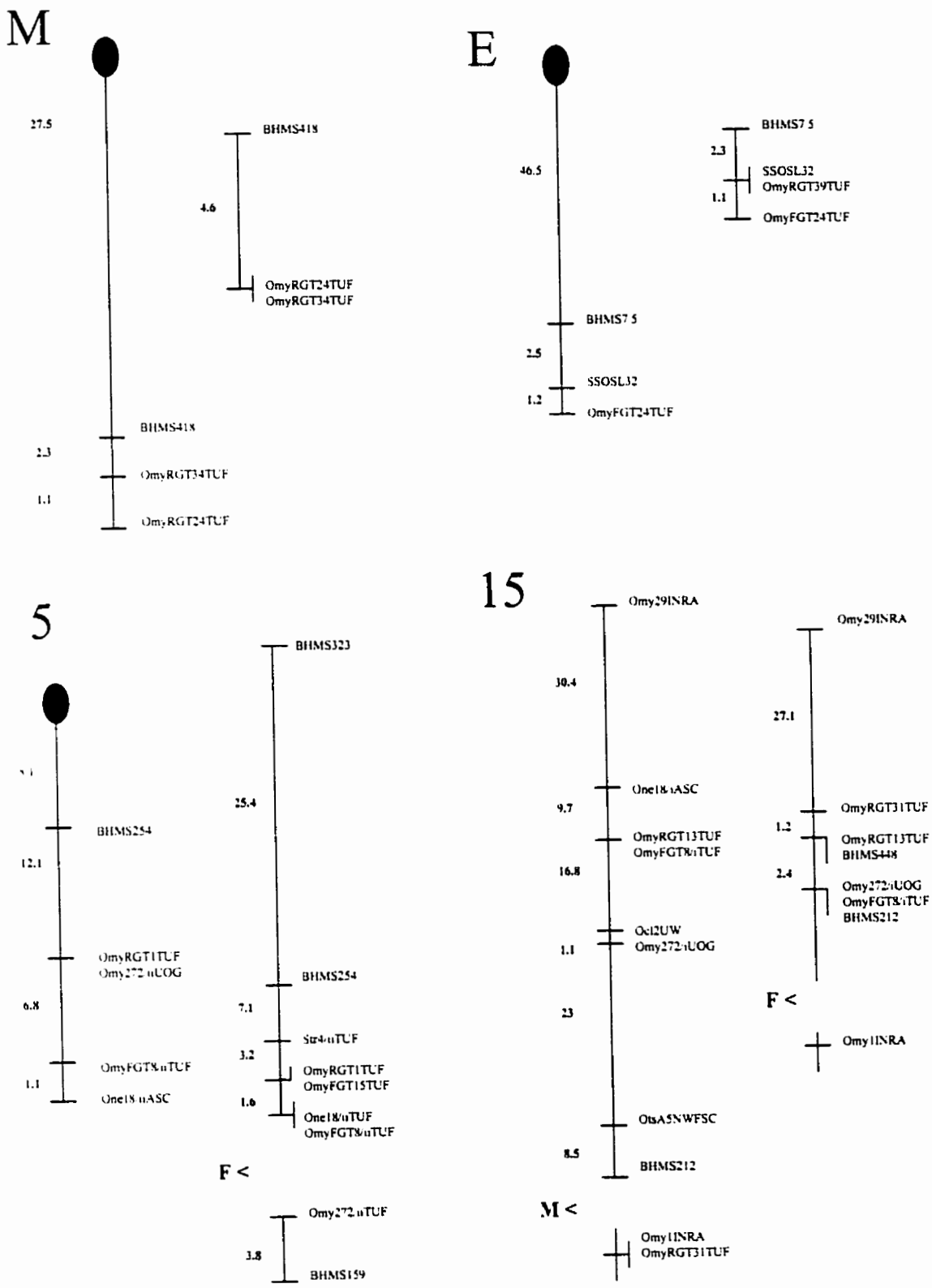
BHMS281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OmyRGT24TUF
E
46.5
BHMS7 5
2.3
SSOSL32
OmyRGT39TUF
1.1
OmyFGT24TUF
BHMS7 5
2.5
SSOSL32
1.2
OmyFGT24TUF
5
BHMS323
25.4
BHMS254
7.1
Str4/uTUF
3.2
OmyRGT1TUF
OmyFGT15TUF
1.6
One18/uTUF
OmyFGT8/uTUF
BHMS254
12.1
OmyRGT1TUF
Omy272/uUOG
6.8
OmyFGT8/uTUF
1.1
One18/uASC
F <
Omy272/uTUF
3.8
BHMS159
15
Omy29INRA
30.4
One18/uASC
9.7
OmyRGT13TUF
OmyFGT8/uTUF
16.8
Oct2UW
1.1
Omy272/uUOG
23
OtsA5NWFSC
8.5
BHMS212
M <
Omy11NRA
OmyRGT31TUF
Omy29INRA
27.1
OmyRGT31TUF
1.2
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BHMS448
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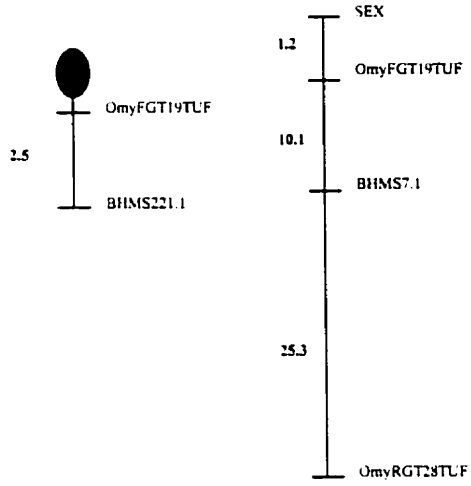
OmyFGT8/uTUF
BHMS212
F <
Omy11NRA

18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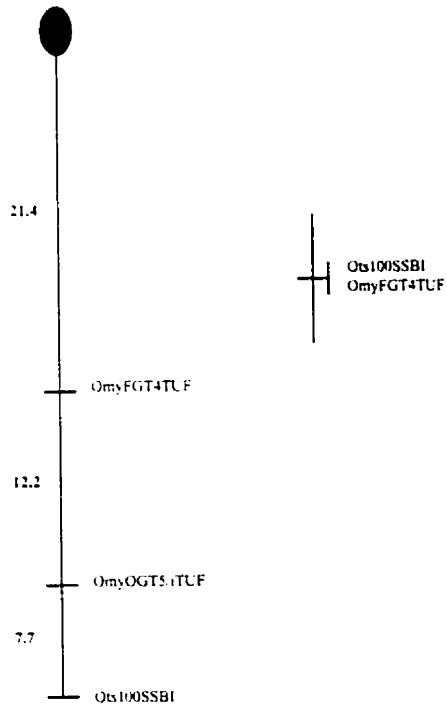
OmyFGT19TUF
2.5
BHMS221.1

Fi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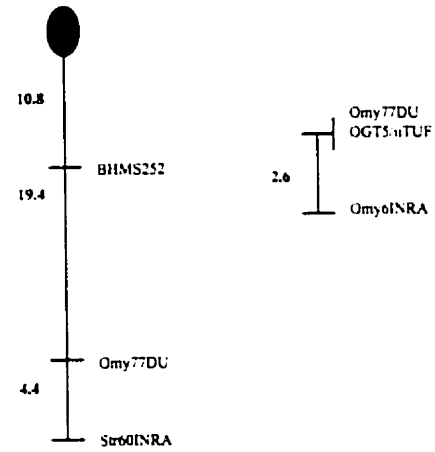
OmyFGT4TUF
12.2
OmyOGT5.1TUF
7.7
Ots100SSBI

Fii


10.8
BHMS252
19.4
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4.4
Str60INRA
Omy77DU
OGT5.1TUF
2.6
Omy6INRA

CHAPTER 3:

DETECTION OF QTL FOR SPAWNING TIME AND BODY WEIGHT IN RAINBOW TROUT (*Oncorhynchus mykiss*)

Introduction

Gene duplication is recognized as an integral component of genome evolution by providing opportunities for the evolution of new gene functions (Haldane 1933; Ohno 1970). Of the many processes that generate gene duplications, polyploidization is the only one in which the entire genome is duplicated. Considerable evidence suggests that two genome-wide duplications occurred early in vertebrate evolution, the most recent approximately 250 myr ago (Ohno et al. 1967). The increase in genetic complexity through genomic elaboration is evident as there are approximately 15 000 genes in nematodes and *Drosophila*, whereas there are thought to be 30 000 – 35 000 in vertebrates (Miklos and Rubin 1996; Simmen et al. 1998). Studies on protein-coding loci of several fishes suggest a more recent genome duplication event after the divergence of the teleost and the tetrapod lineages (Uyenyo and Smith 1972; Allendorf et al. 1975; Bailey et al. 1978).

Under the classical model for the evolution of duplicate genes, one member of the pair will usually become silenced by degenerative mutation with the other retaining the original function (Haldane 1933; Nei and Roychoudhury 1973; Li 1980). Both members of the pair will persist on rare occasions with the fixation of a beneficial mutation at one of the two loci (Ohno 1970). Duplicate gene pairs have been identified in many gene families (Hox clusters, MyoD in mammals, and zebrafish *engrailed* genes) (Ekker et al. 1992; Weintraub 1993; Krumlauf 1994).

Polyploidy is the primary mechanism for generating genomic redundancy as no other process can produce a comparable increase of genetic material on which selection may act. Wendel (2000) suggested that there are three primary possibilities for the

evolutionary fate of duplicated genes generated from a polyploidization event. Functional diversification may arise via selection on both members of the duplicate pair. Alternatively, gene silencing might occur leading to the loss of expression at one of the two duplicated copies. Lastly, both gene copies may retain the original or similar function. Duplicate gene expression is common as rates of gene silencing are much lower than predicted by traditional models (Nadeau and Sankoff 1997). Genetic redundancy may offer a slight fitness advantage that might only be evident in certain life stages or environmental conditions (Cooke et al. 1997). A fourth alternative considers the interaction among duplicate genes as genetic material is exchanged between homeologues, resulting in a loss of independence between pairs (Wendel 2000).

Salmonid fishes are believed to be descended from a single autotetraploid event approximately 25-100 mya (Allendorf and Thorgaard 1984). Immediately following genome duplication, an autotetraploid lineage is expected to demonstrate tetrasomic chromosome segregation. Over time, however, diploidization of the genome occurs and disomic segregation becomes prevalent (Allendorf and Danzmann 1997).

Many homeologous chromosome arms still exchange chromatid segments as a result of multivalent formations in salmonid fishes (Lee and Wright 1981; Wright et al. 1983; Allendorf and Thorgaard 1984). Interestingly, this meiotic event appears to be almost exclusive to males (Allendorf and Thorgaard 1984; Allendorf and Danzmann 1997). Differential crossovers between homeologous chromosomes result in pseudolinkage (the aberrant joint segregation of duplicated loci) and thus may regulate the duplication of some genes and diploidization of others (Sakamoto et al 2000). These forms of residual tetrasomy suggest that diploidization may still be in progress.

Large sex-specific differences in recombination rates have been observed in rainbow trout (Johnson et al. 1987; Sakamoto et al. 2000; Chapter 2). Multivalent formations likely constrain crossover events in males resulting in the repressed rates. However, the observed differences may be conditional upon the chromosomal location of the chiasmata. If chiasmata are localized to the telomeric regions (Wright et al. 1983; Allendorf and Danzmann 1997), then regions proximal to the centromere may experience no crossing over facilitating the diploidization of loci, while telomeric regions may experience an exchange of genetic material with homeologous regions. This would tend to inflate the recombination levels in the telomeric regions of males compared to females which may in turn lead to the increased conservation of duplicated genes due to increased inter homeologue meiotic recombination (Wright et al. 1983; Allendorf and Danzmann 1997; Sakamoto et al. 2000).

Multiple chromosomal segments have been shown to contain quantitative trait loci (QTL) that affect the phenotypic variation in continuously distributed traits (Falconer and Mackay 1996; Lynch and Walsh 1998). Testing the allelic effects on quantitative characters facilitates an understanding of the underlying genetics of these traits, primarily the number of polygenes and magnitude of effect (Barton and Turelli 1989). Two opposing models attempt to elucidate the nature of quantitative variation. Fisher's infinitesimal model proposes that quantitative traits are controlled by a very large number of loci, each with small phenotypic effect (Fisher 1930), while the oligogenic model describes continuous phenotypic variation as the result of a few loci with very large effects (reviewed by Tanksley 1993).

QTL mapping is an empirical approach to distinguish between the two models. Experimental results have shown that at least some traits can be explained by the segregation of a few major QTL, perhaps modified by QTL of minor effect (Mitchell-Olds 1996; Lin 2000). However, it is not always clear whether this outcome is a true reflection of the underlying genetics or a statistical artifact caused by sampling bias (Beavis 1998).

Life history theory seeks to examine and predict how organisms alter reproductive strategy throughout their lifetime in response to environmental and physiological changes (Roff 1992). For instance, the age at first reproduction, amount of reproductive investment, and how to partition the investment all can have profound effects on total fitness of an organism. Life history modeling is based on the hypothesis that trade-offs between fitness-related traits constrain evolution (Roff 1992; Stearns 1992). Evolutionary trade-offs can be viewed as genetic effects of opposed direction that limit genetic variation. The classical theory predicts that life-history traits under strong directional selection should suffer a rapid loss of genetic variation and subsequently show low heritability. However, a study across many taxa found an average value of 0.27 for the heritability of life history traits (Mousseau and Roff 1987), high enough to permit a fairly rapid response to selection (Roff 1992). Therefore, lack of genetic variation is probably not typically a major constraint on life history evolution (Roff 1992).

Antagonistic pleiotropy has become a major component of life history theory as a possible mechanism of balancing selection for the maintenance of genetic variation (Roff 1992; Charlesworth 1994). Rose (1985) first suggested that alleles at a particular locus may result in both high reproduction and low viability, while other alleles result in both

low reproduction and high viability. Antagonistic pleiotropy may, therefore, lead to the maintenance of additive genetic variability for fitness characters (Betran et al. 1998) that would otherwise be eliminated by strong directional selection (Barton 1990). However, antagonistic pleiotropy appears to be an unlikely mechanism when selection is limited to only one sex or when there is a high amount of inbreeding (Hedrick 1999). A stable polymorphism is thus likely to be maintained by antagonistic pleiotropy only when the selective differences are large and somewhat similar in size (Hedrick 1999).

Fitness-traits such as spawning time, body weight, and stress resistance are major factors in the life history of salmonid fishes. Rainbow trout (*Oncorhynchus mykiss*) spawn during a defined time of the year, and each genetic group has a characteristic season of spawning (Bromage et al. 1992). Heritabilities for spawning time and body weight have been reported to be relative large in rainbow trout with most variable levels reported for body weight (Siitonen and Gall 1989; Crandell and Gall 1993a,b; Su et al. 1996). There appears to be little genetic correlation between rainbow trout age at maturity, measured as the day spawned within a season, and body weight at two years of age (Crandell and Gall 1993a).

Salmonid fish are genetically variable and phenotypically flexible in their life history patterns (Hutchings 1993). Once the physiological minimum size has been attained, an individual's reproductive strategy will depend on the relative gain of reproducing early versus reproducing later at a larger size (Hutchings 1993). Reproduction channels energy away from growth and thus reduces survival probability (Roff 1982). Growth is of particular significance in fish as fecundity is generally an increasing function of body size (Ware 1982).

QTL affecting upper temperature tolerance and spawning time have previously been identified in rainbow trout (Jackson et al. 1998; Sakamoto et al. 1999; Perry et al. in press). Furthermore, the localization of these markers on a low resolution microsatellite linkage map (Sakamoto et al. 2000; Chapter 2) now facilitates an examination of duplicate gene function in the polyploid derivative species. Synteny of duplicated microsatellite markers was used to identify eight homeologous chromosome pairs (Sakamoto et al. 2000; Chapter 2). Assimilating this information creates a unique opportunity to test for conserved QTL effects across homeologous pairs and to investigate the genetic architecture of fitness-related traits. The evolutionary fate of homeologous loci has been investigated in a very limited number of cases (e.g. allopolyploid cotton, *Gossypium hirsutum*) (Cronn et al. 1999).

I searched for QTL affecting spawning time and body weight using segregating maternal and paternal alleles at 185 microsatellite loci spanning 26 linkage groups (Sakamoto et al. 2000; Chapter 2). I tested for conservation of QTL affecting spawning time and body weight across eight homeologous chromosome pairs in rainbow trout backcross family selected for spawning time (Table 3.1). As well, ten other linkage groups were investigated for which the homeologous relationships are unknown. The magnitude, average allelic effect, chromosomal location and source of the effect was examined in all QTL regions. I also assessed the effects of individual loci on both traits to study the covariation between spawning time and body weight in a life-history framework.

Materials and methods

Source of fish

A backcross between two strains of rainbow trout that spawn in different seasons was the source material used for this study. Generally, females designated as fall spawners ovulate between September and December, and spring spawners between February and April (Ferguson et al. 1993). In 1990, a spring spawning strain male from a natural population was crossed to a fall spawning strain female from a commercial strain to produce a hybrid family. The male was induced to spawn in the fall through photoperiod manipulation. In 1992, an F_1 hybrid (F x S) male was backcrossed to a fall spawning female to produce the backcross family (details in Sakamoto et al. 1999). Neither the spring spawning nor the fall spawning strain was an inbred line so the genotypes of the backcross parents were not expected to be completely homozygous. Likewise, the F_1 (F x S) parent was not completely heterozygous for the marker loci examined.

Phenotypic data

The spawning dates (ovulation) for 45 female progeny from the backcross family were collected in 1995 and 1996 (3 and 4 years old post-fertilization). Spawning time was calculated as the number of days from August 1st. Females were examined weekly to identify those individuals that had ovulated between August and December in both years. Some females produced batches of eggs in successive weeks. For those females, the spawning date was designated according to the week when the larger volume of eggs was collected. A mid-week spawning date was assigned when egg volumes were approximately equal. In 1995, the recorded spawning dates ranged from 16 – 128 d from

August 1st whereas in 1996, spawning dates ranged from 30 – 114 d. Body weight was measured when the females were two years of age. The average weight of the females was 774.8 (± 151.3 g) with a range of 441.4 g to 1147.6 g. Analysis of male weight was not performed because of the potential limitations on growth that precocious maturation may have rendered.

Microsatellite analysis

Genomic DNA was extracted from muscle, liver, or gill tissue from the backcross progeny and the sire using a standard phenol/chloroform method as described by Bardakci and Skibinski (1994). Microsatellite loci were amplified by PCR as described by Sakamoto et al. (1999) and in Chapter 2. The resulting amplified DNA fragments were separated in a 6% polyacrylamide-7-M urea gel and visualized with a Hitachi FMBIOII fluorescence imaging system. Allele base pair size was determined using 350-Tamra lane standard.

Statistical analysis

QTL analysis was performed separately on the segregating maternal and paternal alleles at each of the 185 microsatellite loci analyzed. Linear probability plots were used to confirm normality of both spawning time and growth distributions in the backcross family prior to analyses. Results from linear regression analysis indicated that there was no correlation between spawning time and body weight (year 3 $R^2 = 0.01$; year 4 $R^2 = 0.03$). Associations of individual marker alleles from each parent with each trait were tested using the following general linear model (PROC GLM, SAS Institute 1996),

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

which considers y_{ij} as the phenotypic value of the jth individual with marker allele i, μ is the family mean, and α_i is the effect of allele i from either the sire or dam, and ε_{ij} is the residual error.

Interaction of maternal and paternal alleles was tested using the following two-factor general linear model,

$$y_{ijk} = \mu + \alpha_j + \beta_k + \alpha\beta_{jk} + \varepsilon_{ijk}$$

which considers y_{ijk} as the phenotypic value of the ith individual inheriting the jth allele at locus α and the kth allele at locus β, μ is the family mean, and α_j is the effect of allele j from the sire, and β_k is the effect of allele k from the dam, $\alpha\beta_{jk}$ is the interaction term, and ε_{ijk} is the residual error.

The phenotypic mean associated with an allele was calculated using least square means. Spawning time alleles associated with a lower mean were denoted as early while alleles associated with a higher mean were designated as late. Likewise, body weight alleles associated with a lower mean were denoted as small, while alleles associated with a higher mean were designated as large (Tables 3.2, 3.3). The R^2 value for each model (genotype SS/total SS) was used to determine the amount of variance attributed to the marker-trait association. The maximum R^2 value calculated at a given marker in the QTL region was used to estimate the magnitude of effect. An arbitrary criterion of $\geq 25\%$ variation was used to define a major QTL (Bradshaw et al. 1998). Significant P-values ($P < 0.05$) obtained from each general linear model test were permuted randomly 10 000 times (PROC MULTTEST, SAS Institute 1996). The permutation method, as described by Churchill and Doerge (1994), determines appropriate significant thresholds to protect against Type I error associated with multiple testing.

Results

Spawning time QTL

A minimum of eleven spawning time QTL were detected on the 26 linkage groups analyzed (U, Oi, Oii, A, K, 8, G, C, I, P, and J) (Figure 2.2). Females inheriting different maternal or paternal alleles at 34 microsatellite loci showed significant differences in spawning time (Table 3.2). Twenty-two of the 34 microsatellite loci showed associations with spawning time in both 3 and 4 year-old females. In addition, a significant association between spawning time and the interaction of segregating parental alleles was detected at Omy7INRA and One1/iASC (Table 3.2).

Spawning time QTL were conserved across two homeologous chromosome pairs (Oi and Oii; A and K) (Figure 2.2). The amount of variance attributed to homeologous QTL was similar across linkage groups A and K (± 3%) (Table 3.2). A QTL with large effect was detected in linkage group Oii (21.4% for OmyRGT42/iiTUF). The parental source (sire or dam) contributing to the allelic effect was conserved across both homeologous pairs (Oi and Oii; A and K). Associations between values for spawning time and alleles at loci on linkage groups A and Oii were observed in both sampling years (Table 3.2).

There was marginal evidence for conserved QTL effects across three additional homeologous pairs (8 and R; 5 and 15; Fi and Fii). A spawning time QTL was detected in the telomeric region on linkage group 8 (Table 3.2). A marginal association ($P = 0.075$, year 3) between maternal alleles at BHMS347.2/i was also detected suggesting the existence of a QTL in the same relative position on linkage group R. Other marginal associations ($0.05 < P < 0.10$) support the suggestion that spawning time QTL exist on

linkage groups 8 (OmyRGT23TUF, dam and sire, year 3) and R (Omy7INRA [dam, both years]; BHMS347.2/i [sire, year 3]). In addition, a marginal association was detected in linkage group Fi between paternal alleles at Ots100SSBI (year 4) while marginal associations between paternal alleles were also detected (year 3) in the same relative position on linkage group Fii (Omy77DU; OmyOGT5/iiTUF; Omy6INRA). Also, marginal associations were also detected across linkage groups 5 (BHMS159 [sire, year 3]; BHMS245/I [sire, year 4]) and linkage group 15 (BHMS212, dam, year 3).

In three cases, spawning time QTL were identified on a single homeologue (G, U, and C) but not on the other homeologue (Q, H, and L, respectively) (Figure 2.2). The proportion of variation accounted for by QTL located in linkage groups G, U, and C were 17.6% (BHMS377), 16.7% (OmyCosB/iTUF) and 10.7% (Ssa289DU), respectively. Associations between spawning date and alleles at loci on linkage groups G and C were detected in both sampling years (Table 3.2).

Spawning time QTL were identified on three linkage groups for which the homeologous relationships are unknown (J, I, and P) (Figure 2.2). Major QTL were detected in linkage groups J and I (33% for BHMS423; 25.5% for BHMS349). Associations between spawning time and alleles at loci on all three linkage groups were observed in both sampling years (Table 3.2).

Body weight QTL

Significant associations between either maternal or paternal and body weight were detected at 21 microsatellite loci (Table 3.3). In addition, a significant association between body weight and the interaction of segregating paternal alleles was detected at three loci on linkage group A (Table 3.3). The distribution of the loci on the linkage

groups analyzed suggests the presence of nine QTL (H, U, Oi, C, G, B, I, P, and N) (Figure 2.2).

Body weight QTL were conserved across a single pair of homeologues (U and H) (Figure 2.2). The magnitude of the effect was similar across QTL regions (± 3%) (Table 3.3). The segregation of maternal alleles contributed to both effects. Interaction between maternal and paternal alleles on body weight was detected at two loci on linkage group H (Table 3.3).

Marginal evidence for conserved QTL effects across two additional homeologous pair exists (G and Q; 5 and 15). First, a QTL with large effect was detected on linkage group G (23.2% for Ots4BML, sire effect), while a marginal association between maternal alleles and body weight was detected on the respective homeologue, linkage group Q (BHMS 230; P = 0.083). Associations between maternal alleles and body weight were detected at four markers in linkage group 5 (OmyRGT1TUF, P = 0.067; Omy272/iiUOG, P = 0.099; OmyFGT8/iiTUF, P = 0.057; One 18/iiASC, P = 0.057). Similarly, associations between maternal allele and body weight were detected at three markers in linkage group 15 (One18/iASC, P = 0.095; BHMS212, P = 0.076; OmyRGT31TUF, P = 0.059).

In two instances, body weight QTL were detected on a single homeologue (C, and Oi) and not on the other homeologue (L, and Oii respectively) (Figure 2.2). A major QTL was detected in linkage group C (25.6% for BHMS422.1). In linkage group Oi, an association between the paternal allele and body weight was detected at OmyFGT29TUF (Table 3.3), while a marginal association between paternal allele and body weight was detected at BHMS184 (P = 0.073). In addition, two marginal associations between

maternal allele and body weight were detected at OmyRGT40/iTUF (P = 0.097) and OmyRGT4TUF (P = 0.104).

Body weight QTL were detected on four linkage groups for which the homeologous relationships are unknown (N, P, B, and I) (Figure 2.2). QTL magnitudes ranged between 8.8% and 19.9% (Table 3.3).

Covariation among QTL

Spawning time QTL and body weight QTL were identified on the same linkage group in six instances (U, Oi, G, C, I and P). Variation at six loci, representing four of these QTL regions (U, G, I, and P), significantly affected both traits (Tables 3.2, 3.3). In most cases, the allelic effects for one trait were not from the same parent as for the other trait. For example, progeny inheriting different maternal alleles at locus BHMS377 had significantly different body weights but not spawning time (the reverse was true for paternal alleles at the same locus). In these instances, it was not possible to look at the direction of the covariation between traits. Such an examination was possible for two cases. Firstly, progeny inheriting allele 259 from the dam at OmyCosB/iTUF were significantly heavier and spawned earlier than those inheriting allele 276. Alternatively, progeny inheriting the 198 bp allele from the sire at One3ASC were significantly larger and spawned later than those inheriting the null allele.

Discussion

I have identified a minimum of nine minor and two major QTL for spawning time in addition to eight minor and one major QTL for body weight in rainbow trout. This concurs with findings from recent QTL studies (Lin and Ritland 1997; Bradshaw et al. 1998; Hurme et al. 2000) indicating that a large proportion of quantitative variation can

be explained by the segregation of a few major QTL (reviewed by Tanksley 1993). The percentage of variation attributed to the major spawning time QTL may exceed 50% while the major body weight QTL accounts for 25% of the variation. The difference in spawning date between segregating alleles at a major spawning time QTL was approximately 28 d whereas the difference in spawning date between segregating alleles at a minor spawning time QTL was approximately 14 d. Likewise, the difference between segregating alleles at the major body weight locus was approximately 150 g whereas the difference in weight between segregating alleles at a minor body weight locus was approximately 90 g. These results suggest that spawning time and body weight are controlled by a few major loci perhaps modified by QTL of minor effect. This conclusion is counter to Fisher's infinitesimal model in which quantitative traits are controlled by a very large number of loci, each with a small phenotypic effect.

A statistical bias towards the detection of genes of larger phenotypic effects may result, however, in an underestimation of the total number of genes affecting a trait. Also, distinguishing between single gene versus multigene composition of individual QTL is difficult when QTL mapping resolution is limited to 10 – 20 cM (reviewed by Tanksley 1993). Additional QTL analysis will further elucidate the true number and magnitude of genes affecting these two fitness-related traits in rainbow trout.

Spawning time QTL were conserved across two pairs of homeologues with a strong indication for an additional three pairs out of the eight pairs examined. Moreover, body weight QTL were conserved across a single homeologous pair with compelling evidence for an additional two pairs. The recent report of a growth QTL at OmyRGT1TUF in a hatchery strain of rainbow trout strongly supports the marginal

associations detected at linkage group 5 in this study (Martyniuk 2001). These results correspond with recent reports suggesting a much larger proportion of duplicate gene preservation than predicted by the classical model (Ahn and Tanksley 1993; Hughes and Hughes 1993).

Recent empirical evidence indicates that gene silencing rates are low (Nadeau and Sankoff 1997) and maintenance of gene function is a common fate of homeologous genes (Cronn et al. 1999). Under the classical model, the only mechanism by which duplicate gene pairs may persist is when a rare beneficial mutation arises to cause divergence in function prior to mutational decay (Ohno 1970). It has been estimated that about 99% of duplicate genes would devolve into pseudogenes by this process (Walsh 1995). Subsequently, additional mechanisms have been proposed to explain the high proportion of duplicate gene pairs observed in eukaryotic genomes. For example, positive selection for the maintenance of multiple gene copies may exist as genetic redundancy may offer a slight fitness advantage (Clark 1994; Wagner 1999). Alternatively, preservation of duplicate gene pairs may result from degenerative mutations that cause a partial loss-of-function, a process known as subfunctionalization (Force et al. 1999). By this process, degenerative mutations can increase the probability of duplicate gene preservation through the partitioning of ancestral functions (Force et al. 1999). Therefore, the joint levels and patterns of activity of both members of the pair is reduced to that of the single ancestral gene thus reducing pleiotropic constraints (Lynch and Force 2000). However, subfunctionalization appears to be an unlikely mechanism of duplicate gene preservation when the effective population size is low (Lynch and Force 2000). In contrast,

pseudogene formation and positive selection for genetic redundancy appear to be probable when the effective population sizes are large (Wagner 1999).

The underlying mechanism for the maintenance of duplicate gene pairs in salmonid fish is uncertain. The fact that duplicate genes often differ in the timing of expression and/or the pattern of expression in different tissues is consistent with the subfunctionalization model (Ferris and Whitt 1979; Otto and Whitton 2000). When a locus is uniquely expressed in a particular tissue or developmental stage it is unlikely to become fixed for a null allele because the other locus can no longer protect it from natural selection (Allendorf and Thorgaard 1984). There is direct allozyme evidence for tissue-specific expression of loci in salmonids (Wright et al. 1975; Allendorf et al. 1982). However, positive selection for the maintenance of duplicate gene pairs is also a plausible mechanism. The rate of adaptation can potentially be faster for polyploids thus resulting in a broader ecological tolerance (Otto and Whitton 2000). Subsequently, genetic redundancy may provide a slight fitness advantage in specific stages throughout the complex life history of salmonid fish.

Although it is clear that considerable conservation of QTL effects across homeologues exists in rainbow trout, it remains difficult to estimate the propensity for such conservation at the genome level overall. Those cases where QTL effects were detected in only one of the two homeologues might be due to the inadequate density of markers on those linkage groups leading to limited statistical power. Furthermore, only one family was examined in this study resulting in a lack of tested variation in the species. In addition, tests were not possible for those linkage groups where homeologous

relationships are unknown. Ultimately, a high-density map will reveal additional homeologous relationships and provide a robust estimate of QTL conservation.

The distribution of duplicated loci along chromosome arms was used to identify corresponding segments between homeologous pairs. Interestingly, spawning time QTL detected across three homeologous pairs (A and K; Fi and Fii; 8 and R) and body weight QTL detected across a single pair (5 and 15) map to analogous regions. For instance, associations between maternal alleles and body weight were detected at both One18/iiASC in linkage group 5 and at One18/iASC in linkage group 15. These results suggest the presence of a duplicate gene pair exerting similar effects on the trait rather than independent, non-related genes affecting the same trait. Comparison across other homeologous pairs was restricted due to limited distribution of duplicated markers proximal to QTL regions. Also, the genetic background confounded further examination as a few QTL were mapped in the opposite sex across homeologous pairs.

Consideration of meiotic configurations in rainbow trout suggest that QTL in telomeric regions of homeologues should have a greater probability of being conserved than those located close to the centromere. In female rainbow trout, duplicated loci exhibit random assortment whereas in males, an aberrant pattern of nonrandom segregation results in an excess of nonparental progeny types relative to the parental types. Wright et al. (1983) developed a meiotic segregation model to explain this apparent pseudolinkage. Essentially, two nonhomeologous acrocentric chromosomes pair to form a metacentric chromosome. Homeologous acrocentric pairs of chromosomes may then randomly pair with their homeologous arms in the metacentrics to form multivalents. Multivalent formation in male salmonids likely constrains crossovers

proximal to the centromere thus facilitating the diploidization of loci located in this region (Sakamoto et al. 2000). Alternatively, crossovers between homeologous chromosome segments in telomeric region preserve duplicated gene regions. Similarly, homeologous QTL located in the telomeric region would likely be conserved at a higher degree relative to QTL proximal to the centromere. Therefore, we would expect analogous regions in which QTL effects were identified to map to the telomeric region.

The association between degree of conservation and chromosomal location could be evaluated in two cases and was restricted to marginal associations. First, the QTL located in linkage group 8 is distal to the centromere whereas associations were detected at a locus distal to the centromere and at a locus proximal to the centromere in linkage group R. Second, associations between spawning time and paternal alleles were detected across homeologues Fi and Fii. Based upon female map data, one could infer that these QTL regions are telomeric. A high-resolution map will permit a more detailed evaluation of this prediction.

The candidate genes responsible for the QTL effects are unknown. However, circumstantial evidence suggests that the associations between body weight and maternal alleles detected on linkage group Oi may be attributable to growth hormone I. Growth hormone I promotes growth in salmonid fishes (Agellon et al. 1988) and was mapped to linkage group Oi by Sakamoto et al. (2000). Interestingly, I detected marginal associations between maternal alleles and body weight at OmyRGT4TUF which is approximately 0.08 cM from GH1 in the female map (Sakamoto et al. 2000). Also, marginal associations were detected at OmyRGT40/iTUF, approximately 6.0 cM from GHI, as an effect from the dam. However, the QTL effect on body weight detected at

OmyFGT29TUF is unlikely to be attributed to GH1 as this locus maps approximately 14 cM from GH1 in the male map (Sakamoto et al. 2000). The association detected at OmyFGT29TUF does coincide, however, with previous studies where significant associations between paternal alleles and body size were detected at three loci (OmyRGT40/iTUF, OmyFGT18/iTUF, One14ASC) on linkage group Oi in a hatchery strain of rainbow trout (Martyniuk 2001).

Quantitative variation is based on the overlapping effects of many genes on many characters, and selection on one character will strongly influence that on any other (Charlesworth 1994). I have mapped QTL for different traits to the same six linkage groups. In four of these linkage groups, the QTL for each trait were mapped in the opposite sex (Oi, P, G, C). In two of these linkage groups, however, associations have been detected at similar loci (G, C). This observation suggests that the same genes may be affecting both traits. On the other hand, QTL detected on linkage groups Oi and P map to different regions and thus are likely to be controlled independently by different genes. There were only two cases where I could examine the covariation between traits at a particular locus as an effect from the same parent. Progeny inheriting allele 259 from the dam at OmyCosB/iTUF were significantly heavier and spawned earlier than those inheriting allele 276. Alternatively, progeny inheriting the 198 bp allele from the sire at One3ASC were significantly heavier and spawned later than those inheriting the null allele. These results suggest that the QTL detected may represent a pleiotropic gene or a number of tightly linked genes, influencing both body weight and spawning time.

The covariation observed between spawning date and body weight at OmyCosB/iTUF corresponds to the predictions about salmonid life-history where large

fish tend to breed earlier in the season while smaller fish will delay breeding (Hendry et al.1999). Large females dig deeper nests which have a decreased probability of egg disturbance by late spawning females (van den Berghe and Gross 1986). Also, large females are capable of defending their nest from most intruders for a longer time relative to small individuals (Foote 1990). Thus, large females could potentially dominate limited resources, forcing smaller individuals to breed later. In an investigation of the correlation between spawning date and life-history traits within a salmon population, Hendry et al. (1999) reported that early-spawning females were slightly larger than late-spawning females. While females can delay spawning under very high densities, most fish begin spawning shortly after maturation to avoid egg deterioration (Foote 1990). Alternatively, early spawning may impose direct selection for larger body size (Hendry et al. 1999).

In contrast, the covariation observed between spawning date and body weight at One3ASC may represent an antagonistic pleiotropic interaction between these two traits. Life-history theory is based on the hypothesis that trade-offs exist between fitness-related traits. Evolutionary trade-offs can be viewed as genetic effects of opposed direction that give rise to antagonistic pleiotropy (Betran et al. 1998). For example, alleles at a particular locus may result in both high reproduction and low viability, while other alleles result in both low reproduction and high viability. A commonly assumed trade-off is that between size and age at maturity. While a decreased age to maturity will increase fitness (Fisher 1930), the reduced time for growth may result in a decreased body size. This trade-off may be operating on a smaller scale within spawning season of salmonid fish. Fecundity is generally an increasing function of body size yet reproduction channels

energy away from growth. Therefore, an individual must assess the relative gain of reproducing early versus reproducing later at a larger size.

In conclusion, QTL affecting spawning time and body weight in rainbow trout have been conserved across homeologous chromosome pairs. However, the propensity for such conservation at the genome level remains difficult to measure. These results concur with recent empirical evidence suggesting that a larger proportion of duplicate gene copies have been preserved than predicted by the classical model. Furthermore, the number and magnitude of QTL detected suggest that both spawning time and body weight may be controlled by a few loci with large effects. Additional research will elucidate the nature of the covariation between these two traits to determine if antagonistic pleiotropy is an important factor maintaining polymorphism.

Table 3.1. Eight pairs of linkage groups identified as showing some homeology to one another because of the presence of duplicated markers (Sakamoto et al. 2000, and unpublished).

Homeologous pair	Duplicated markers
H and U	OmyCosB/iTUF and OmyCosB/iiTUF
Oi and Oii	OmyFGT18/iTUF and OmyFGT18/iiTUF; OmyFGT32/iTUF and OmyFGT32/iiTUF; OmyRGT40/iTUF and OmyRGT40/iiTUF; OmyRGT42/iTUFand OmyRGT42/iiTUF
A and K	Ogo2/iUW and Ogo2/iiUW; OmyFGT21/iTUF and OmyFGT21/iiTUF
8 and R	OmyRGT15/iTUF and OmyRGT15/iiTUF
G and Q	Omy27/iINRA and Omy27/iiINRA
C and L	OmyRGT6/iTUF and OmyRGT6/iiTUF
5 and 15	Omy272/iiOUG and Omy272/iUOG; OmyFGT8/iiTUF and OmyFGT8/iTUF; One18/iiASC and One18/iASC;
Fi and Fii	OmyOGT5/iTUF and OmyOGT5/iiTUF

Table 3.2. Results of general linear model testing for associations between parental alleles and spawning date in rainbow trout. Significant interactions terms were detected using two-factor general linear model.

Linkage Group	Marker	Year	Source	Alleles (bp)	Mean ± SE	P-values	R^2
U	OmyRGT12TUF	3	sire	137	85.91 ± 5.96	0.006**	0.167
				139	61.50 ± 5.96		
	OmyCosB/iTUF	3	sire	259	60.90 ± 6.10	0.006**	0.167
				310	85.39 ± 5.83		
		3	dam	259	66.86 ± 5.52	0.046*	0.092
				276	85.69 ± 7.30		
Oi	One14ASC	3	dam	152	82.50 ± 5.92	0.033*	0.104
				157	63.15 ± 6.49		
	OmyFGT32/iTUF	3	interaction	76, 80	87.00 ± 8.60	0.035	0.109
				85, 80	63.50 ± 7.69		
Oii	OmyFGT18/iiTUF	3	dam	208	57.53 ± 7.49	0.010*	0.157
				214	82.92 ± 5.69		
		4	dam	208	56.67 ± 5.32	0.035*	0.109
				214	71.23 ± 4.04		
	OmyFGT32/iiTUF	3	dam	80	81.67 ± 5.55	0.026*	0.112
				85	61.05 ± 7.00		
	OmyRGT42/iiTUF	3	dam	97	59.79 ± 6.08	0.002**	0.214
				143	86.91 ± 5.52		
		4	dam	97	58.63 ± 4.89	0.035*	0.103
				143	72.88 ± 4.35		
A	OmyFGT17TUF	3	sire	151	62.95 ± 6.10	0.017*	0.129
				154	84.45 ± 6.10		
		4	sire	151	57.34 ± 4.34	0.009**	0.149
				154	74.36 ± 4.42		
	Ogo1UW	3	sire	224	62.95 ± 6.10	0.017*	0.129
				229	84.45 ± 6.10		
		4	sire	224	57.35 ± 4.33	0.009**	0.149
				229	74.36 ± 4.42		
	Ssa4DU	3	sire	191	84.45 ± 6.10	0.017*	0.130
				213	62.95 ± 6.10		
		4	sire	191	74.36 ± 4.42	0.009**	0.150
				213	57.34 ± 4.34		
	BHMS267.1	3	sire	277	86.19 ± 7.21	0.040*	0.121
				282	65.32 ± 6.61		
		4	dam	270	56.35 ± 4.94	0.025*	0.139
				282	72.26 ± 4.67		
	OmyRGT41TUF	3	sire	139	84.45 ± 6.10	0.017*	0.129
				143	62.95 ± 6.10		
		4	sire	139	74.36 ± 4.42	0.009**	0.150
				143	57.35 ± 4.34		
K	OmyFGT21/iiTUF	4	sire	143	72.50 ± 4.57	0.042*	0.092
				201	59.13 ± 4.47		
	OmyRGT7TUF	4	sire	139	57.95 ± 4.51	0.021*	0.118
				190	73.04 ± 4.41		
	SSOSL34	4	sire	1	72.50 ± 4.57	0.042*	0.092
				2	59.13 ± 4.47		
8	OmyRGT21TUF	3	dam	148	64.48 ± 6.05	0.033*	0.104
				150	83.80 ± 6.33		

Linkage Group	Marker	Year	Source	Alleles (bp)	Mean ± SE	P-values	R^2
R	Omy7INRA	3	interaction	244, 255	95.30 ± 9.00	0.014	0.137
				244, 260	58.71 ± 7.25		
	One1/iASC	4	interaction	115, 119	73.40 ± 6.92	0.044	0.077
				117, 119	57.10 ± 6.60		
G	OmyRGT36TUF	3	dam	158	62.59 ± 6.06	0.013*	0.138
				164	83.81 ± 6.33		
	One2ASC	3	dam	223	83.40 ± 6.00	0.025*	0.115
				243	63.10 ± 6.29		
		4	dam	223	72.04 ± 4.37	0.038*	0.096
				243	58.38 ± 4.67		
	Ssa85DU	3	dam	112	63.10 ± 6.48	0.032*	0.105
				157	82.54 ± 5.92		
		4	dam	112	57.70 ± 4.76	0.030*	0.105
				157	72.04 ± 4.26		
	BHMS377	3	dam	126	86.90 ± 6.36	0.009**	0.164
				140	62.29 ± 6.21		
		4	dam	126	74.70 ± 4.51	0.006**	0.176
				140	56.10 ± 4.51		
C	Ssa289DU	3	sire	111	82.24 ± 5.80	0.030*	0.107
				113	62.47 ± 6.64		
		4	sire	111	71.62 ± 4.19	0.034*	0.100
				113	57.53 ± 4.90		
	SSOSL439	4	sire	125	72.28 ± 4.29	0.046*	0.093
				131	58.67 ± 5.05		
	OmyOGT4TUF	3	sire	222	62.47 ± 6.64	0.030*	0.107
				239	82.24 ± 5.79		
		4	sire	222	57.53 ± 4.90	0.034*	0.100
				239	71.62 ± 4.19		
I	OmyFGT34TUF	3	sire	161	81.79 ± 5.97	0.050*	0.088
				188	64.00 ± 6.54		
		4	sire	161	73.80 ± 4.10	0.005**	0.171
				188	55.50 ± 4.58		
		3	dam	157	81.80 ± 5.82	0.040*	0.096
				188	63.05 ± 6.68		
		4	dam	157	72.81 ± 4.08	0.010*	0.140
				188	55.89 ± 4.77		
	One3ASC	4	sire	null	56.55 ± 4.67	0.012*	0.137
				198	72.96 ± 4.18		
	BHMS349	3	sire	88	83.75 ± 5.76	0.021*	0.129
				98	62.29 ± 6.84		
		4	sire	88	76.16 ± 3.91	P < 0.001***	0.255
				98	53.41 ± 4.74		
	E2TUF	3	interaction	163, 180	86.58 ± 8.55	0.050	0.092
				163, 156	60.60 ± 9.37		
P	Otsc15NWFSC	3	sire	174	62.44 ± 6.61	0.017*	0.139
				179	84.57 ± 5.85		
		4	sire	174	57.00 ± 4.78	0.013*	0.146
				179	73.87 ± 4.35		
J	OmyFGT12TUF	3	sire	131	82.20 ± 5.09	0.005**	0.173
				140	55.50 ± 7.45		
		4	sire	131	72.39 ± 3.60	0.0027**	0.207

Linkage Group	Marker	Year	Source	Alleles (bp)	Mean ± SE	P-values	R^2
				140	50.79 ± 5.35		
		3	dam	140	62.13 ± 5.84	0.006**	0.164
				188	86.38 ± 6.11		
		4	dam	140	56.75 ± 4.14	0.003**	0.188
				188	75.86 ± 4.42		
	One5ASC	3	dam	350	86.64 ± 5.93	0.003**	0.198
				370	59.60 ± 6.22		
		4	dam	350	74.41 ± 4.51	0.011*	0.147
				370	52.24 ± 4.62		
	Ogo8UW	4	sire	98	70.72 ± 3.97	0.039*	0.096
				100	56.50 ± 5.35		
	SSOSL311	3	sire	150	59.67 ± 7.45	0.025*	0.114
				160	80.97 ± 5.36		
		4	sire	150	53.07 ± 5.31	0.006**	0.164
				160	71.97 ± 3.76		
		3	dam	131	86.38 ± 6.11	0.006**	0.164
				150	62.13 ± 5.84		
		4	dam	131	72.86 ± 4.42	0.003**	0.188
				150	56.75 ± 4.14		
	BHMS426	3	dam	103	88.12 ± 6.40	0.008**	0.176
				111	63.00 ± 6.23		
		4	dam	103	75.89 ± 4.83	0.009**	0.166
				111	57.75 ± 4.60		
	BHMS415	3	dam	125	88.11 ± 6.47	0.007**	0.168
				134	63.30 ± 5.88		
		4	dam	125	75.89 ± 4.80	0.009**	0.156
				134	58.21 ± 4.27		
	BHMS145	3	dam	213	87.40 ± 6.24	0.007**	0.162
				217	63.30 ± 5.82		
		4	dam	213	75.70 ± 4.62	0.008**	0.157
				217	58.20 ± 4.21		
	BHMS423	3	sire	143	54.14 ± 6.96	0.004**	0.330
				145	91.13 ± 6.35		
		4	sire	143	53.00 ± 7.70	0.006**	0.307
				145	81.19 ± 5.90		
		3	dam	143	54.14 ± 9.60	0.004**	0.330
				145	91.13 ± 6.35		
		4	dam	143	53.00 ± 7.70	0.006**	0.307
				145	81.19 ± 5.09		

Marker represents the microsatellites used in this study

Year indicates spawning season (Year 3 = 1995; Year 4 = 1996)

Source indicates the parental effect; interaction terms represent a significant genotype (male allele, female allele)

Alleles represents the size (in base pairs) of the two segregating alleles from a particular parent

Mean indicates significantly different spawning times (days) and the standard error between two alleles within a season

*= Significant differences between mean spawning time after permutation with a threshold value of 0.05

** = Significant differences between mean spawning time after permutation with a threshold value of 0.01

*** = Significant differences between mean spawning time after permutation with a threshold value of 0.001

Table 3.3. Results of general linear model testing for associations between parental alleles and body weight in rainbow trout. Significant interaction terms were detected using a two-factor general linear model.

Linkage Group	Marker	Source	Alleles (bp)	Mean ± SE	P-values	R^2
H	BHMS356	dam	128	818.80 ± 29.94	0.022*	0.127
			150	708.07 ± 35.57		
	OmyFGT11TUF	interaction	152, 156	884.73 ± 72.36	0.015	0.133
			166, 156	711.28 ± 40.14		
	OmyRGT2TUF	interaction	157, 153	825.93 ± 35.89	0.012	0.142
			157, 155	706.68 ± 39.82		
U	OmyCosB/iTUF	dam	259	815.05 ± 27.14	0.020*	0.119
			276	708.48 ± 34.83		
Oi	OmyFGT29TUF	sire	null	848.53 ± 37.44	0.029*	0.111
			263	743.48 ± 27.40		
A	Ogo1UW	interaction	224, 222	707.21 ± 41.11	0.015	0.130
			229, 222	878.04 ± 45.04		
	Ssa4DU	interaction	191, 207	878.04 ± 45.45	0.027	0.109
			213, 207	718.50 ± 39.86		
	OmyRGT41TUF	interaction	139, 122	878.04 ± 44.64	0.009	0.148
			143, 122	696.18 ± 42.56		
G	OmyFGT16TUF	sire	157	824.94 ± 31.71	0.036*	0.098
			231	730.91 ± 29.66		
	OmyRGT36TUF	sire	166	845.56 ± 28.92	0.001**	0.214
			175	707.10 ± 28.29		
	One19ASC	sire	114	707.10 ± 28.29	0.001**	0.214
			122	845.56 ± 28.92		
	BHMS238	sire	178	719.17 ± 25.29	0.005**	0.190
			186	831.66 ± 27.96		
	OmyPuPuPy	sire	380	845.56 ± 29.16	0.002**	0.204
			388	710.48 ± 29.16		
	One2ASC	sire	243	845.56 ± 28.92	0.001***	0.214
			270	707.10 ± 28.29		
	Ots4BML	sire	121	701.16 ± 28.60	P < 0.001***	0.232
			125	845.22 ± 27.97		
	Ssa85DU	sire	104	725.45 ± 29.23	0.018*	0.124
			145	831.18 ± 31.25		
C	BHMS422.1	dam	107	849.51 ± 29.33	P < 0.001***	0.256
			122	699.58 ± 26.66		
B	OmyFGT27TUF	dam	166	731.27 ± 28.22	0.022*	0.116
			168	834.34 ± 33.01		
I	One3ASC	sire	null	725.22 ± 32.68	0.048*	0.088
			198	814.45 ± 29.23		
P	Otsc15NWFSC	dam	170	717.23 ± 29.35	0.003**	0.197
			179	853.77 ± 32.29		
	Omy2DU	dam	142	727.53 ± 32.60	0.042*	0.095
			148	820.10 ± 29.76		
N	Ocl4UW	dam	1	863.26 ± 33.44	0.004**	0.199
			3	727.43 ± 28.14		
	OmyRGT14TUF	dam	139	851.87 ± 32.73	0.004**	0.177
			188	723.41 ± 26.72		
	OmyRGT32TUF	dam	187	851.87 ± 32.73	0.004**	0.177
			226	723.41 ± 26.72		

Linkage Group	Marker	Source	Alleles (bp)	Mean ± SE	P-values	R^2
	OmyRGT47TUF	dam	133	838.12 ± 33.85	0.020*	0.119
			137	732.57 ± 27.64		
	OmyRGT51TUF	dam	119	732.57 ± 27.64	0.020*	0.119
			137	838.12 ± 33.85		

Marker represents the microsatellites used in this study

Source indicates the parental effect; interaction terms indicate a significant genotype (male allele, female allele)

Alleles represents the size (in base pairs) of the two segregating alleles from a particular parent

Mean indicates significantly different weight (grams) and the standard error between two alleles within a season

* = Significant differences between mean body weight after permutation with a threshold value of 0.05

** = Significant differences between mean body weight after permutation with a threshold value of 0.01

*** = Significant differences between mean body weight after permutation with a threshold value of 0.001

CHAPTER 4:

SUMMARY AND FINAL REMARKS

Summary and final remarks

While polyploidy is a frequent and successful evolutionary transition in many plant and some animal lineages its impact on evolution has yet to be determined. Data from a variety of ancient polyploids suggests that a much larger proportion of duplicate gene copies are preserved than predicted by the classical model (Otto and Whitton 2000). Evolutionary biologists have begun to reassess the forces acting on duplicate genes to understand why genes with overlapping functions have been retained, in some cases for hundreds of millions of years.

Conservation of some QTL effects across homeologues in rainbow trout, *Oncorhynchus mykiss*, suggests that a proportion of the duplicate gene pairs arising from the tetraploid event have been preserved. The underlying mechanism for the maintenance of multiple gene copies in salmonids remains uncertain. The fact duplicated loci often differ in the timing and/or the pattern of expression in different tissues is consistent with the subfunctionalization model. Partitioning of ancestral functions via degenerative mutations could potentially reduce the pleiotropic constraints at a given locus. Subsequently, variation in expression (i.e. in a particular tissue or developmental stage) would likely decrease the probability that a locus will be silenced since the other locus can no longer shield it from natural selection (Allendorf and Thorgaard 1984). On the other hand, situations may exist where there is selection for the maintenance of multiple gene copies. Genetic redundancy may offer a fitness advantage under certain environmental conditions or at various life-history stages. While there is considerable evidence for conserved QTL effects across ancestral homeologues in rainbow trout,

additional molecular mapping is required to obtain a more accurate genome-wide estimation.

The results suggest that both spawning time and body weight are controlled by a few major QTL with large effects. There is, however, a statistical bias towards the detection of genes with large phenotypic effects. Increasing the marker density will provide a more accurate measure of the total number of polygenes and the magnitude of effect. The detection of QTL with major effects on spawning time and body weight concur with the oligogenic model. However, the "neo-Darwinian" view of evolution argues that major QTL are generally not important factors in the evolution of quantitative traits because of their deleterious pleiotropic effects on fitness (Fisher 1930; Orr and Coyne 1992). This view is supported by studies in animal populations (Falconer and Mackay 1996). Recent molecular mapping in natural plant populations have detected loci with large effect and at least some of the QTL did not have significant deleterious pleiotropic effects on fitness (Mitchell-Olds 1996; Lin 2000). In fact, these major QTL are considered important factors in the adaptive evolution of these plant species. Thus, detecting QTL and estimating the magnitude of their effects is only the first step towards understanding their role in adaptive evolution.

QTL mapping is a powerful approach towards understanding the variation of fitness-related traits in a quantitative genetic context. One can identify the genetic regions throughout the genome that jointly influence a trait and evaluate their effects in a range of environments and genetic backgrounds. It has been demonstrated that environmental variation can lead to a differential expression of quantitative traits (Leips and Mackay 2000). Two mechanisms have been proposed to explain the genetic basis

underlying this plastic response. Firstly, alleles may vary in effect in response to different environmental conditions ("allelic sensitivity"). Alternatively, regulatory genes responsible for turning on the expression of structural genes that directly influence the phenotype may respond to specific environmental cues ("gene regulation") (Via et al. 1995). Furthermore, it is critical to understand the complex interactions of the genes that contribute to variation in a trait. For example, a gene affecting a given trait may also have pleiotropic effects on other traits and even interact epistatically with other genes.

Spawning time QTL and body weight QTL were identified on the same linkage group in six cases. QTL affecting each trait mapped to the same chromosomal region in four cases. Further fine-scale mapping is needed to determine whether candidate genes within the regions to which QTL map are actually responsible for the observed variation in both these traits. Identifying such genes will determine whether the variation in phenotype results from a pleiotropic effect of a single gene or from several tightly-linked genes affecting each trait separately. Identifying the gene is only the first step towards understanding its activity throughout the lifetime of an organism. For instance, a single gene may exert antagonistic pleiotropic effects on a trait at different life-history stages with alleles enhancing fitness at early ages, but resulting in deleterious effects at later ages (Rose 1985).

Identifying QTL and estimating their effects will allow us to better understand the genetic interactions of fitness-related traits. In salmonid fish, certain life-history traits vary among individual that spawn at different times within a season. Two ecological mechanisms have been proposed to explain this relationship: (1) spawning date within a season is an effect of variation in life history traits, (2) spawning date is a cause of such

variation (Hendry et al. 1999). Identifying the loci affecting spawning time and associated fitness traits will elucidate the genetic covariation of these traits relative to the environment.

References

Agellon, L.B., Emery, C.J., Jones, J.M., Davies, S.L., Dingle, A.D., and Chen, T.T. 1988. Promotion of rapid growth of rainbow trout (*Salmo gairdneri*) by a recombinant fish growth hormone. Canadian Journal of Fisheries and Aquatic Sciences **45**: 146-151.

Ahn, S., and Tanksley, S.D. 1993. Comparative linkage maps of the rice and maize genomes. Proceedings of the National Academy of Sciences of the USA **90**: 7980-7984.

Allendorf, F.W., and Thorgaard, G.H. 1984. Tetraploidy and the evolution of salmonid fishes, pp. 1-53 in *Evolutionary Genetics of Fishes*, edited by B. Turner. Plenum Publishing Corporation, New York.

Allendorf, F.W., and Danzmann, R.G. 1997. Secondary tetrasomic segregation of MDH-B and preferential pairing of homeolgoues in rainbow trout. Genetics **145**: 1083-1092.

Allendorf, F.W., Utter, F.M., and May, B.P. 1975. Gene duplication within the family Salmonidae: II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations, pp. 415-432 in *Isozymes*, edited by C.L. Markert. Academic Press, New York.

Allendorf, F.W., Knudsen, K.L., and Phelps, S.R. 1982. Identification of a gene regulating the tissue expression of a phosphoglucomutase locus in rainbow trout. Genetics **102**: 259-268.

Andersson, L., Haley, C.S., Ellegren, H., Knott, S.A., Johansson, M., Andersson, K., Andersson-Eklund, L., Edfors-Lilja, I., Fredholm, M., Hansson, I., Hakansson, J., and Lundstrom, K. 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science **263**: 1771-1774.

Aparicio, S. 2000. Vertebrate evolution; recent perspectives from fish. Trends in Genetics **16**: 54-56.

Aparicio, S., Hawker, K., Cottage, A., Mikawa, Y., Zuo, L., Venkatesh, B., Chen, E., Krumlauf, R., and Brenner, S. 1997. Organization of the *Fugu rubripes* Hox clusters: evidence for continuing evolution of vertebrate Hox complexes. Nature Genetics **16**: 79-83.

Arranz, J.J., Coppieters, W., Berzi, P., Cambisano, N., Grisart, B., Karim, L., Marcq, F., Moreau, L., Mezer, C., Riquet, J., Simon, P., Vanmanshoven, P., Wagenaar, D., and Georges, M. 1998. A QTL affecting milk yield and composition maps to bovine chromosome 20: a confirmation. Animal Genetics **29**: 107-115.

Bailey, G.S., Poulter, R.T., and Stockwell, P.A. 1978. Gene duplication in tetraploid fish: model for gene silencing at unlinked duplicated loci. Proceedings of the National Academy of Sciences of the USA **75**: 5575-5579.

Bailey, W.J., Kim, J., Wagner, G.P., and Ruddle, F.H. 1997. Phylogenetic reconstruction of vertebrate Hox cluster duplications. Molecular Biology and Evolution **14**: 843-853.

Banks, M.A., Blouin, M.S., Baldwin, B.A., Rashbrook, V.K., and Fitzgerald, H.A. 1999. Isolation and inheritance of novel microsatellites in Chinook salmon (*Oncorhynchus tschawytscha*). Journal of Heredity **90**: 281-288.

Bardakci, F., and Skibinski, D.O.F. 1994. Application of the RAPD technique in tilapia fish: species and subspecies identification. Heredity **73**: 117-123.

Barendse, W., Armitage, S.M., Kossarek, L.M., Shalom, A., Kirkpatrick, B.W., Ryan, A. M., Clayton, D., Li, L., Neibergs, H. L., Zhang, N., Grosse, W. M., Weiss, J., Creighton, P., McCarthy, F., Ron, M., Teale, A. J., Fries, R., McGraw, R. A., Moore, S. S., Georges, M., Soller, M., Womack, J. E., and Hetzel, D. J. S. 1994. A genetic linkage map of the bovine genome. Nature Genetics **6**: 227-235.

Barton, N.H. 1990. Pleiotropic models of quantitative variation. Genetics **124**: 773-782.

Barton, N.H., and Turelli, M. 1989. Evolutionary quantitative genetics: how little do we know? Annual Review of Genetics **23**: 337-370.

Beavis, W.D. 1998. QTL analyses: power, precision, and accuracy, pp. 145-162 in *Molecular Dissection of Complex Traits*, edited by A.H. Paterson. CRC Press, Boca Raton, Florida.

Bernatchez, L. 1996. Reseau de suivi environmental du complex la grande. Caracterisation genetique des formes naines et normales de grande coregone de grand coregone du reservoir Caniapiscau et du lac Serigny a l'aide de marqueurs microsatellites. Rapport presente par l'universite Laval a la vice-presicance Environnement et Collectivites d' Hydro-Quebec.

Betran, E., Santos, M., and Ruiz, A. 1998. Antagonistic pleiotropic effects of second chromosome inversions on body size and early life-history traits in *Drosophila buzzatti*. Evolution **52**: 144-154.

Billard, R. 1985. Environmental factors in salmonid culture and control of reproduction, pp. 70-87 in *Salmonid Reproduction*, edited by S.S. Iwamoto. Washington Sea Grant Program, Seattle, Washington.

Botstein, D., White, R.L., Skolnick, M., and Davis, R.W. 1980. Construction of a genetic map in man using restriction fragment length polymorphisms. American Journal of Human Genetics **32**: 314-331.

Bradshaw, H., Otto, K., Frewen, B., McKay, J., and Schemske, D.W. 1998. Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (*Mimulus*). Genetics **149**: 367-382.

Bromage, N., Jones, J., Randell, C., Thrush, M., Davies, J., Springate, J., Duston, J., and Barker, G. 1992. Broodstock management, fecundity, egg quality and timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). Aquaculture **100**: 141-166.

Charlesworth, B. 1994. *Evolution in Age-Structured Populations*, 2nd Ed. Cambridge Univ. Press, Cambridge, UK.

Cheverud, J.M., and Routman, E. 1993. Quantitative trait loci: individual gene effects on quantitative characters. Journal of Evolutionary Biology **6**: 463-480.

Churchill, G.A., and Doerge, R.W. 1994. Empirical threshold values for quantitative trait mapping. Genetics **138**: 963-971.

Clark, A.G. 1994. Invasion and maintenance of a gene duplication. Proceedings of the National Academy of Sciences of the USA **91**: 2950-2954.

Cooke, J.M.A., Nowak, M.A., Boerlijst, and Maynard Smith, J. 1997. Evolutionary origins and maintenance of redundant genes expression during metazoan development. Trends in Genetics **13**: 360-364.

Condrey, M.J., and Bentzen, P. 1998. Characterization of coastal cutthroat trout (*Oncorhynchus clarki clarki*) microsatellites and their conservation in other salmonids. Molecular Ecology **7**: 787-789.

Crandell, P. A., and Gall, G.A.E. 1993a. The effect of sex on heritability estimates of body weight determined from data on individually tagged rainbow trout (*Oncorhynchus mykiss*). Aquaculture **113**: 47-55.

Crandell, P.A., and Gall, G.A.E. 1993b. The genetics of body weight and its effect on early maturity based on individually tagged rainbow trout (*Oncorhynchus mykiss*). Aquaculture **117**: 77-93.

Crawford, A.M., Dodds, K.G., Ede, A.J., Pierson, G.W., and Montgomery, G.W. 1995. An autosomal linkage map of the sheep genome. Genetics **140**: 703-724.

Cronn, R.C., Small, R.L., and Wendel, J.F. 1999. Duplicated genes evolve independently after polyploidy formation in cotton. Proceedings of the National Academy of Sciences of the USA **96**: 14406-14411.

Danzmann, R.G. LINKMFEX. www.uoguelph.ca/zoology/~rdanzmann/software/

De Gortari, M.J., Freking, B.A., Cuthbertson, R.P., Kappes, S.M., and Keele, J. 1998. A second-generation linkage map of the sheep genome. Mammalian Genome **9**: 204-209.

Dib, C., Faure, S., Fizames, C., Samson, D., and Drouot, N. 1996. A comprehensive map of the human genome based on 5,264 microsatellites. Nature **380**: 152-154.

Dietrich, W.F., Miller, J., Steen, R., Merchant, M.A., and Damron-Boles, D. 1996. A comprehensive genetic map of the mouse genome. Nature **380**: 149-152.

Ekker, M., Wegner, J., Akimenko, M.-A., and Westerfield, M. 1992. Coordinate expression of three zebrafish *engrailed* genes. Development **116**: 1001-1010.

Falconer, D.S., and MacKay, T.F.C. 1996. *Introduction to Quantitative Genetics*. 4th Ed. Longman Sci. and Tech., Harlow, UK.

Ferguson, M.M., and Danzmann, R.G. 1998. Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? Canadian Journal of Fisheries and Aquatic Sciences **55**: 1553-1563.

Ferguson, M.M., Danzmann, R.G., and Arndt, S.K.A. 1993. Mitochrondrial DNA and allozyme variation in Ontario culture rainbow trout spawning in different seasons. Aquaculture **117**: 237-259.

Ferris, S.D., and Whitt, G.S. 1979. Evolution of the differential regulation of duplicate genes after polyploidization. Journal of Molecular Evolution **12**: 267-317.

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford Univ. Press, Oxford, UK.

Foote, C.J. 1990. An experimental comparison of male and female spawning terroriality in a Pacific salmon. Behaviour **115**: 283-314.

Force, A., Lynch, M., Pickett, B.F., Amores, A., Yan, Y., and Postlethwait, J. 1999. Preservation of duplicate genes by complementary, degenerative mutations. Genetics **151**: 1531-1545.

Gates, M.A., Kim, L., Egan, E.S., Cardozo, T., Sirotkin, H.I., Dougan, S. T., Lashkari, D., Abagyan, R., Schier, A. F., and Talbot, W. S. 1999. A genetic linkage map for zebrafish: comparative analysis and localization of genes and expressed sequences. Genome Research **9**: 334-337.

Haldane, J. B. S. 1933. The part played by recurrent mutation in evolution. American Naturalist **67**: 5-9.

Haley, C.S. 1995. Livestock QTLs - bringing home the bacon? Trends in Genetics **11**: 487-492.

Hedrick, P.W. 1999. Antagonistic pleiotropy and genetic polymorphism: a perspective. Heredity **82**: 126-133.

Hendry, A.P., Berg, O.K., and Quinn, T.P. 1999. Condition dependence and adaption-by-time: breeding date, life history, and energy allocation within a population of salmon. Oikos **85**: 499-514.

Holm, L.-E., and Brusgaard, K. 1999. Two polymorphic dinucleotide repeats in rainbow trout (*Oncorhynchus mykiss*). Animal Genetics **30**: 162-163.

Hughes, M.K., and Hughes, A.L. 1993. Evolution of duplicate genes in a tetraploid animal, *Xenopus laevis*. Molecular Biology and Evolution **10**: 1360-1369.

Hurme, P., Sillanpaa, M.J., Arjas, E., Repo, and T., Savolainen, O. 2000. Genetic basis of climatic adaptation in Scots pine by Bayesian quantitative trait locus analysis. Genetics **156**: 1309-1322.

Hutchings, J.A. 1993. Adaptive life histories effected by age-specific survival and growth rate. Ecology **74**: 673-684.

Iwabe, N., Kuma, K., and Miyata, T. 1996. Evolution of gene families and relationship with organismal evolution: rapid divergence of tissue-specific genes in the early evolution of chordates. Molecular Biological Evolution **13**: 483-493.

Jackson, T.R., Ferguson, M.M., Danzmann, R.G., Fishback, A.G., Ihssen, P.E., O'Connell, M., and Crease, T.J. 1998. Identification of two QTL influencing upper temperature tolerance in three rainbow trout (*Oncorhynchus mykiss*) half-sib families. Heredity **80**: 143-151.

Johnson, K.R., Wright, J.E., and May, B. 1987. Linkage relationships reflecting ancestral tetraploidy in salmonid fish. Genetics **116**: 579-591.

Kappes, S.M., Keele, J.W., Stone, R.T., McGraw, R.A., Sonstegard, T.P.L., Smith, N. L., Lopez-Corrales, and Beattie, C. W. 1997. A second-generation linkage map of the bovine genome. Genome Research **7**: 235-249.

Knapik, E.W., Goodman, A., Ekker, M., Chevrette, J., Delgado, J., Neuhauss, S., Shimoda, N., Driever, W., Fishman, M.C., and Jacob, H.J. 1998. A microsatellite genetic linkage map for zebrafish (*Danio rerio*). Nature Genetics **18**: 338-343.

Kocher, T.D., Lee, W., Sobolewska, H., Penman, D., and McAndrew, B. 1998. A genetic linkage map of the cichlid fish, the tilapia (*Oreochromis niloticus*). Genetics **148**: 1225-1232.

Krumlauf, R. 1994. Hox genes in vertebrate development. Cell **78**: 191-201.

Lander, E.S., and Botstein, D. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics **121**: 185-199.

Lee, W.-J., and Kocher, T.D. 1996. Microsatellite DNA markers for genetic mapping in *Oreochromis niloticus*. Journal of Fish Biology **49**: 169-171.

Lee, G.M., and Wright, J.E. 1981. Mitotic and meiotic analyses of brook trout, *Salvelinus fontinalis*. Journal of Heredity **72**: 321-327.

Leips, J. and Mackay, T.F.C. 2000. Quantitative trait loci for life span in *Drosophila melanogaster*: interactions with genetic background and larval density. Genetics **155**: 1773-1788.

Li, W.-H. 1980. Rate of gene silencing at duplicate loci: a theoretical study and interpretation of data from tetraploid fish. Genetics **95**: 237-258.

Lin, J.-Z. 2000. The relationship between loci for mating system and fitness-related traits in *Mimulus* (Scrophulariaceae): A test for deleterious pleiotropy of QTL with large effects. Genome **43**: 628-633.

Lin, J.-Z., and Ritland, K. 1997. Quantitative trait loci differentiating the outbreeding *Mimulus guttatus* from the inbreeding *Mimulus platycalyx*. Genetics **146**: 1115-1121.

Lynch, M., and Force, A. 2000. The probability of duplicate gene preservation by subfunctionalization. Genetics **154**: 459-473.

Lynch, M., and Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Assoc., Inc., Sunderland, Massachusetts.

Martyniuk, C.J. 2001. A microsatellite DNA analysis examining the relationship between growth and maturation rate genes in rainbow trout. M.Sc. Thesis. University of Guelph, Guelph, Ontario, Canada.

May, B., and Johnson, K.R. 1990. Composite linkage map of salmonid fishes (*Salvelinus, Salmo, Oncorhynchus*), pp. 151-159 in *Genetic maps: Locus Maps of Complex Genomes*, edited by S.J. O'Brien. Cold Springs Harbor Laboratory Press, Cold Springs Harbor, New York.

Miklos, G.L., and Rubin, G.M. 1996. The role of the genome project in determining gene function: insights from model organisms. Cell **86**: 521-529.

Mitchell-Olds, T. 1996. Genetic constraints on life-history evolution: Quantitative trait loci influencing growth and flowering in *Arabidopsis thaliania*. Evolution **50**: 140-145.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Bhatia, C.R., and Sasaki, T. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. Molecular Breeding **3**: 87-103.

Montgomery, G.W., and Kinghorn, B.P. 1997. Recent developments in the gene mappping and progress towards marker-assisted selection in sheep. Australian Journal of Agricultural Research **48**: 729-741.

Morris, D.B., Richard, K.R., and Wright, J.M. 1996. Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. Canadian Journal of Fisheries and Aquatic Sciences **53**: 120-126.

Morton, N. 1955. Sequential tests for the detection of linkage. American Journal of Human Genetics **7**: 277-318.

Mousseau, T.A., and Roff, D.A. 1987. Natural selection and the heritability of fitness components. Heredity **59**: 181-197.

Nadeau, J.H., and Sankoff D. 1997. Comparable rates of gene loss and functional divergence after genome duplications early in vertebrate evolution. Genetics **147**: 1259-1266.

Nei, M., and Roychoudhury, A.K. 1973. Probability of fixation of nonfunctional genes at duplicate loci. American Naturalist **107**: 362-372.

Nelson, R.J., and Beacham, T.D. 1999. Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. Animal Genetics **30**: 228-229.

Nowak, M.H., Boerlijst, M.C., Cooke, J., and Maynard Smith, J. 1997. Evolution of genetic redundancy. Nature **388**: 167-171.

O'Brien, S. J., Menotti-Raymond, M., Murphy, W. J., Nash, W. G., Wienberg, J., Stanyon, R., Copeland, N. G., Jenkins, N. A., Womack, J. E., and Marshall Graves, J. A. 1999. The promise of comparative genomics in mammals. Science **286**: 458-481.

O'Connell, M., and Wright, J.M. 1997. Microsatellite DNA in fishes. Reviews in Fish Biology and Fisheries **7**: 331-363.

Ohno, S. 1970. The enormous diversity in genome sizes of fish as a reflection of nature's extensive experiments with gene duplication. Transactions of the American Fisheries Society **99**: 120-130.

Ohno, S., Wolf, S.U., and Atkin, N.B. 1967. Evolution from fish to mammals by gene duplication. Hereditas **59**: 169-187.

Olsen, J.B., Bentzan, P., and Seeb, J.E. 1998. Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology **7**: 1087-1090.

Orr, H.A., and Coyne, J.A. 1992. The genetics of adaptation: a reassessment. American Naturalist **140**: 725-742.

Otto, S. P., and Whitton, J. 2000. Polyploid incidence and evolution. Annual Review of Genetics **34**: 401-437.

Perry, G., Danzmann, R., Ferguson, M.M., and Gibson, J. In press. Quantitative trait loci for upper thermal tolerance in outbred strains of rainbow trout (*Oncorhynchus mykiss*). Heredity

Poompuang, S., and Hallerman, E.M. 1997. Toward detection of quantitative trait loci and marker-assisted selection in fish. Reviews in Fisheries Science **5**: 253-277.

Postlethwait, J.H., Johnson, S.L., Midson, C.N., Talbot, W.S., Gates, M., Ballinger, E.W., Africa, D., Andrews, R., Carl, T., Eisen, J.S., Horne, S., Kimmel, C.B., Hutchinson, M., Johnson, M., Rodriguez, M.A. 1994. A genetic linkage map for the zebrafish. Science **264**: 699-703.

Prince, V.E., Moens, C.B., Kimmel, C.B., and Ho, R.K. 1998. Zebrafish hox genes: genomic organization and modified collinear expression patterns in the trunk. Development **125**: 407-420.

Queller, D.C., Strassmann, J.E., and Hughes, C.R. 1993. Microsatellite and kinship. Trends in Ecology and Evolution **8**: 285-288.

Roff, D.A. 1982. An allocation model of growth and reproduction in fish. Canadian Journal of Fisheries and Aquatic Sciences **40**: 1395-1404.

Roff, D. A. 1992. *The Evolution of Life Histories*. Theory and Analysis. Chapman & Hall, New York.

Rohrer, G.A., Alexander, L.J., Hu, Z., Smith, T.P., Keele, J.W., and Beattie, C.W. 1996. A comprehensive map of the porcine genome. Genome Research **6**: 371-391.

Rose, M.R. 1985. Life history evolution with antagonistic pleiotropy and overlapping generations. Theoretical and Population Biology **28**: 342-358.

Sakamoto, T., Okamoto, N., and Ikeda, Y. 1996. Application of PCR primer pairs from rainbow trout to detect polymorphisms of CA repeat DNA loci in five confamilial species. Fisheries Science **62**: 552-555.

Sakamoto, T., Danzmann, R.G., Okamoto, N., Ferguson, M.M., and Ihssen, P.E. 1999. Linkage analysis of quantitative trait loci associated with spawning time in rainbow trout (*Oncorhynchus mykiss*). Aquaculture **173**: 33-43.

Sakamoto, T., Danzmann, R.G., Gharbi, K., Howard, P., Ozaki, A., Sokkean, K., Woram, R., Okamoto, N., Ferguson, M.M., Holm, L.-E., Guyomard, R., and Hoyheim, B. 2000. A microsatellite linkage map of rainbow trout (*Oncorhynchus mykiss*) characterized by large sex-specific differences in recombination rates. Genetics **155**: 1331-1345.

SAS Institute 1996. SAS version 6.12. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina.

Sax, K. 1923. The relation between chromosome number, morphological characters and rust resistance in segregates of partially sterile wheat hybrids. Genetics **8**: 301-321.

Schultz, R.J. 1980. Role of polyploidy in the evolution of fishes, pp. 313-340 in *Polyploidy: Biological relevance,* edited by W.H. Lewis. Plenum Press, New York.

Scribner, K.T., Gust, J.R., and Fields, R.L. 1996. Isolation and characterization of novel microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences **53**: 833-841.

Seoighe, C., and Wolfe, K. H. 1999. Yeast genome evolution in the post-genome era. Current Opinions Microbiology **2**: 548-554.

Shimoda, N., Knapik, E.W., Ziniti, J., Sim, C., Yamada, E., Kaplan, S., Jackson, D., De Sauvage, F., Jacob, H., and Fishman, M.C. 1999. Zebrafish genetic map with 2000 microsatellite markers. Genomics **58**: 219-232.

Siitonen, L., and Gall, G.A.E. 1989. Response to selection for early spawn data in rainbow trout, *Salmo gairdneri.* Aquaculture **78**: 153-161.

Simmen, M.W., Leitgeb, S., Clark, V.H., Jones, S.J., and Bird, A. 1998. Gene number in an invertebrate chordate, *Ciona intestinalis.* Proceedings of the National Academy of Sciences of the USA **95**: 4437-4440.

Soller, M., and Brody, T. 1976. On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theoretical and Applied Genetics **47**: 35-39.

Sokal, R.R. and Rohlf, F.J. 1981. *Biometry.* W.H. Freeman and Co., New York.

Stearns, S.C. 1992. *The Evolution of Life Histories.* Oxford Univ. Press, Oxford, UK.

Stebbins, G.L. 1971. *Process of Organic Evolution.* Prentice Hall, Englewood Cliffs, New Jersey.

Stickney, R. 1991. *Culture of Salmonid Fishes.* CRC Press, Boca Raton, Florida.

Sturtevant, A. H. 1913. The linear arrangement of six sex-linked factors in *Drosophila,* as shown by their mode of association. Journal of Experimental Zoology **14**: 43-59.

Su, G.-S., Liljedahl, L.-E., and Gall, G.A.E. 1996. Genetic and environmental variation in body weight in rainbow trout. Aquaculture **143**: 71-80.

Tanksley, S.D. 1993. Mapping polygenes. Annual Review of Genetics **27**: 205-233.

Tautz, D., and Renz, M. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research **12**: 4127-4138.

Uyenyo, T., and Smith, G.R. 1972. Tetraploid origin of the karyotype of catostomid fishes. Science **175**: 644-646.

Vallejo, R.L., Bacon, L.D., Liu, H.-C., Witter, R.L., Groenen, M., Hillel, J., and Cheng, H.H. 1998. Genetic mapping of quantitative trait loci affecting susceptibility to Marek's disease virus induced in F_2 intercross chickens. Genetics **148**: 349-360.

van den Berghe, E.P., and Gross, M.R. 1986. Length of breeding life in coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Zoology **64**: 1482-1486.

Via, S., Gomulkiewicz, R., Dejong, G., Scheiner, S.M., Schlichting, C.D, and Van Tienderen, P.H. 1995. Adaptive phenotypic plasticity: consensus and controversy. Trends in Ecology and Evolution **10**: 212-217.

Vos, P., Hogers, R., Bleeker, M., Rijans, M., Van Der Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research **23**: 4407-4414.

Wagner, A. 1999. Redundant gene functions and natural selection. Journal of Evolutionary Biology **12**: 1-16.

Walsh, J. B. 1995. How often do duplicated genes evolve new functions? Genetics **139**: 421-428.

Ware, D.M. 1982. Power and evolutionary fitness of teleosts. Canadian Journal of Fisheries and Aquatic Sciences **39**: 3-13.

Weintraub, H. 1993. The MyoD family and myogensis: redundancy, networks, and thresholds. Cell **75**: 1241-1244.

Wendel, J.F. 2000. Genome evolution in polyploids. Plant Molecular Biology **42**: 225-249.

Wright, J.E., Heckman, J.R., and Atherton, L.M. 1975. Genetic and developmental analyses of LDH isozymes in trout, pp. 375-399 in *Isozymes* III: *Developmental Biology*, edited by C.L. Markert. Academic Press, New York.

Wright, J. E., Johnson, K., Hollister, A., and May, B. 1983. Meiotic models to explain classical linkage, pseudolinkage, and chromosome pairing in tetraploid derivative salmonid genomes, pp. 239-260 in *Isozymes: Current topics in Biological and Medical Research*, edited by M.C. Rattazzi, J.G. Scandalios, and G. S. Whitt. Alan R. Liss, Inc., New York.

Wright, J.M. 1993. DNA fingerprinting in fishes, pp. 58-91, in *Biochemistry and Molecular Biology of Fishes*, edited by P.W. Hochachka and T. Mommsen. Elsevier, Amsterdam.

Young, W.P., Wheeler, P.A., Coryell, V.H., Keim, P., and Thorgaard, G.H. 1998. A detailed linkage map of rainbow trout produced using doubled haploids. Genetics **148**: 839-850.

Appendix 1. A list of all the microsatellite primers used in this study and corresponding author.
Fifty-three microsatellite markers in italics have been mapped as part of this project.
BHMS and Ots primers have recently been renamed and the current nomenclature is also listed.

Locus	Linkage Group	Reference	Revised name
BHMS7.15	2	Hoyheim (unpublished)	Ssa118NVH
BHMS159	5	Hoyheim (unpublished)	Ssa142NVH
BHMS254/ii	5	Hoyheim (unpublished)	Ssa125/iiNVH
BHMS323	5	Hoyheim (unpublished)	Ssa142NVH
BHMS339	8	Hoyheim (unpublished)	Ssa102NVH
BHMS212	15	Hoyheim (unpublished)	Ssa94NVH
BHMS448	15	Hoyheim (unpublished)	Ssa73NVH
BHMS221.1	18	Hoyheim (unpublished)	Ssa96NVH
BHMS7.1	18	Hoyheim (unpublished)	Ssa1NVH
BHMS267.1	A	Hoyheim (unpublished)	Ssa38NVH
BHMS328	C	Hoyheim (unpublished)	Ssa86NVH
BHMS422.1	C	Hoyheim (unpublished)	Ssa68NVH
BHMS281	D	Hoyheim (unpublished)	Ssa44NVH
BHMS7.5/I	E	Hoyheim (unpublished)	Ssa79/iNVH
BMS252	Fii	Hoyheim (unpublished)	Ssa36NVH
BHMS238	G	Hoyheim (unpublished)	Ssa32NVH
BHMS377	G	Hoyheim (unpublished)	Ssa57NVH
BHMS117B	H	Hoyheim (unpublished)	Ssa10NVH
BHMS337	H	Hoyheim (unpublished)	Ssa107NVH
BHMS356	H	Hoyheim (unpublished)	Ssa109NVH
BHMS349	I	Hoyheim (unpublished)	Ssa103NVH
BHMS145	J	Hoyheim (unpublished)	Ssa136NVH
BHMS415	J	Hoyheim (unpublished)	Ssa104NVH
BHMS423	J	Hoyheim (unpublished)	Ssa70NVH
BHMS426	J	Hoyheim (unpublished)	Ssa80NVH
BHMS250	K	Hoyheim (unpublished)	Ssa35NVH
BHMS486	K	Hoyheim (unpublished)	Ssa81NVH
BHMS418	M	Hoyheim (unpublished)	Ssa67NVH
BHMS184	Oi	Hoyheim (unpublished)	Ssa24NVH
BHMS188	Oi	Hoyheim (unpublished)	Ssa150NVH
BHMS230	Q	Hoyheim (unpublished)	Ssa155NVH
BHMS124/ii	R	Hoyheim (unpublished)	Ssa133/iiNVH
BHMS347B/i	R	Hoyheim (unpublished)	Ssa174/iNVH
BHMS205	UA	Hoyheim (unpublished)	Ssa29/iiNVH
BHMS253	UA	Hoyheim (unpublished)	Ssa218NVH
BHMS347B/ii	UA	Hoyheim (unpublished)	Ssa174/iiNVH
BHMS373	UA	Hoyheim (unpublished)	Ssa55NVH
BHMS425	UA	Hoyheim (unpublished)	Ssa112NVH
Ocl3	UA	Bernatchez 1996	
E2(TUF)	I	Sakamoto et al. 1996	
Hsc71(DIAS)	Oi	Holm and Brusgaard 1999	
Ocl1UW	P	Olsen et al. 1998; Condrey and Bentzen 1998	
Ocl2UW	15	Olsen et al. 1998; Condrey and Bentzen 1998	
Ocl4UW	N	Olsen et al. 1998; Condrey and Bentzen 1998	
Ogo1UW	A	Olsen et al. 1998; Condrey and Bentzen 1998	
Ogo4UW	R	Olsen et al. 1998; Condrey and Bentzen 1998	

Locus	Linkage Group	Reference	Revised name
Ogo8UW	J	Olsen et al. 1998; Condrey and Bentzen 1998	
Omy1INRA	15	Gharbi and Guyomard (unpublished)	
Omy1UOG	UA	Woram et al. (unpublished)	
Omy272/iiUOG	5	Jackson et al. 1998	
Omy272/iUOG	15	Jackson et al. 1998	
Omy27INRA	Q	Gharbi and Guyomard (unpublished)	
Omy29INRA	15	Gharbi and Guyomard (unpublished)	
Omy2DU	P	Morris et al. 1996	
Omy301UoG	B	Jackson et al. 1998	
Omy325UoG	B	Jackson et al. 1998	
Omy335UoG	H	O'Connell et al. 1997	
Omy38DU	H	Morris et al. 1996	
Omy3DIAS	R	Holm and Brusgaard 1999	
Omy6INRA	Fii	Gharbi and Guyomard (unpublished)	
Omy77DU	Fii	Morris et al. 1996	
Omy7INRA	R	Gharbi and Guyomard (unpublished)	
OmyCosB/iiTUF	H	Sakamoto et al. 1996	
OmyCosB/iTUF	U	Sakamoto et al. 1996	
OmyFGT10TUF	H	Sakamoto et al. 1996	
OmyFGT11TUF	H	Sakamoto et al. 1996	
OmyFGT12TUF	J	Sakamoto et al. 1996	
OmyFGT13TUF	J	Sakamoto et al. 1996	
OmyFGT14TUF	P	Sakamoto et al. 1996	
OmyFGT15TUF	5	Sakamoto et al. 1996	
OmyFGT16TUF	G	Sakamoto et al. 1996	
OmyFGT17TUF	A	Sakamoto et al. 1996	
OmyFGT18/iiTUF	Oii	Sakamoto et al. 1996	
OmyFGT18/iTUF	Oi	Sakamoto et al. 1996	
OmyFGT19TUF	18	Sakamoto et al. 1996	
OmyFGT20TUF	UA	Sakamoto et al. 1996	
OmyFGT21/iiTUF	K	Sakamoto et al. 1996	
OmyFGT23TUF	Q	Sakamoto et al. 1996	
OmyFGT24TUF	E	Sakamoto et al. 1996	
OmyFGT25TUF	Oii	Sakamoto et al. 1996	
OmyFGT26TUF	R	Sakamoto et al. 1996	
OmyFGT27TUF	B	Sakamoto et al. 1996	
OmyFGT28/i(F)TUF	Oi	Sakamoto et al. 1996	
OmyFGT28/i(M)TUF	Oi	Sakamoto et al. 1996	
OmyFGT28/iiTUF	N	Sakamoto et al. 1996	
OmyFGT29TUF	Oi	Sakamoto et al. 1996	
OmyFGT2TUF	B	Sakamoto et al. 1996	
OmyFGT32/iiTUF	Oii	Sakamoto et al. 1996	
OmyFGT32/iTUF	Oi	Sakamoto et al. 1996	
OmyFGT34TUF	I	Sakamoto et al. 1996	
OmyFGT3TUF	2	Sakamoto et al. 1996	
OmyFGT4(F)TUF	Fi	Sakamoto et al. 1996	
OmyFGT4(M)TUF	Fi	Sakamoto et al. 1996	
OmyFGT5TUF	A	Sakamoto et al. 1996	

Locus	Linkage Group	Reference	Revised name
OmyFGT6TUF	UA	Sakamoto et al. 1996	
OmyFGT7TUF	B	Sakamoto et al. 1996	
OmyFGT8/iiTUF	5	Sakamoto et al. 1996	
OmyFGT8/iTUF	15	Sakamoto et al. 1996	
OmyFGT9TUF	H	Sakamoto et al. 1996	
OmyGT23DTUF	2	Sakamoto et al. 1996	
OmyJTUF	N	Sakamoto et al. 1996	
OmyOGT4TUF	C	Sakamoto et al. 1996	
OmyOGT5/iiTUF	Fii	Sakamoto et al. 1996	
OmyOGT5/iTUF	Fi	Sakamoto et al. 1996	
OmyP9-2TUF	B	Sakamoto et al. 1996	
OmyPuPuPyDU	G	Morris et al. 1996	
OmyRGT10TUF	Q	Sakamoto et al. 1996	
OmyRGT12TUF	U	Sakamoto et al. 1996	
OmyRGT13TUF	15	Sakamoto et al. 1996	
OmyRGT14TUF	N	Sakamoto et al. 1996	
OmyRGT15/iiTUF	R	Sakamoto et al. 1996	
OmyRGT15/iTUF	8	Sakamoto et al. 1996	
OmyRGT17TUF	R	Sakamoto et al. 1996	
OmyRGT18TUF	Q	Sakamoto et al. 1996	
OmyRGT19TUF	2	Sakamoto et al. 1996	
OmyRGT1TUF	5	Sakamoto et al. 1996	
OmyRGT21TUF	8	Sakamoto et al. 1996	
OmyRGT23TUF	8	Sakamoto et al. 1996	
OmyRGT24TUF	M	Sakamoto et al. 1996	
OmyRGT26TUF	B	Sakamoto et al. 1996	
OmyRGT27TUF	L	Sakamoto et al. 1996	
OmyRGT28TUF	18L	Sakamoto et al. 1996	
OmyRGT2TUF	H	Sakamoto et al. 1996	
OmyRGT30TUF	Oi	Sakamoto et al. 1996	
OmyRGT31TUF	15	Sakamoto et al. 1996	
OmyRGT32TUF	N	Sakamoto et al. 1996	
OmyRGT33TUF	Oi	Sakamoto et al. 1996	
OmyRGT34TUF	M	Sakamoto et al. 1996	
OmyRGT36TUF	G	Sakamoto et al. 1996	
OmyRGT38TUF	Oi	Sakamoto et al. 1996	
OmyRGT39TUF	E	Sakamoto et al. 1996	
OmyRGT40/iiTUF	Oii	Sakamoto et al. 1996	
OmyRGT40/iTUF	Oi	Sakamoto et al. 1996	
OmyRGT41TUF	A	Sakamoto et al. 1996	
OmyRGT42/iiTUF	Oii	Sakamoto et al. 1996	
OmyRGT42/iTUF	Oi	Sakamoto et al. 1996	
OmyRGT43(F)TUF	D	Sakamoto et al. 1996	
OmyRGT43(M)TUF	D	Sakamoto et al. 1996	
OmyRGT47TUF	N	Sakamoto et al. 1996	
OmyRGT4TUF	Oi	Sakamoto et al. 1996	
OmyRGT51TUF	N	Sakamoto et al. 1996	
OmyRGT52TUF	R	Sakamoto et al. 1996	
OmyRGT53TUF	UA	Sakamoto et al. 1996	

Locus	Linkage Group	Reference	Revised name
OmyRGT6/iiTUF	C	Sakamoto et al. 1996	
OmyRGT6/iTUF	L	Sakamoto et al. 1996	
OmyRGT7TUF	K	Sakamoto et al. 1996	
OmyRGT8TUF	8	Sakamoto et al. 1996	
One1/iASC	R	Scribner et al. 1996	
One1/iiASC	R	Scribner et al. 1996	
One10/iASC	H	Scribner et al. 1996	
One11ASC	G	Scribner et al. 1996	
One14ASC	Oi	Scribner et al. 1996	
One18/iASC	15	Scribner et al. 1996	
One18/iiASC	5	Scribner et al. 1996	
One19ASC	G	Scribner et al. 1996	
One2ASC	G	Scribner et al. 1996	
One3ASC	I	Scribner et al. 1996	
One5ASC	J	Scribner et al. 1996	
Ots100SSBI	Fi	Nelson and Beacham 1999	
Ots1BML	8	Banks et al. 1999	
Ots4BML	G	Banks et al. 1999	
Otsa5/iNWFSC	15	Nash (unpublished)	Ots502NWFSC
Otsb5NWFSC	Q	Nash (unpublished)	Ots507NWFSC
Otsc15NWFSC	P	Nash (unpublished)	Ots513NWFSC
Otse2NWFSC	Q	Nash (unpublished)	Ots522NWFSC
Otsh4NWFSC	Oi	Nash (unpublished)	Ots534NWFSC
Sal12UOG	B	Woram et al. (unpublished)	
Sal2UOG	UA	Woram et al. (unpublished)	
Sal8/iUOG	R	Woram et al. (unpublished)	
SEX	18	May and Johnson 1990	
Ssa14DU	D	Morris et al. 1996	
Ssa197DU	B	Morris et al. 1996	
Ssa289DU	C	Morris et al. 1996	
Ssa4DU	A	Morris et al. 1996	
Ssa6.33NUIG	C	Powell (unpublished)	
Ssa85DU	G	Morris et al. 1996	
SSOSL311	J	Slettan et al. 1996	
SSOSL32	E	Slettan et al. 1996	
SSOSL34	K	Slettan et al. 1996	
SSOSL439	C	Slettan et al., 1996	
Str4/iilNRA	5	Gharbi and Guyomard (unpublished)	
Str58CNRS	C	Poteaux (unpublished)	
Str60INRA	Fii	Gharbi and Guyomard (unpublished)	
Str73INRA	Oi	Gharbi and Guyomard (unpublished)	
Str7/iINRA	UA	Gharbi and Guyomard (unpublished)	