GENETIC ANALYSIS OF TRAITS ASSOCIATED WITH DOMESTICATION IN RAINBOW TROUT

By

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The members of the Committee appointed to examine the dissertation of ROBERT EDWARD DREW find it satisfactory and recommend that it be accepted.

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GENETIC ANALYSIS OF TRAITS

ASSOCIATED WITH DOMESTICATION

IN RAINBOW TROUT

Abstract

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Domestication is a complex process by which populations evolve in the presence of humans and in response to rearing in captivity. Several behavioral and physiological differences have been detected between wild and domesticated salmonid populations. In particular, reduced stress physiology has been documented in salmonids and other domesticated species. Negative impacts of chronic stress on growth, survival, and reproduction suggest that reduced responsiveness to stress may reflect an adaptation to captivity. Growth itself is an economically trait, under complex genetic and environment control, and is the focus of continued research. This dissertation examines the genetic factors underlying these two traits using rainbow trout clonal lines. In the analysis of stress physiology, stress hormone levels, in response to handling stress, were reduced in a heavily domesticated clonal line (Arlee) relative to another heavily domesticated line (OSU) and a less domestication line (Swanson). Analysis using doubled haploids derived from clonal lines with divergent stress responses (OSU and Arlee) revealed two QTL with opposing additive effects on stress cortisol levels, and no genetic link between stress physiology and growth rate in this cross. A more detailed analysis of juvenile growth was performed using doubled haploids derived from two clonal lines with divergent growth rates and domestication histories (OSU and Swanson). Body length, mass, and condition factor were

measured at two time points (89 and 194 days postfertilization), and specific growth rate was calculated for the interval between these time points. Twenty-two QTL were detected in at least 14 locations on 11 linkage groups. Several QTL affecting different growth traits mapped to similar locations suggesting the presence of loci with pleiotropic effects or closely linked loci affecting different traits. The analysis also suggests possible genotype x environment and genotype x age interactions but this could not be reliably evaluated. Finally comparisons with QTL analyses of embryonic and adult growth in rainbow trout reveals that, in some case, the same linkage groups harbor QTL affecting growth at different levels. Our findings reveal that QTL analyses of traits associated with salmonid domestication can reveal complex genetic and environmental interactions.

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CHAPTER ONE

GENERAL INTRODUCTION

Understanding the genetic effects of domestication is critical to success in salmonid aquaculture and conservation. Evolutionary change is unavoidable when a wild population is taken into captivity. Beyond the random effects of genetic drift, captive environments have different combinations of selection forces, including artificial selection, relaxed selection, and natural selection leading to adaptation to captivity (Price, 1998; 1999; Waples, 1999). These selection forces result from goal-directed breeding, and fundamental environmental and ecological differences between natural and captive environments. Domestication eventually leads to changes in the gene pool of the captive population and divergence from the wild progenitor. If these changes produce phenotypes that are maladaptive in the wild, then natural populations could be severely impacted by gene flow from hatchery supplementation and escapes from aquaculture facilities (Waples, 1991). Characterization of the genetic factors underlying traits associated with domestication would be useful for monitoring the effects of aquaculture escapes on wild populations. Also, conservation programs using captive breeding must be carefully planned to minimize the effects of domestication (Arnold, 1995; Flagg and Nash, 1999). Conversely, information about the genetics underlying adaptation to captivity could allow faster incorporation of traits, such as disease resistance, from wild stocks that may have poor survival in captivity.

A growing number of studies examined the effects of domestication in salmonids by comparing wild and captive-bred populations. Most of these comparisons involve traits that are continuous in nature and under complex environmental and genetic controls. Differences in behavior patterns are often reported in comparisons of wild and captive-bred salmonid

populations. Captive-bred salmonids display a greater willingness to feed in the presence of a predator (Johnsson and Abrahams, 1991; Johnsson et al., 1996; Yamamoto and Reinhardt, 2003), shorter durations of hiding and flight behavior patterns (Vincent, 1960; Einum and Fleming, 1997; Fleming and Einum, 1997; Johnsson et al., 2001), higher surface orientation (Vincent, 1960), and reduced ability to avoid predation (Berejikian, 1995). Changes in aggression level have also been reported but findings have been inconsistent. Some studies report higher levels of aggression in captive-bred populations (Swain and Riddell, 1990; Einum and Fleming, 1997; Rhodes and Quinn, 1998; Deverill et al., 1999; Metcalfe et al., 2003), others report higher levels in wild populations (Berejikian et al., 1996; Petersson et al., 1996; Mork et al., 1999), and still others report no significant relationship with captive breeding (Lahti et al., 2001; Yamamoto and Reinhardt, 2003).

Domesticated populations also tend to show physiological changes that may underlie behavioral differences. Captive-bred populations are often under artificial selection and exhibit faster growth rates than wild populations (Vincent, 1960; Einum and Fleming, 1997; Fleming and Einum, 1997). Enhanced growth in captive populations can result in increased metabolic demand and may explain higher feeding motivation and willingness to feed under risk of predation (Johnsson et al., 1996). Captive populations also show changes in physiological processes associated with attenuated fear-like behaviors, such as reductions in cortisol levels (Woodward and Strange, 1987; Salonius and Iwama, 1993), brain serotonergic activity (Lepage et al., 2000), and heart rate (Johnsson et al., 2001) in response to stressors. Reduced brain size in captive rainbow trout has also been reported (Marchetti and Nevitt, 2003).

Quantitative trait locus (QTL) analysis is a powerful tool that can aid in the understanding of the genetic mechanisms underlying domestication in salmonids and other

species. QTL analyses can provide valuable information about the inheritance of a trait by estimating the number of loci involved and the distribution of effects (Mackay, 2001). By this means, control of a trait can be narrowed to a few loci with strong effects or many loci with weak effects. Also, estimation of the location of QTL in the genome is a first step towards identification and characterization of the actual genes responsible for variation in the trait. Although this is a difficult goal to attain, generally requiring intensive and expensive genetic research (Mackay, 2001), successful identifications of genes underlying QTL are increasing in frequency (e.g., Van Laere et al., 2003; Oliver et al., 2005). Identification of markers closely associated with a QTL can be used in marker-assisted selection, although this has proven difficult in practice (Young, 1999). Simultaneous analysis of multiple traits can reveal pleiotropic effects of QTL and identify sources of correlated responses to selection (Jiang and Zeng, 1995). Finally, proper experimental design and statistical models can allow the examination of epistasis among QTL (Carlborg et al., 2005), and interactions with environment and sex (Mackay, 2001).

Rainbow trout (*Oncorhynchus mykiss*) clonal lines are an invaluable tool for investigating the genetics of quantitative trait differences between wild and domesticated populations. Rainbow trout clonal lines are populations of genetically-identical, completely homozygous individuals, analogous to mouse inbred lines. Within just two generations of chromosome set manipulation through androgenesis or gynogenesis, genetic variation can be captured in a research population and made available for research (Parsons and Thorgaard, 1984; Young et al., 1996; Robison and Thorgaard, 2004). Because of the relatively recent domestication of salmonids, clonal lines have been derived from a variety of populations with different histories of domestication, ranging from complete life cycle captive rearing to partial life cycle captive

rearing to fully wild populations. Additionally, androgenesis allows the use of doubled haploid populations (Young et al., 1998; Robison et al., 2001) for QTL mapping, which provide a more powerful experimental design than traditional backcrosses (Carbonell et al., 1993) and allow the use of powerful analytical techniques such as composite interval mapping (Zeng, 1994). In this dissertation, I apply these techniques in two studies to examine genetic variation in responsiveness to stress and growth rate, two traits of considerable economic importance.

Stress is a serious issue in aquaculture, particularly in regard to fish health, production, and propagation. In intensive culture, fish are subject to many stressors that are often chronic and may include poor water quality, crowding, handling and transport, competition for food, and social stress (Wedemeyer, 1997). These chronic stressors impair growth, health, disease resistance, and reproduction in many cultured fishes. Measurement of cortisol levels in response to stress is generally a reliable indicator of sensitivity to stress (Barton, 2002). Variation in cortisol levels has a heritable component (Fevolden et al., 1992; Fevolden et al., 1999; Pottinger and Carrick, 1999) and domesticated populations of many species show reduced levels of glucocorticoids in response to stressors (Treiman and Levine, 1969; Martin, 1978; Woodward and Strange, 1987; Salonius and Iwama, 1993; Künzl and Sachser, 1999). Taken as a whole, these points suggest that reduced sensitivity to stress is an adaptation to captivity (Fevolden et al., 1999). I examine this by first characterizing the stress response of three clonal lines with different histories of domestication. Clonal lines derived from populations with long histories of captive breeding are expected to have reduced cortisol levels relative to lines derived from populations more recently taken into captivity. Then, the underlying genetics of stress responsiveness are examined through QTL analysis of a doubled haploid population descended from a cross of two lines with divergent stress responses. Finally, QTL influencing sensitivity to

stress are compared to QTL for body mass detected in the same population and in other studies, to test the hypothesis that there is a genetic link between sensitivity to stress and growth rate.

In the second study, growth rate is examined in greater detail with additional QTL analyses. Growth rate is the primary production trait of salmonid culture, is the focus of considerable research (Gjerde, 1986; Gjedrem, 1992; Gjerde, 1993; Gjedrem, 2000), and QTL analyses have been performed on embryonic growth (Robison et al., 2001) and growth in adult fish (O'Malley et al., 2002; Martyniuk et al., 2003). In this study, growth was measured in two replicate groups of a doubled haploid population descended from a cross of two clonal lines with divergent growth rates. Over the course of approximately three months, body mass and length were measured for a total of three time points. This study focuses on the first and last time points. QTL are estimated for body mass, length, condition factor and specific growth rate, and comparisons are made with previous studies of embryonic and adult growth.

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ATTRIBUTION

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CHAPTER TWO

DETECTION OF QTL INFLUENCING CORTISOL LEVELS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Abstract

Captive-rearing of fish populations introduces many chronic stressors that can impact growth and reproduction. Variation in stress responsiveness has a genetic component and reduction of the stress response in domesticated populations may reflect an adaptation to captivity. Stress physiology is also linked with variations in fish growth, an economically and ecologically important trait. In this study, we examine the genetic factors underlying variation in stress physiology in three rainbow trout clonal lines derived from populations of differing levels of domestication. Comparison of cortisol levels among three clonal lines revealed that one heavily domesticated line (AR) had a reduced stress response compared to a second heavily domesticated line (OSU) and a less domesticated line (SW). A quantitative trait loci analysis (QTL) was also performed using doubled haploid offspring produced from clonal lines with divergent stress cortisol levels (OSU and AR). Two significant QTL with opposing additive effects on stress cortisol levels were detected on linkage groups OA-XXIII and OA-XXVII. Finally, a single QTL explaining 34% of variation in body mass mapped to linkage group OA-XXXI. Our findings suggest that reduction in stress physiology was the result of complex genetic control and that there was no genetic link between stress cortisol levels and growth in this cross.

1. Introduction

Management of stress is an important component of successful fish culture. Fish routinely are subjected to brief, acute stressors and can adjust their physiology and behavior to cope with these momentary disturbances. Repeated and chronic stressors, however, may be inescapable and can adversely affect fish health, growth, survival, and reproduction (Wedemeyer, 1997). The captive environments of hatcheries and aquaculture facilities can present many chronic stressors to captive fish, including poor water quality, overcrowding, and frequent handling by and interaction with humans (Mommsen et al., 1999). The adverse effects of chronic stressors may represent selection forces that are inherent to the captive environment, favoring individuals with reduced sensitivity to stressors (Fevolden et al., 2002). Identification of genetic factors influencing responsiveness to stress would therefore be beneficial for developing new domesticated stocks for aquaculture production (Pankhurst and van der Kraak, 1997). Conversely, such knowledge would be useful for monitoring quantitative trait variation in captive brood stocks reared for conservation purposes (Arnold, 1995).

One of the primary physiological systems through which fish respond to stressors is the hypothalamic-pituitary-interrenal axis, or HPI axis (reviewed by Wendelaar Bonga, 1997), homologous to the hypothalamic-pituitary-adrenal axis in birds and mammals. When presented with stressful stimuli, the hypothalamus releases corticotropin-releasing factor (CRF) into the hypothalamic-hypophysial portal system. CRF stimulates the release of adrenocorticotropic hormone (ACTH), which, in turn, stimulates the release of cortisol from the interrenal cells of the fish head kidney. Cortisol is transported throughout the body and has numerous effects, including mobilization of glucose through glycogenolysis and gluconeogenesis. Because of its relative reliability, cortisol is the most commonly used indicator of stress in fish (Barton, 2002).

Elevated blood cortisol levels have been associated with reductions in growth (Pankhurst and van der Kraak, 1997), gamete and offspring quality (Schreck et al., 2001), and resistance to disease (Balm, 1997). Cortisol levels are also negatively correlated with the ability to compete with conspecifics (Sloman et al., 2000; 2001). Variation in stress-induced cortisol levels has a heritable component (Fevolden et al., 1992; 1999; Pottinger and Carrick, 1999) and can be altered through artificial selection (Fevolden et al., 1991; Pottinger and Carrick, 1999; Fevolden et al., 2002). Additionally, reduced stress responses are associated with domestication in many species, including salmonids (Woodward and Strange, 1987; Salonius and Iwama, 1993), rats (Treiman and Levine, 1969), guinea pigs (Künzl and Sachser, 1999), and mallard ducks (Martin, 1978), suggesting that reduction of stress responsiveness may be an adaptation to captivity.

Of particular interest to aquaculture are the negative effects of stress responsiveness on growth. Chronic stress has long been associated with inhibition of growth, primarily through increased energetic demand (Pankhurst and van der Kraak, 1997). Evidence suggests that cortisol, as an indicator of sensitivity to stress, is associated with decreased growth in rainbow trout. Treatment with exogenous cortisol decreases appetite, growth rate, condition factor, and food conversion efficiency in rainbow trout (Gregory and Wood, 1999; De Boeck et al., 2001). Also, Weil et al. (2001) found that the duration of elevated cortisol levels in response to stress, not the amplitude of the response, was negatively associated with growth rate. Finally, a rainbow trout line selected for reduced stress cortisol levels had higher growth rates than a high responding line, although this was not seen at all ages (Fevolden et al., 2002). These findings suggest that loci affecting sensitivity to stress may also be associated with differences in growth.

Rainbow trout clonal lines are a unique tool for examining the genetic architecture of the stress response. A rainbow trout clonal line is a population of genetically-identical, completely

homozygous individuals created through two generations of androgenesis and/or gynogenesis (Parsons and Thorgaard, 1984; Young et al., 1996). Through these techniques, genetic variation for traits can be captured in completely inbred populations and made available for study. Numerous rainbow trout clonal lines have been developed at Washington State University (WSU) from populations from throughout the natural species range, with varying levels of domestication. These clonal lines offer a wide range of phenotypes that can be investigated.

In this study, we first characterized the cortisol stress response in three clonal lines to test the hypothesis that clonal lines derived from source populations with long histories of domestication have reduced sensitivity to handling stress. Second, we mapped quantitative trait loci (QTL) influencing stress cortisol levels in doubled haploid offspring of an F_1 hybrid of two clonal lines with divergent stress cortisol levels. Finally, we mapped QTL influencing body mass and compared these with QTL influencing stress cortisol levels to test the hypothesis that there is a genetic link between sensitivity to stress and growth rate.

2. Materials and methods

2.1. Parental lines

The Arlee (AR) clonal line was derived from a population of rainbow trout reared at the Arlee Hatchery (Montana) for stocking lakes for sporting purposes. Evidence suggests that the Arlee Hatchery population originated from a mixture of *O. mykiss* stocks, including steelhead trout, from the McCloud River (California) from which many domesticated rainbow trout populations are believed to be derived (Needham and Behnke, 1962). The OSU clonal line was established from a research population reared at Oregon State University. This population is also believed to have originated from wild populations in the McCloud River. Both source

populations for the AR and OSU clonal lines have been reared in captivity for more than 100 years. A third clonal line, Swanson (SW), was generated from a population from the Swanson River (Alaska) that had been reared in captivity for approximately three generations at the time this clonal line was created. Behavioral analyses suggest that the SW clonal line is more timid and less aggressive than the AR clonal line (Lucas et al., 2004). The AR and SW are homozygous male (YY) lines while the OSU is homozygous female (XX).

In February 2003, eggs from a single outbred (i.e. not homozygous) hatchery female (Trout Lodge, Inc., Sumner, WA) were divided into three groups, each fertilized by a male from each of the YY male clonal lines and a sex-reversed (XX) male from the OSU line. Fertilized eggs were reared in a Heath-style incubator in a 10°C cold room until the age of emergence (67 days postfertilization), when the fish had completely absorbed the yolk sac and were ready to begin exogenous feeding. During development, the fish were disturbed as minimally as possible, only to verify health and remove inviable eggs. At emergence, each family was transferred to a separate 19 L tank (30-cm diameter) in the same flow-through, recirculating system (approximately 10°C), with approximately 100 fish per tank. Fish were given commercial starter feed and reared indoors under an approximately natural photoperiod. The outbred progeny of these families were used to assess the stress phenotypes of the parental clonal lines. Use of outbred x clonal line progeny has been used to successfully characterize variation among rainbow trout clonal lines for development rate (Robison et al., 1999) and behavior (Lucas et al., 2004).

2.2 Stress treatments

Stress trials were conducted in a fish behavior facility at 10°C. Fifteen plastic test aquaria (26.5-cm L x 16.0-cm W x 17-cm H) were distributed one per cubicle in a 3 (row) x 6 (column) shelving unit. To minimize disturbance, each cubicle was individually aerated, lit with an incandescent light bulb and secluded by black curtains to the front and back of each aquarium. In addition, the back and lateral sides of each test aquarium were painted solid white to further minimize disturbance. Each aquarium was covered with a mesh lid.

Between 1800 and 1900 hours on the day before stress treatments, 5 individuals from each family were collected from the rearing tanks and transported to the behavior facility. One fish was randomly assigned to each test aquarium. One individual from each family was randomly assigned to each poststress recovery period: Control (no stress treatment), 30 min, 1 h, 3 h, and 12 h following stress. Fish were allowed to acclimatize to the test aquaria overnight. Between 0900 and 1100 hours on the following morning, each individual assigned to stress treatments was captured in a small net, suspended above the water for 30 s, and then returned to the water. At the assigned collection time, 25 ml of tricaine methanosulfate (MS222—44mg/ml) was injected through tubing into the sampled aquarium resulting in a final concentration of approximately 200 mg/L MS222, as recommended by the manufacturer for rapid anesthesia (1-2 min). Delivery of anesthesia by this method has less effect on cortisol levels than other methods (Gerwick et al., 1999). Following MS222 injection, the tubing was immediately flushed by injection of 30 ml of water. After reaching an anesthetized state, the sampled individual was quickly removed from the test aquarium, measured for total body length, placed in a 1.5-ml centrifuge tube, and frozen in liquid nitrogen. The test aquarium was then thoroughly rinsed with hot tap water, soaked in hot tap water for 2 h, rinsed again, drained, and then refilled with dechlorinated water for the next day's trials. Stress trials were performed over three consecutive

days in June 2003 for a total of 3 individuals from each family at each recovery period. Frozen samples were stored at -20°C until cortisol extractions were performed.

2.3. Cortisol extraction and assay

Cortisol was extracted following methods adapted from Hiroi et al. (1997). Wet mass for each individual was measured to the nearest mg. Each fish was then homogenized on ice in 5 volumes (w/v) phosphate-buffered saline (PBS) with a Tissue-Tearor homogenizer (Biospec Products, Bartlesville, OK). A 300 μl sample of homogenate was then extracted twice with 3 ml diethyl ether and evaporated to dryness in a 37°C water bath. Defatting with tetrachloromethane was necessary to remove assay interference detected during assay validation. Extracts were resuspended in 300 μl of tetrachloromethane, vortexing for 4 min. Then, 300 μl of assay buffer was added and samples were vortexed for 2 min. Samples were then centrifuged at 3000 rpm at 4°C and the assay buffer was transferred to a clean tube. Extracts were then diluted 1:10 and assayed with the Cortisol Enzyme Immunoassay kit (Cayman Chemical Co., Ann Arbor, MI) following the manufacturer's protocol. Extraction efficiency (90.1%) was estimated during assay validation by including two duplicate extractions with 100 pg/ml cortisol added. Interassay variation (10.0%) was estimated by replicating four samples in every assay.

2.4. Statistical analysis

Whole body cortisol levels were analyzed using Analysis of Variance (ANOVA) using PROC GLM in SAS version 9.1 (SAS Institute, Inc., Cary, NC). Clonal line, recovery period, and their interaction were included as factors in the model, while body mass, sampling time of day, and water temperature were included as covariates and column in the shelving unit and day

as cofactors. Examination of Studentized residuals from preliminary analyses suggested that error variances were not equal so all analyses reported here were performed on log-transformed cortisol levels.

2.5. Doubled haploid mapping population

In December 2004, an OSU x AR doubled haploid mapping population was created by androgenesis (Parsons and Thorgaard, 1984; Young et al., 1998). Briefly, eggs from an outbred female were treated with γ-radiation to destroy the maternal nuclear genetic component. Eggs were then fertilized with sperm from an OSU x AR hybrid male and a temperature shock was used to restore diploidy, resulting in a population of completely homozygous, recombinant individuals, or doubled haploids (DHs). DHs were reared as described above except that the entire DH family was reared in a single 19 L tank following the age of emergence.

2.6. Stress Treatments and Cortisol Assay

In April 2005, after 8 weeks on exogenous feed (119-122 days postfertilization), stress treatments were applied to the doubled haploids. Experimental protocols were identical to those described above for characterizing the clonal lines except that all individuals were sampled 30 min following stress treatment, as this recovery time resulted in the greatest difference in cortisol levels between OSU and AR. Stress treatments and sampling occurred between 0900 and 1200 h each day over four consecutive days. Cortisol extraction and assay procedures were performed as described above. In total, 52 DHs were used in this experiment.

2.7. Genotyping

Before homogenization for cortisol extraction, a portion of the caudal fin was removed and stored in 95% ethanol for genetic analysis. This sample was small and did not include fleshy portions of the caudal peduncle and so was unlikely to impact determination of whole body cortisol levels. Genomic DNA was extracted using the Puregene® Genomic DNA Purification Kit (Gentra Systems, Minneapolis, MN). Individuals were then genotyped for Amplified Fragment Length Polymorphisms (AFLPs) (Vos et al., 1995) following methods adapted by Robison et al. (2001). Briefly, 500 ng of genomic DNA was digested with EcoRI and MseI and adapter sequences were ligated to the sticky ends of restriction fragments. First selective PCR amplifications (+1 reactions) were performed using primers that recognize the adapter sequence, the restriction site, and one specified nucleotide. Second selective PCR amplifications (+3 reactions) were performed using the +1 reaction products as template for primers that selectively amplified fragments with three specified nucleotides beyond the restriction site. Primers recognizing the EcoRI adapter and restriction sites were labeled with Cy-5. The +3 reactions were separated using denaturing polyacrylamide gel electrophoresis and visualized using a Typhoon 9400 variable mode imager (Amersham Biosciences, Piscataway, NJ). AFLP markers shared with our dense reference linkage map (Young et al., 1998; Nichols et al., 2003) were identified by comparing gel images between the studies.

2.8. Linkage analysis

Initial linkage groups were constructed and ordered using Mapmaker for Mac version 2.0 (Dr. Scott Tingey, Dupont Experimental Station, Wilmington, DE, U.S.A.). Only markers genotyped in 70% or more of the DHs were included in the linkage analysis. Linkage groups were built using the ‘group’ command using the Kosambi mapping function, a minimum LOD of

3.0, and a maximum θ of 0.35. Each linkage group was then ordered using the 'compare' command on a maximum of eight markers. Once the best possible order was determined, positions of additional markers in a linkage group were determined using the 'try' command. Genotyping errors were identified by comparing with linkage patterns from the reference map which was generated from independent families of the same OSU x AR cross (Nichols et al., 2003) and by using the 'errorlod' function of R/qtl (Broman et al., 2003). In order to utilize R/qtl, the DH cross was coded as a backcross but the results were still applicable. Genotypes of markers identified by these means were checked and corrected or removed from the analysis if the marker was low quality. Marker orders were then checked using the 'ripple' function of R/qtl with a window size of 6 markers and using the likelihood method. Error detection and order testing with 'ripple' was repeated until the best orders were established. Final distances between markers were then estimated using Mapmaker for Mac v. 2.0.

2.9. QTL analysis

The effect and position in the genome of QTL affecting whole body cortisol levels were estimated by composite interval mapping (Zeng, 1994) using Model 6 in the program Windows QTL Cartographer Version 2.5 (Basten et al., 1994; Wang et al., 2005). The genetic map was scanned for QTL at 2.0 cM intervals. Background control markers were added to the model through stepwise linear regression with forward addition and backward elimination. The Type I error rate for marker inclusion/elimination was set at 0.1. The five most significant background control markers within a 10.0 cM window were retained in the model. Because analysis of cortisol levels in the clonal lines suggested the necessity of data transformation to reduce inequality of error variances, QTL analyses were performed on raw cortisol levels and after log-

transformation. Significance thresholds were determined using permutations with 1000 replications (Churchill and Doerge, 1994; Doerge and Churchill, 1996). Linkage groups and 1-LOD support intervals were drawn using MapChart v. 2.1 (Voorrips, 2002).

2.10 Relationship between stress responsiveness and body mass

A QTL analysis of body mass was performed as described above and results were compared to the analysis of cortisol levels. Identification of QTL affecting cortisol levels and body mass in the same location would suggest the presence of a locus affecting both traits. Also Pearson correlation coefficients were calculated between cortisol levels (raw and log-transformed) and body mass using PROC CORR in SAS System version 9.1 (SAS Institute, Cary, NC).

3. Results

3.1 Cortisol stress response in clonal lines

Mean whole body cortisol levels are summarized for each clonal line in Figure 1. The stress treatment resulted in a two-fold increase in whole body cortisol levels for the progeny of OSU and SW clonal lines while the progeny of AR clonal line showed only a modest increase. Cortisol levels returned to control levels (or lower) 12 h after the stress treatment.

ANOVA revealed significant differences in log-transformed cortisol levels among clonal lines and recovery periods; the interaction was not significant (Table 1). Body mass was a significant covariate, inversely proportional to log-transformed cortisol levels. Time of day, water temperature, column, and day did not significantly influence cortisol levels. Analyses including clonal line and body mass in the model were repeated within each recovery period to

determine when differences among clonal lines were occurring. When significant differences among clonal lines were detected, Fisher's LSD was performed using the LSMEANS statement of PROC GLM. When analyzed within each recovery period, log-transformed cortisol levels for the AR progeny were significantly less than for the OSU and SW progeny after a 30-min recovery period and significantly less than for the SW progeny after 60 min of recovery (Figure 1). Cortisol levels did not differ among the clonal lines in the unstressed controls and after 180 min and 720 min of recovery post-stress.

3.2 QTL analysis of stress cortisol levels

The DHs had a mean whole body cortisol level of 18.9 ng/g fish (SD 11.9), intermediate between the parental lines sampled at 30 min (Figure 2). The range of cortisol levels was wide (3.2–56.5 ng/g fish) and encompassed the parental means. Body mass ranged from 0.3656 to 1.7569 g with a mean of 0.8711 g (SD 0.2447).

A total of 504 polymorphic markers were generated from 57 AFLP primer combinations. Thirty-one linkage groups were formed from 432 markers; 68 markers remained unassigned. By matching markers between studies, we identified all 31 linkage groups from the reference map (Young et al., 1998; Nichols et al., 2003). Only those linkage groups with significant QTL are depicted in Figure 3. Two linkage groups (OA-V and OA-XXV) were each formed of two fragment groups which could not be reliably combined and ordered as a single linkage group. One linkage group (OA-XXVIII) from the reference map was identified with only one AFLP marker (Etccctt470a); however, this linkage group is not believed to represent an entire chromosome on its own (Nichols et al., 2003). The total map length was 1187.7 cM with an average interval distance of 10.0 cM (1187.7 cM /119 nonzero map intervals).

Suggestive QTL ($p < 0.1$) influencing whole body cortisol levels were detected on OA-XX, OA-XXIII, and OA-XXX but none of these QTL reached significance thresholds. Significant QTL influencing log-transformed cortisol levels (*logcort*-1 and *logcort*-2) were detected on OA-XXIII and OA-XXVII and had negative and positive effects, respectively (Table 2 and Figure 3). These QTL combined to explain 43.0% of the total variance in log-transformed cortisol levels.

3.3 QTL analysis of body mass

A single significant QTL influencing body mass (*bodymass-1*) was detected on OA-XXXI and explained 34.2% of the total trait variance. This QTL mapped to a different linkage group than the QTL detected in the analysis of cortisol levels. There was no significant correlation between body mass and cortisol levels ($r = -0.04$, $p = 0.78$) or log-transformed cortisol levels ($r = 0.09$, $p = 0.53$) in the DHs.

4. Discussion

4.1 Characterization of stress response in clonal lines

Stress responsiveness of the clonal lines did not show a consistent association with the level of domestication of the source population. The heavily domesticated AR clonal line clearly showed a significantly reduced stress response compared to the SW clonal line, which has a much shorter history of domestication. These differences in stress physiology seem consistent with observations that the AR clonal line was bolder and more aggressive than the SW clonal line (Lucas et al., 2004). However, the OSU clonal line, also derived from a population with a long history of domestication, did not have a reduced stress response. This illustrates that

captive rearing, even for many generations, need not result in the same evolutionary outcome. The stress responsiveness of each captive population will depend upon genetic variation for the trait in the source population, genetic variation for the trait in the founding population (founder effect), subsequent effects of genetic drift in future captive generations, the rate of introduction of new alleles via mutation or gene flow, and the strength of selection for reduced stress responsiveness in the captive environment. Genetic drift through small population size and founder effect can quickly fix a population despite any selection forces acting to the contrary. That reduced responsiveness is adaptive to captivity has not been conclusively proven, but is supported by evidence that routine procedures, such as handling, result in elevated cortisol levels (Mazur and Iwama, 1993; Sharpe et al., 1998; Barton, 2000; Dunlop et al., 2004), clear negative effects of chronic stressors on fitness traits such as growth, health and reproduction (Pankhurst and van der Kraak, 1997; Wendelaar Bonga, 1997; Schreck et al., 2001), and a tendency for domesticated populations to have reduced responsiveness to stress (Treiman and Levine, 1969; Martin, 1978; Woodward and Strange, 1987; Salonius and Iwama, 1993; Künzl and Sachser, 1999). An alternative prediction is also possible: natural selection in captivity will favor individuals with normal functioning stress responses needed to successfully cope with chronic stressors is also possible (Fevolden et al., 2002). Evaluation of stress responsiveness as an adaptive trait will require monitoring of experimental populations across generations to test for the presence of selection and quantify its strength (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

It is important to remember that a clonal line is a population of completely homozygous, genetically identical individuals and that each clonal lines represents a single haploid genome captured from a single individual taken from a source population. Comparisons of the clonal line

to its source population must be made before reliable inferences can be made about the effects of domestication. Investigations of another physiological trait, development rate, in clonal lines (Robison et al., 1999) and outbred populations (Robison and Thorgaard, 2004) show that a clonal line can reflect the phenotype of its source population. However, true inferences about the nature of stress responsiveness in the source populations of these clonal lines are beyond the scope of the present study.

4.2 QTL for stress cortisol levels

This study is one of the few that have identified QTL influencing stress hormone levels and, to our knowledge, the first to do so in a fish species. Previous studies have analyzed QTL for endocrine stress responses in pigs (Désautés et al., 2002; Ousova et al., 2004) and rats (Potenza et al., 2004). In our study, three suggestive QTL were detected for whole body stress cortisol levels and two significant QTL were detected for log-transformed stress cortisol levels. It is curious that, although OSU showed higher cortisol levels in response to handling stress in the comparison of parental clonal lines, an OSU allele at *logcort-1* (on OA-XXIII) was associated with a reduced stress response (negative additive effect) while at *logcort-2* (on OA-XXVII) an OSU allele was associated with an increase (positive additive effect). Stated another way, the AR allele at *logcort-1* is associated with increased cortisol levels despite findings that this line showed little or no increase in cortisol levels in response to a handling stressor. One hypothesis is that the AR clonal line has reduced activation of the HPI axis in response to handling stressors and also has a reduced rate of cortisol clearance. Considering that cortisol has additional functions in fish, such as maintaining osmotic balance (Wendelaar Bonga, 1997), it is plausible that a concurrent decrease in cortisol clearance could mitigate possible detrimental

effects of reduced cortisol production on other life functions. It is also possible that epistatic interactions between *logcort-1*and *logcort-2*, or another as yet undetected locus, may interfere with action of this locus in the AR clone line.

Another possibility is that the stress response measured in the DHs is more complex than that measured for the characterization of the parental clonal lines. Despite repeated efforts, we have been unable to replicate experiments further characterizing the stress response of OSU, AR, and additional clonal lines (unpublished results). Elevated cortisol levels in control groups, including AR families, suggest that an additional, unidentified stressor has been introduced to the experimental system. One possibility is that the test aquaria became contaminated from previous experiments and this contaminant is elevating cortisol levels. Indeed, the presence of chemical cues produced by stressed rainbow trout results in elevated cortisol levels in otherwise untreated individuals (Toa et al., 2004). The unfortunate consequence of this inability to as yet replicate the original experiment is that cortisol levels measured for the detection of QTL in the DHs may be a complex response to a combination of the handling treatment and an additional, unknown stressor, rather than a response to handling alone. If variations in the response to these stressors are the result of variation at different loci, a complex pattern of QTL would be detected. This could be an alternative explanation for the detection of QTL with both positive and negative additive effects in this study. We are currently working to identify and eliminate this unknown stressor and plan to replicate QTL analyses in additional crosses to further examine and verify the results of this study. Despite this important caveat, it is certain that we have identified QTL influencing cortisol levels in response to one or more stressors, and these new findings provide important evidence that will aid further genetic dissection of variation in stress responsiveness.

The ultimate goal of QTL analyses is the characterization of the sequence variation underlying genetic variation in a trait, but this is a difficult goal to attain (Mackay, 2001). A great deal of information on the genetic control of endocrine function has been collected from analyses of mutant, knockout, and transgenic research lines of mice (Steckler, 2001). However, loss of function and overexpression mutations may not be adequate models of subtle mutations that may underlie quantitative traits, such as small changes in gene expression (Yalcin et al., 2004; Oliver et al., 2005). Successful identification of the underlying genes has been rare but successes are becoming more common. Evidence suggests that allelic variation in cortisol-binding globulin (CBG) is associated with variation in cortisol levels between Large White and Meishan pigs (Ousova et al., 2004), and strong evidence supports regulatory differences in *Rgs2*, a gene encoding a G protein regulator, as the source of variation in behavioral anxiety in mice (Yalcin et al., 2004). Thus, the goal of identifying genes is attainable but often requires intensive genetic information and research tools, such as dense comparative maps and whole genome sequences, that are currently unavailable in salmonids (Thorgaard et al., 2002). An alternate but less powerful approach to identifying genes is to test for associations between QTL regions and genes that are known to be involved in the control of the trait. Unfortunately, few genes have been mapped in rainbow trout, though efforts to remedy this situation are in progress. As yet, the only gene related to the stress response that has been mapped is 20β-hydroxysteroid dehydrogenase, also known as carbonyl reductase (CBR1), which is one of the enzymes involved in cortisol clearance in rainbow trout (Truscott, 1979). This gene mapped to OA-I (Nichols et al., 2003), to which no QTL for stress cortisol were associated.

4.3 QTL for body mass and correlates to stress

A single major QTL affecting juvenile body mass was detected on OA-XXXI. No QTL were associated with linkage groups to which growth hormone genes GH1 (OA-IX) and GH2 (OA-II) were previously mapped (Nichols et al., 2003), suggesting that the growth hormone genes are not directly responsible for differences in growth observed between the parental clonal lines (P. Wheeler, unpublished data). O'Malley et al. (2002) and Martyniuk et al. (2003) detected QTL affecting body mass in outbred rainbow trout populations of adult rainbow trout. The QTL was associated with a linkage group (5) that has synteny to OA-XXXI (Nichols et al., 2003), where a body mass QTL was detected in the present study. The relative locations of these QTL on the linkage group are not known due to incomplete alignment of the maps, but it is possible that the same locus is affecting body mass in these studies.

Previous evidence suggests that variation in stress responsiveness may influence growth rate. A rainbow trout line selected for high stress responsiveness showed reduced growth compared to line selected for low responsiveness, although this was not significant throughout the life of the fish (Fevolden et al., 2002). Research in a domestic chicken x red junglefowl F_2 population suggests that QTL associated with fear-like behavior also influence production traits, such as body weight (Schütz et al., 2004). In contrast, there is no evidence for a genetic link between responsiveness to stress and growth in our study. The lack of QTL influencing both stress cortisol levels and body mass suggests that different genes are independently influencing each trait in this cross. It is possible that the relationship between growth and stress responsiveness may be age-specific. QTL for body mass in juveniles (< 1 year old) (Drew *et al. in prep*) and 2-year old females (O'Malley et al., 2002) have been detected on linkage groups syntenic to OA-XXVII, the linkage group containing *logcort-2*. Interestingly, this linkage group is homeologous with OA-XXXI (Nichols et al., 2003), which was associated with body mass in

our study. Also, because both of the parent clonal lines in this study (OSU and AR) were derived from domesticated populations, there may be less relationship between stress responsiveness and growth. Analysis of a cross with a clonal line derived from a less domesticated or completely wild population may detect a stronger genetic relationship between these traits. Finally, it is possible that the power of our analysis was insufficient for detecting all QTL influencing these traits. Through both simulation (Beavis, 1998) and empirical research (Melchinger et al., 1998), it has been found that sample sizes of 100 or fewer individuals have low power for detecting QTL of low to moderate effect. Sample sizes of 500 or more are generally recommended (Beavis, 1998). Thus, our study should be considered a preliminary analysis of the relationship between QTL affecting stress responsiveness and growth.

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Table 1: ANOVA analysis of log-transformed whole body cortisol levels in juvenile rainbow trout in response to a handling stressor. (**P < 0.01, *P < 0.05)

Source of Variation	**DF**	**Type III SS**	**Mean Square**	**F Value**	**P**
Clonal Line	2	3.77977200	1.88988600	15.46	0.0012**
Recovery Time	3	2.54057931	0.84685977	6.93	0.0103*
Clonal Line x Recovery	6	1.57645674	0.26274279	2.15	0.1453
Temperature	1	0.45563692	0.45563692	3.73	0.0855
Anesthesia Time	1	0.07173574	0.07173574	0.59	0.4632
Catch Time	1	0.15165671	0.15165671	1.24	0.2941
Body Mass	1	1.05802781	1.05802781	8.66	0.0164*
Column	5	0.44132110	0.08826422	0.72	0.6234
Day	2	0.21958350	0.10979175	0.90	0.4408
Error	9	1.09984014	0.12220446		

Table 2: QTL influencing cortisol levels and body mass in OSU x AR doubled haploids.

QTL	Linkage Group	Position[a]	LOD[b]	a[c]	r^{2d}
logcort-1	OA-XXIII	45.6 (acacag01)	5.68	–0.368	0.270
logcort-2	OA-XXVII	0.01 (agactc06)	3.62	0.488	0.160
bodymass-1	OA-XXXI	41.4	6.23	–0.149	0.342

[a]Position is the distance of the QTL from the top of the linkage group. If the position coincided with a marker, the marker name is reported in parentheses. [b]LOD is Log of Odds that calculated additive effects are significantly different from zero. Significance thresholds were calculated using permutations with 1000 replications. All QTL were significant ($p < 0.01$). [c]Additive effect or effect of replacing an AR allele with an OSU allele at the QTL. [d]r^2 is the proportion of the phenotypic variance explained by the QTL using background markers as cofactors.

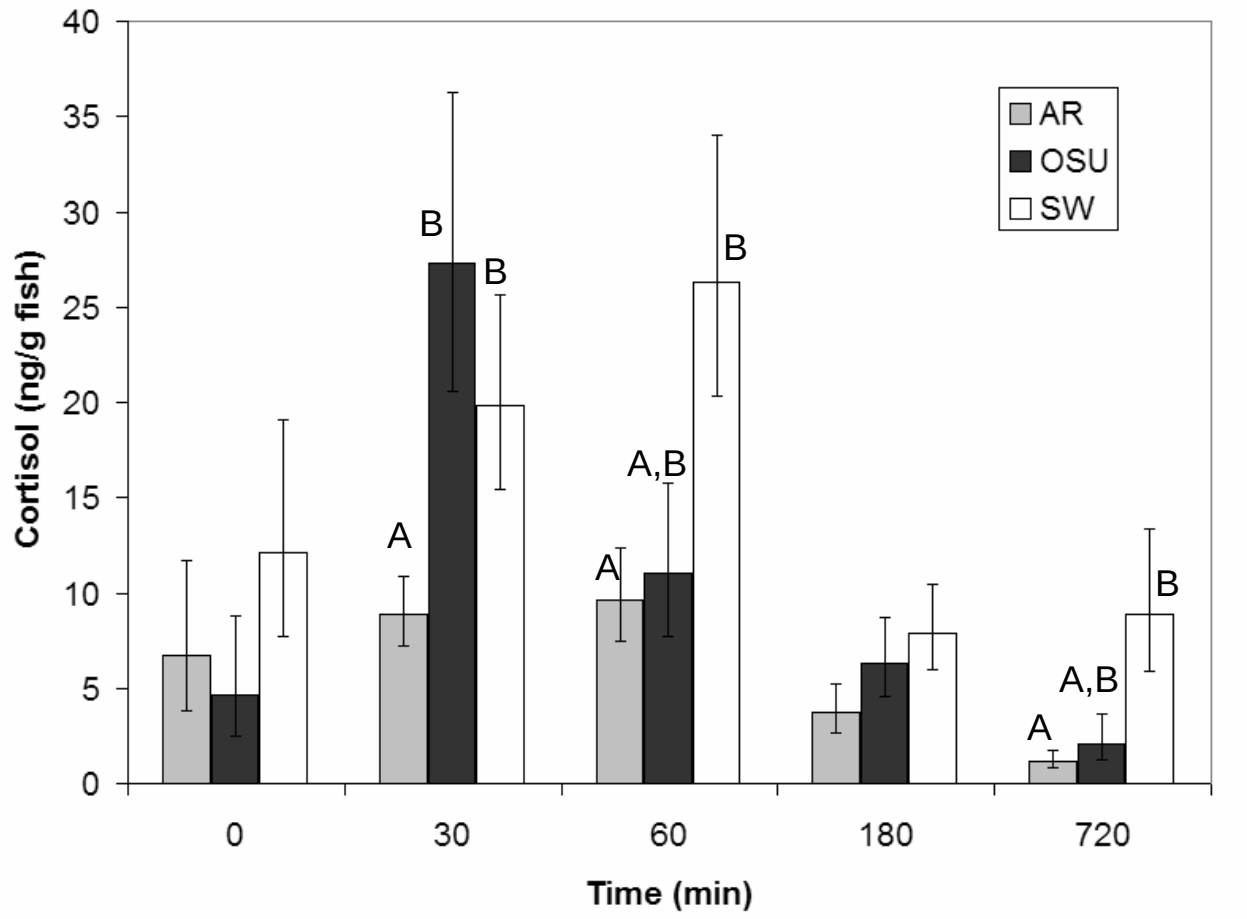
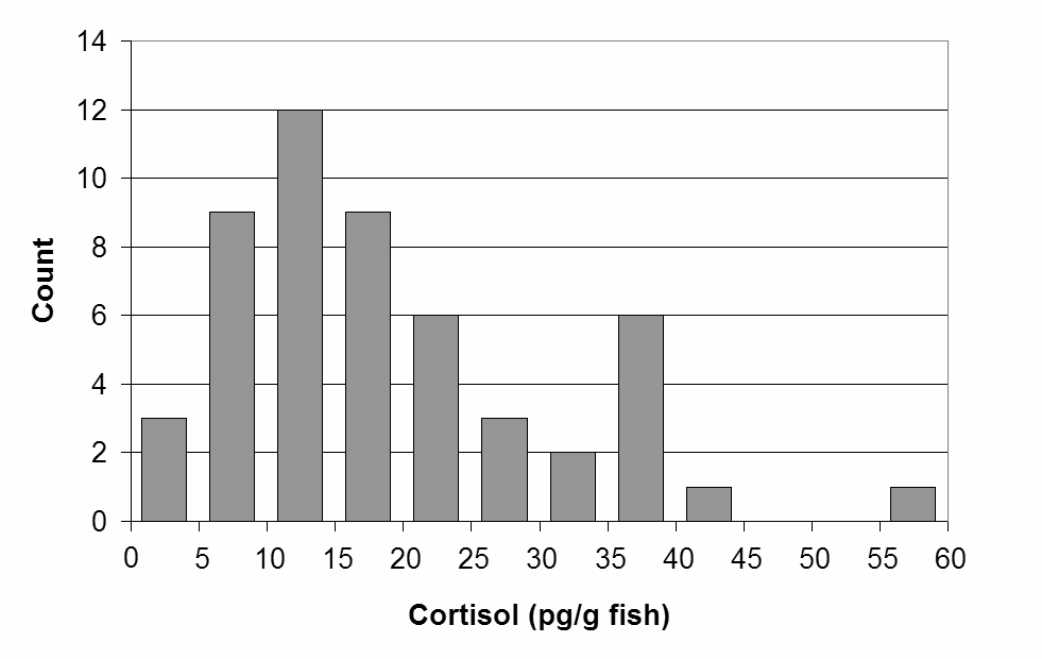


Figure 1: Adjusted mean whole body cortisol levels in rainbow trout, in response to a handling stressor. Error bars represent the standard error around the means. Columns with different letters were significantly different in pairwise comparisons using Fisher's Protected LSD ($P < 0.05$) after ANOVA within each recovery period.

Figure 2: Distributions of (a) whole body cortisol levels and (b) body mass in OSU x AR doubled haploids.

a.



b.

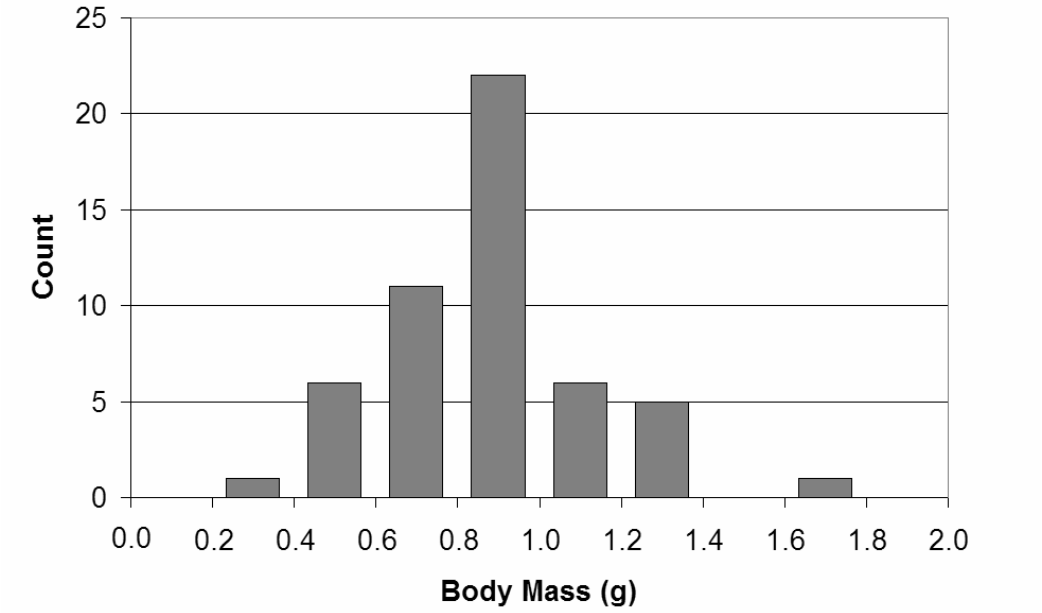
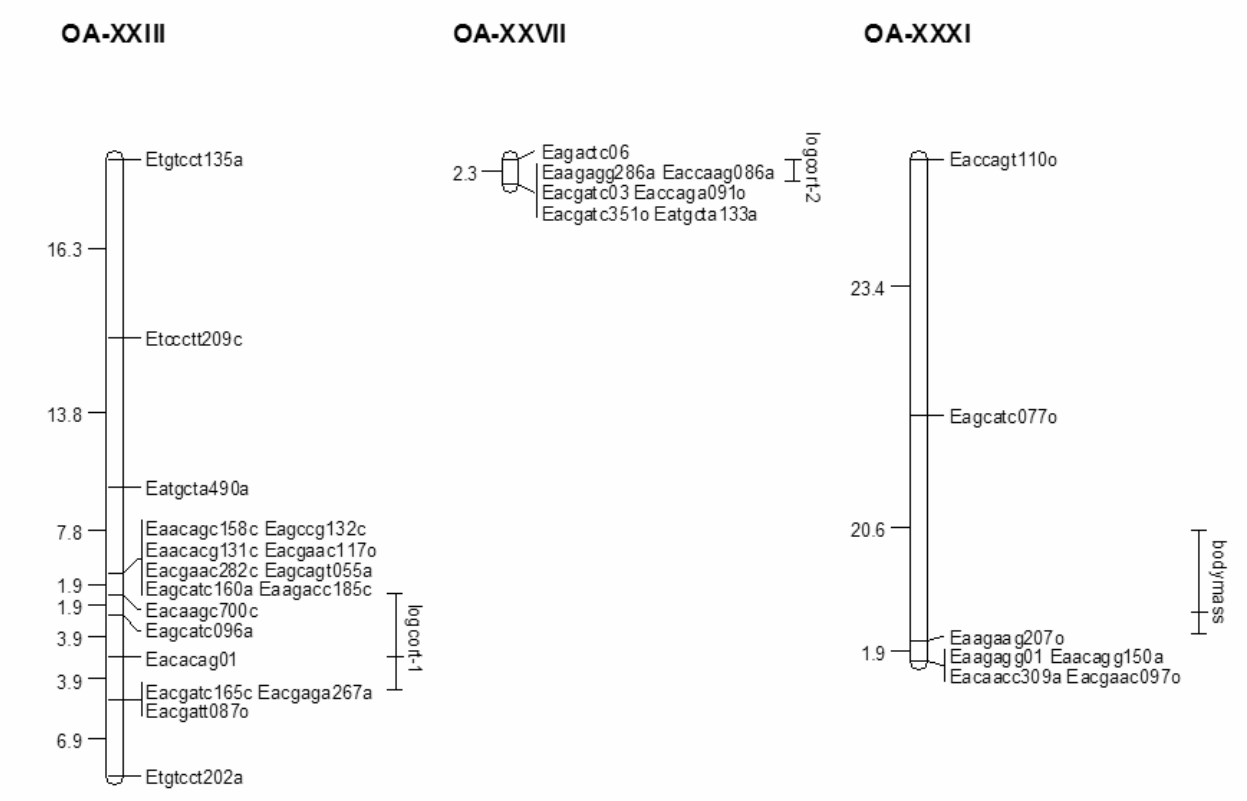


Figure 3: OSU x AR doubled haploids linkage groups. Only those linkage groups with significant stress response and body mass QTL are shown. Linkage groups are named according to the reference map (Nichols et al. 2003). Numbers to the left of each linkage group are the interval distances between markers in cM. The position of QTL and 1-LOD intervals are represented to the right of each linkage group.



CHAPTER THREE

QTL ANALYSIS OF GROWTH DIFFERENCES ASSOCIATED WITH DOMESTICATION OF RAINBOW TROUT

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Abstract

Fish growth is an economically important trait under complex genetic and environmental control. In this study we estimate the number of genetic factors underlying juvenile growth, using quantitative trait loci (QTL) analysis, in a population of rainbow trout doubled haploids. At three sampling periods over the course of three months, fork length, body mass, and condition factor were monitored in two groups of juvenile doubled haploids produced from a cross of two clonal lines with divergent growth rates (OSU and SW). Data points from the first and last sampling times were matched using genetic markers in order to calculate specific growth rate for this interval. Pooled analyses and separate analyses of each group revealed 22 QTL mapping to at least 14 locations on 11 different linkage groups. QTL affecting different growth traits frequently mapped to similar locations suggesting pleiotropy or the presence of closely linked loci. Differences in QTL detected in separate analyses of each population may be the result of genotype x environment interactions but this could not be reliably evaluated. Comparisons with independent QTL studies of embryonic and adult growth in rainbow trout reveal several linkage groups that contain QTL affecting growth across studies; however, due to incomplete alignment of different genetic maps, the relative locations of these QTL could not be determined so it is not known if these are the same QTL across studies. Our analyses of growth suggest the presence of complex genetic and environmental interactions, in agreement with analyses of growth in other species.

1. Introduction

The genetics of growth has been the focus of many studies for the purpose of improving the productivity of domesticated lines (Gjedrem, 1992; Gjerde, 1993; Gjedrem, 2000). In salmonids, the genetics of growth has been well studied and is known to be under complex genetic and environmental influences (Sumpter, 1992). Heritability of body mass as an indicator of growth rate has been estimated in populations of rainbow trout (Klupp, 1979; McKay et al., 1986; Crandell and Gall, 1993b; 1993a; Su et al., 1996; Martyniuk et al., 2003), coho salmon (Hershberger et al., 1990; Myers, 1990), chinook salmon (Winkelman and Peterson, 1994), and Arctic charr (Nilsson, 1992). For rainbow trout, heritability estimates had a mean $h^2 = 0.23$ (range 0.17–0.38) for fish more than one year of age. Estimates are more variable for fish less than one year of age, with a mean $h^2 = 0.21$ (0.06–0.52) (Gjedrem, 1992). While the presence of additive genetic variance for growth has been identified, further improvement of stocks would benefit from more detailed examination of the genetic factors underlying this trait.

Analysis of quantitative trait loci (QTL) offers an opportunity to improve understanding of growth in salmonids. QTL are loci associated with variation in a quantitative trait (Lynch and Walsh, 1998). Through statistical analysis, the number, location, and effect of loci influencing a trait can be estimated from recombinant descendants of a cross between divergent lines (Lander and Botstein, 1989; Zeng, 1993; 1994). With refinements of statistical techniques for QTL detection (Lander and Botstein, 1989; Zeng, 1994) and rapid increase in modern computing power, there has been a recent explosion of QTL analyses of growth, particularly in important model species such as mice (Cheverud et al., 1996; Christians et al., 2004; Ishikawa and Namikawa, 2004; Rocha et al., 2004; Allan et al., 2005; Carlborg et al., 2005; Ishikawa et al., 2005; Oliver et al., 2005; Rance et al., 2005; Wu et al., 2005), and several economically

important species, including pigs (Andersson et al., 1994; Knott et al., 1998; Bidanel et al., 2001; Nonneman and Roher, 2002; Varona et al., 2002; Evans et al., 2003; Nagamine et al., 2003; Sato et al., 2003; Van Laere et al., 2003; Nagamine et al., 2004; Houston et al., 2005; Kim et al., 2005; Pérez-Enciso et al., 2005; Stearns et al., 2005b; 2005a), cattle (Kim et al., 2002; Li et al., 2002; Casas et al., 2003; Kim et al., 2003; Casas et al., 2004; Li et al., 2004; Mizoshita et al., 2004), sheep (Johnson et al., 2005), and chickens (Sewalem et al., 2002; Deeb and Lamont, 2003; Carlborg et al., 2004; de Koning et al., 2004; Sasaki et al., 2004; Siwek et al., 2004), but also more obscure species such as American bison (Schnabel et al., 2003). The eventual goals of QTL analyses of growth are to identify and isolate the nucleotide polymorphisms (QTN) within genes that are responsible for variation in the trait (Mackay, 2001).

QTL analyses of growth have been attempted in salmonids with some success, particularly in rainbow trout. Rainbow trout are the most well-studied fish species in many areas of research (Thorgaard et al., 2002), and genetic tools available for this species include isogenic (clonal) research lines (Young et al., 1996) and dense genetic linkage maps (Young et al., 1998; Sakamoto et al., 2000; Nichols et al., 2003b). Robison et al (2001) detected four QTL influencing embryonic length and mass on three linkage groups using two clonal lines divergent for embryonic development rate. A single QTL affecting juvenile body mass at 120 days postfertilization was detected on a different linkage group in a cross involving different clonal lines (Drew *et al., in prep*). Several analyses have also detected QTL for body size of rainbow trout in outbred populations (O'Malley et al., 2002; Martyniuk et al., 2003). In each of these studies, body size at one instant in time was used as an indicator of growth; growth rate itself was not studied directly.

Rainbow trout clonal lines provide a powerful tool for analyzing the genetic architecture of complex traits (Robison et al., 2001). Rainbow trout clonal lines are populations of genetically-identical, completely homozygous individuals, and are analogous to mouse inbred lines. Within just two generations of chromosome set manipulation through androgenesis or gynogenesis, genetic variation from an outbred population can be captured in a homozygous research population and made available for research (Young et al., 1996; Robison and Thorgaard, 2004). In this manner, variation among several clonal lines has been detected for traits such as development rate (Robison et al., 1999), behavior (Lucas et al., 2004), meristic characters (Nichols et al., 2004), and stress physiology (Drew, *et al. in prep*). Additionally, crosses of clonal lines can be used to generate doubled haploid (DH) mapping populations (Young et al., 1998). The doubled haploid design has greater statistical power for QTL detection than traditional backcross designs (Carbonell et al., 1993), and allows the use of powerful analytical techniques such as composite interval mapping (Zeng, 1994). Experiments using rainbow trout DHs have successfully detected QTL influencing development rate (Robison et al., 2001; Nichols, 2002), meristic characters (Nichols et al., 2004; Zimmerman et al., 2005), disease resistance (Nichols et al., 2003a), immune function (Zimmerman et al., 2004), and responsiveness to stress (Drew et al., *in prep*).

In this study, we apply the rainbow trout DH design to the analysis of juvenile growth. Two populations of DHs were generated from a cross of a slow-developing, rapid-growing female clonal line (OSU) and a rapid-developing, slow-growing male clonal line (SW). The slow-growing line was derived from a source that was only recently brought into captivity. Thus, this study may provide insights into changes associated with the domestication process. Body length, mass, and condition factor were monitored during the first summer of growth and used to

estimate specific growth rates. QTL analyses were performed on these traits and then compared with the findings of other QTL analyses of growth in rainbow trout (Robison et al., 2001; O'Malley et al., 2002; Martyniuk et al., 2003).

2. Materials and methods

2.1. Mapping population

The parental lines consisted of the OSU and Swanson (SW) clonal lines. The OSU clonal line is a female line derived from a research population at Oregon State University (OSU—Corvallis, OR, USA). The population at OSU has been reared in captivity for over 100 years and is believed to be derived from the McCloud River in California, from which most domesticated rainbow trout populations are thought to originate (Needham and Behnke, 1962). The SW clonal line is a male (YY) clonal line derived from a captive-reared population founded from the Swanson River, Alaska (Robison and Thorgaard, 2004). At the time that this clonal line was developed, the hatchery population had been in captivity for approximately three generations. The OSU clonal line has a slower development rate (Robison et al., 1999) but is faster growing than the SW clonal line (Wheeler, unpublished data).

In February 2003, doubled haploid (DH) offspring of an F_1 OSU x SW hybrid male were generated as previously described (Parsons and Thorgaard, 1984; Young et al., 1998) using eggs from six outbred females (Trout Lodge, WA). Hereafter, this population will be referred to as Group A. Two weeks later, another population of OSU x SW DHs (Group B) was generated using eggs from six additional females. Embryos were reared at Washington State University (WSU) in Heath-style trays at approximately 10° C until the age of emergence (total absorption of the yolk sac and onset of exogeneous feeding). Offspring from one group A family were

lightly anesthesized in 2-phenoxyethanol and a tissue sample was taken for the purpose of matching development rate with postembryonic growth. Unfortunately, despite careful work, the sampling resulted in heavy mortality in this family. This sampling was not repeated with any of the other families in groups A or B.

2.2. Growth study

At the age of emergence, one population of 100 individuals was formed within each group by pooling the approximately equal numbers of offspring of each of three viable DH families. The populations were then shipped overnight to the Hagerman Fish Culture Experiment Station (Hagerman, ID). Mortality occurred during transit resulting in initial population sizes of 65 and 96 for Groups A and B, respectively. Each group was reared separately in a 152-liter indoor tank at approximately 10°C under a seasonal photoperiod. Once each day, each tank was provided with commercial trout food by hand, one pellet at a time until feeding ceased.

Body length and mass were measured at three time points over the course of a four-month growth experiment. Each individual was anesthetized with tricaine methanosulfate (MS222) and measured for body mass (to the nearest 0.1 g) and fork length (to the nearest mm). At the same time, a 2-3 mm tissue sample was cut from the tip of the caudal fin and stored in a 1.5-ml centrifuge tube of 95% ethanol for later genetic analysis. Fish were allowed to recover from anesthesia and then returned to the rearing tank. Fish were not fed on the day they were measured.

Some mortality occurred over the course of the experiment. Prior to the start of the experiment, the initial source of mortality for group A was the attempt to collect tissue samples

at the age of emergence and then subsequent stress during transit from WSU to the Hagerman facility. During transit, group A was reduced from 100 to 65 individuals. Group B had a little mortality in transit, reducing the population from 100 to 96. Some mortality also occurred during the course of the experiment (Table 1).

2.3. AFLP genotyping

Genomic DNA was extracted using the Puregene® DNA purification kit (Gentra Systems, Minneapolis, MN) following the manufacturer's protocol. Samples were then genotyped at Amplified Fragment Length Polymorphisms (Vos et al., 1995), as adapted by (Robison et al., 2001). Briefly, approximately 500 ng of genomic DNA was digested with EcoRI and MseI, and adapter sequences specific to each restriction enzyme were ligated to sticky ends of the restriction fragments. First selective amplifications (+1 reactions) were then performed using primers recognizing the adapter sequence, the restriction site, and then one base into the genomic sequence. Second selective amplifications (+3 reactions) were then performed using +1 reaction products (diluted 1:20 in TE) as template for primers recognizing the adapter sequence, the restriction site, and then three bases into the genomic fragment. The +3 primer specific to the EcoRI adapter sequences was labeled with Cy-5. Next, +3 reactions were separated with denaturing polyacrylamide gel electrophoresis and visualized using a Typhoon 9400 variable mode imager (Amersham Biosciences, Piscataway, NJ). Markers are named by the three selective bases of the EcoRI +3 primer, the three selective bases of the MseI +3 primer, and a marker identification number or the estimated fragment size in base pairs.

Samples from the final time point (194-195 dpf) were genotyped at 27 AFLP primer combinations to produce markers for the linkage map. Thirteen of these primer combinations

were used to generate the linkage map for analysis of development rate using independent families from the same cross (Robison et al., 2001), while twenty-four were used to generate the reference rainbow trout linkage map in a cross between OSU and an independent clonal line (Nichols et al., 2003b).

Samples from the first time point (89 dpf) were genotyped at three different AFLP primer combinations for purposes of matching individual data from the first time point to data from the final time point. Genotypes were matched using a simple PERL program which calculated pairwise genotype similarity between each individual from the first time point and all individuals from the last. Comparisons were also performed in the opposite direction (final time point to first time point) to verify assignments. Disagreements between genotypes from the best matches were investigated for genotyping errors. Generally, matches between time points were considered reliable if both samples were genotyped at 16 or more AFLP markers and had >90% similarity without ambiguous secondary matches to other samples. Data from 89 dpf samples that could not be reliably matched to 194 dpf samples were removed from the experiment. The experiment focused on samples collected at 89 and 194-195 dpf because these were the most extreme time points in the experiment. Samples collected at 126-129 dpf were not genotyped.

2.4. Statistical analyses

Fork length (FL) and body mass (mass) were used to calculate condition factor (K) at each time point and specific growth rate (SGR) when samples were reliably matched between time points. Condition factor (K) was calculated as $100{,}000(\text{mass}/\text{FL}^3)$. SGR was calculated as $100[\ln(\text{mass194}) - \ln(\text{mass89})]/(t_3 - t_1)$, where mass194 and mass89 are body masses at 89 and 194 dpf, respectively, and $(t_3 - t_1)$ is the number of days between measurements. Traits were

identified by the age of the sampling (e.g. FL89 = fork length at 89 dpf). All statistical analyses were performed using SAS System version 9.1 (SAS Institute, Cary, NC). Because the trait variances between groups were often significantly different, differences of trait means between groups were investigated assuming unequal variance between populations using PROC TTEST. The PROC CORR procedure in SAS was used to estimate Pearson correlation coefficients among traits within and between time points 89 and 194 dpf. Correlation coefficients were also estimated after z-transformation of traits to eliminate possible effects of unequal variances.

2.5. Linkage analysis

Linkage groups and initial marker orders were determined using Mapmaker for Mac v. 2.0 (Dr. Scott Tingey, Dupont Experimental Station, Wilmington, DE, U.S.A.). Only markers that were genotyped in at least 70% of the DHs were used in the analysis. Linkage groups were formed using the 'group' command, with the Kosambi mapping function, minimum LOD of 4.0, and maximum θ of 0.40. Orders of no more than 8 markers were determined using the 'compare' command and the positions of remaining markers were determined using 'try.' Clustering markers that had identical genotyping patterns in all DHs were then identified, and the marker with the best quality and highest percentage of DHs genotyped was selected as a representative of the cluster. Initial orders were then evaluated using R/qtl (Broman et al., 2003), coding the cross as a backcross in order to use the software. 'Ripple' was used to test for better marker orders, using the 'likelihood' method with a window size of 6 markers. Then error detection was performed using 'errorlod.' Markers identified by error detection were evaluated for genotyping errors and corrected or removed if poor quality. 'Ripple' and error detection was repeated until the best orders were obtained and no further errors were identified. Final distances

between markers was then determined using Mapmaker for Mac. Synteny of this map with those of Robison et al. (2001) and Nichols et al.(2003b) were determined by matching bands between gel images from each study.

2.6. QTL analyses

Composite interval mapping (Zeng, 1994) was performed on all traits using Windows QTL Cartographer Version 2.5 (Basten et al., 1994; Wang et al., 2005). Markers were evaluated as cofactors in the Model 6 using forwards addition/backwards removal with probability of adding or removing a cofactor set at 0.1 and a window size of 10.0 cM. Logarithm of odds (LOD) scores for the main effects were calculated at 2.0 cM intervals and significance thresholds were determined by permutations with 1000 replications (Churchill and Doerge, 1994; Doerge and Churchill, 1996). Traits were analyzed in each group separately and in a combined analysis after z-transformation within each group. Linkage groups and 1-LOD support intervals were drawn using MapChart v. 2.1 (Voorrips, 2002). Significant QTL were named with the trait in question, a letter indicating the analysis (a = A only, b = B only, z = pooled analysis), and an identification number.

3. Results

3.1. Summary of growth traits

Mean trait values are reported in Table 1. Genotypes of 110 of 136 individuals (81%) collected at 194 dpf were reliably matched with samplings taken at 89 dpf, allowing specific growth rate to be calculated. Means of FL89, mass89, mass126, K126, and K194 differed significantly between groups A and B. Analyses indicated that group variances were unequal for

three measures at 89 dpf [FL89 ($p = 0.03$), mass89 ($p < 0.0001$), and K89 ($p < 0.0001$)] but for no measures at the other time points.

Growth traits were highly correlated in the DH groups with general agreement between analyses of raw and z-transformed data (Table 2). Fork length and body mass within each time point were the most highly correlated. Fork length and body mass were also significantly correlated between time points. Within each time point, condition factor had a significantly negative correlation with fork length. SGR was negatively correlated with body mass and condition factor at 89 dpf and positively correlated with fork length and body mass at 194-195 dpf.

Given the significant correlation coefficients between fork length and body mass between time points, simple linear regression using PROC GLM in SAS was performed to investigate the ability to predict body size at 194 dpf given body size at 89 dpf. FL89 was a significant predictor of FL194 ($p < 0.0001$) and the same relationship held for mass ($p < 0.0001$). However, the relationships had low to moderate R^2 (0.27 for FL and 0.15 for mass) suggesting that size at 89 dpf was an insufficient predictor of size at 194-195 dpf.

3.2. Linkage analysis

136 individuals were genotyped at 287 AFLP loci. Linkage analysis produced 29 linkage groups consisting of 274 markers. Linkage groups with significant QTL are shown in Figure 1. The total map distance was 1289.8 cM, with an average intermarker distance of 8.2 cM (1289.8 cM / 158 marker intervals).

Map synteny with the linkage map of Robison et al. (2001) was unambiguously determined for 21 linkage groups with 2 or more shared markers and 5 linkage groups with only

one shared marker each. Synteny for one linkage group was questionable and two others did not share markers with linkage groups of Robison et al (2001). Two linkage groups from Robison et al. (2001) were not identified. The linkage group designated R2 (Robison et al., 2001) was previously found to have synteny with OA-XIV and OA-XX from the reference rainbow trout linkage map (Young et al., 1998; Nichols et al., 2003b); in the present map, R2 was split into two linkage groups coinciding with the previously determined synteny.

Synteny with the reference map (Nichols et al., 2003b) was unambiguously determined for 10 linkage groups with 2 or more markers and 10 with only one shared marker. Six linkage groups showed disagreement among shared markers. Because co-migration of AFLPs from the paternal lines may not always accurately indicate homology, tentative synteny for four of these ambiguous linkage groups was assigned based upon alleles from the maternal (OSU) line which were shared between studies. Each linkage group for which synteny could be determined was named with "OS" to identify the OSU x SW cross and a number indicating the matching linkage group from the reference map (e.g. OS-01 is syntenic with OA-I). The remaining two ambiguous linkage groups and three others which lacked synteny markers were named using the nomenclature of Robison et al. (2001).

3.3. QTL analyses

At least one significant QTL was identified for each growth trait (Tables 3 and 4). In total 22 QTL were identified, mapping to at least 14 discrete locations in the genome (Figure 1). When analyses were performed separately within each group, 4 significant QTL were detected in group A and 14 in group B. No QTL within a single trait at a given time point overlapped between groups. Seven QTL were detected in combined analyses of z-transformed growth traits,

three of which were identified in separate analyses of group B. No QTL within a trait detected in the combined analyses were detected in the separate analyses of group A. QTL for each trait are described in further detail in the following sections. QTL affecting different traits were frequently associated with the same or similar locations in the linkage map.

3.3.1. Fork length

In total, 6 QTL with significant influence on fork length were identified. Analyses of FL89 in group A detected a single QTL with positive additive effects (replacement of a SW allele with an OSU allele resulted in an increase in FL89), accounting for 15.9% of the phenotypic variation. Two QTL were detected in group B, one with positive additive effects and one with negative, combining to explain 40.1% of the phenotypic variation. The combined analysis detected both QTL found in analysis of group B only, explaining 20.6% of the variation.

No QTL affecting FL194 were detected in the analysis of group A only. Analysis of FL194 revealed three significant QTL in group B, totaling to account for 41.3% of the phenotypic variation. One of these QTL (on OS-09) had positive additive effects while the other two had negative additive effects. A suggestive QTL in group B was also detected on OS-07. The combined analysis detected a single suggestive QTL coinciding with *fl194-b-1* on R7c. 1-LOD intervals for *fl89-b-2* and *fl194-b-3*, both QTL with negative additive effects, overlapped considerably on R17b (Figure 1), suggesting the presence of a single locus on this linkage group affecting fork length at both ages.

3.3.2. Body mass

A total of 11 QTL were detected with significant influence on body mass. At 89 dpf, analysis of group A detected 2 significant QTL influencing body mass, one with positive additive effects and one negative, accounting for 36.3% of the phenotypic variation. Three significant QTL affecting mass89 were detected in analysis of group B, two with positive additive effects and one negative, explaining 46.5% of the phenotypic variation. No QTL for mass89 were detected on the same linkage group in the separate analyses. The combined analysis detected a suggestive QTL on R17b, the same linkage group as a QTL detected in group B but not within its 1-LOD interval.

QTL analysis of mass194 revealed four significant QTL in group B, combining to explain 67.5% of the phenotypic variation. Two of these QTL had positive additive effects while the other two were negative. The combined analysis detected two significant QTL with opposite additive effects, accounting for 21.9% of the phenotypic variation. One of these QTL coincided with a QTL detected on R7c in the analysis of group B. The other mapped to the same linkage group (OS-20) as a suggestive QTL detected in the analysis of group A, but mapped to opposite ends of the linkage group and had opposing additive effects. The combined analysis also revealed suggestive QTL on OS-09, within the 1-LOD interval for *mass194-b-2*, and OS-16.

There was evidence suggesting that some QTL influenced body mass at both 89 and 194-195 dpf. On R7c, *mass89-a-2*, a QTL affecting body mass at 89 dpf in group A, localized to the same position as QTL influencing body mass at 194-195 dpf in separate analysis of group B and the combined analysis; all loci had negative additive effects on body mass. Also the 1-LOD intervals of *mass89-b-3* and *mass194-b-3* overlapped on R17b and both QTL had negative additive effects on body mass.

Body mass and fork length were highly correlated within each time point and overlap among QTL for these traits suggests that these correlations may be the result of pleiotropy. Overlapping QTL influencing both mass and FL were detected on three linkage groups: OS-09, R16, and R7c (Figure 1). QTL affecting mass and FL were also associated with linkage group R17b but, although there is considerable overlap of 1-LOD intervals, appear to localize to opposite ends of the linkage group. In each of these cases, corresponding QTL had the same directionality of additive effect on fork length and body mass.

3.3.3. Condition factor

Combined analysis of condition factor revealed three significant QTL influencing this trait. At 89 dpf, a single significant QTL with negative additive effects on K89 was detected on OS-10. Suggestive QTL were detected in the separate analyses, on OS-17 in group A and on OS-10 in group B at agcagt12, just outside the 1-LOD of *k89z-1*. At 194-195 dpf, two QTL were detected in the combined analysis, one with positive and one with negative additive effects, accounting for 18.3% of the phenotypic variation. No QTL influencing K194 were detected in the separate analysis of group A, and the analysis of group B had problematic results that require further troubleshooting.

Because condition factor is a function of body mass and length, it is not surprising that there would be some correspondence among QTL for these traits. In the combined analyses, QTL influencing both K194 and mass194 mapped in close proximity on OS-20 but their 1-LOD intervals did not overlap. Both QTL had positive additive effects on body mass and condition factor in agreement with correlation coefficient estimates between these traits. Finally, a QTL influencing K194 mapped to the same position on R7b as a QTL affecting FL89 detected in the

separate analysis of group A. The QTL had opposite effects on the traits, as would be expected considering the inverse correlation between body length and condition factor.

3.3.4. Specific growth rate

Two QTL were detected with significant effects on specific growth rate occurring between 98 and 194-195 dpf. Separate analysis of group A revealed a single QTL on OS-18 with positive additive effects on SGR and explaining 26.2% of the phenotypic variation. Another QTL was detected on OS-20 in the separate analysis of group B, also with positive additive effects and accounting for 25.6% of the phenotypic variation. This QTL mapped to the same linkage group as QTL influencing body mass and condition factor at 194 dpf. Finally, a suggestive QTL was detected on OS-01 in the combined analysis.

4. Discussion

Our findings suggest that variation in growth is complex, resulting from a large number of loci with possible environmental interactions. Differences in the number and position of growth QTL detected in the separate analyses of groups A and B in this study might indicate genotype x environment (GxE) interactions, but proper evaluation was inhibited by low sample size. Groups A and B differed in density and may have experienced different temporal environments because their fertilization dates differed by two weeks. Interactions between strain and production facility suggest that GxE interactions impact growth traits in rainbow trout (Sylvén and Elvingson, 1992). Heritability estimates for growth of rainbow trout in the first year vary widely and tend to increase with age (Gjedrem, 1992; Su et al., 1996), suggesting that growth rates of juvenile rainbow trout may be particularly sensitive to environmental effects.

GxE effects have been detected in several QTL analyses, including lifespan in *Drosophila* (Vieira et al., 2000), but such analyses require a much higher sample size that of than the present study.

Lack of detection of the same QTL in both groups is most likely the result of insufficient sample size in the individual groups (Beavis, 1998; Melchinger et al., 1998; Young, 1999). Through simulations, Beavis (1998) found that studies with 100 individuals or less have low power to detect QTL of low to moderate effect and therefore may only detect a fraction of the QTL that are influencing a trait. Insufficient sample size also leads to overestimation of the percentage of phenotypic variance explained by QTL that are detected. His results suggest that power is vastly improved with mapping populations of greater than 500 individuals. This phenomenon, known as the Beavis effect, has been verified with experimental evidence in maize. Melchinger et al. (1998) performed QTL analyses of grain yield in two populations of maize (N = 107 and 344). They detected 107 QTL in the large population and only 39 in the small, with only 20 shared between the analyses. The positive side of Beavis's (1998) findings is that, although not all QTL may be detected, those that are detected are likely to be real.

Embryonic and postembryonic growth are generally believed to be under different genetic control. Postembryonic growth is controlled by insulin-like growth factor I (IGFI), which is regulated by growth hormone, and embryonic growth is influenced by IGFII, which is not (Sumpter, 1992). However, the results of the present study suggest that growth QTL identified in this study may have pleiotropic effects on embryonic growth. Using independent families from the same cross used in the present study (OSU x SW), Robison et al. (2001) identified 7 QTL influencing development rate and body size at swim up localizing to four linkage groups (Table 5). All four linkage groups were identified in the present study, two of

which contained QTL for growth traits. A QTL designated *wtR11*, influencing body mass at swim up (approximately 60 dpf), mapped to the same linkage group (OS-03) as a QTL influencing body mass at 89 dpf. Likewise a QTL influencing time to hatch (*tthR9*) localized to the same linkage group (OS-10) as a QTL influencing condition factor at 89 dpf. The relative positions of the two QTL could not be determined because of incomplete determination of synteny for this linkage group. However, assignment to the same linkage group and a relatively small difference in age between the measures (approximately 29 dpf) suggests that these QTL could be the same locus, though further investigation is needed. No QTL for growth traits were detected on the remaining two linkage groups, including a QTL (*tth-1* on OS-08) that had the largest influence on time to hatch and embryonic length in two independent crosses (Robison et al., 2001; Nichols, 2002). This agrees with a lack of association between microsatellites closely linked to this QTL and body size at years one and two in advanced backcrosses (Sundin et al., 2005).

Comparison across studies suggests that QTL on at least 17 linkage groups are involved in postembryonic growth (Table 5). Linkage groups that were associated with growth traits in our study were also identified in the studies of body mass QTL in outbred rainbow trout populations (O'Malley et al., 2002; Martyniuk et al., 2003). Relative locations of these shared QTL could not be determined due to limited alignment of the linkage maps. Four more linkage groups from our experiment contained growth QTL, but synteny has not yet been determined and may overlap with these other studies. Additional QTL detected in these studies on five other linkage groups for which synteny could be determined were not detected in our study. Likewise, we detected new QTL on three additional linkage groups that were not previously detected. The large number of QTL detected across these studies suggests that genetic variation in body size

traits in rainbow trout is the product of many loci with small additive effects, following a polygenic model of quantitative trait variation (Lynch and Walsh, 1998). QTL analyses of growth in mice have also detected large numbers of QTL (Cheverud et al., 1996; Rocha et al., 2004; Ishikawa et al., 2005). On the other hand, variation in specific growth rate itself appears to be the result of two QTL explaining a good portion of the phenotypic variance. These observations are intriguing and deserve further study.

Sex has considerable effect on growth and failing to account for this can bias heritability estimates of growth, especially after individuals begin to reach sexual maturity (Crandell and Gall, 1993a). Genes with effects on growth have been identified on sex chromosomes in salmonids (Allendorf et al., 1994; Forbes et al., 1994), as have QTL influencing thermotolerance and fork length (Perry et al., 2003; 2005). Additionally, epistatic interactions between sex-linked and autosomal QTL have been detected (Perry et al., 2003). While no sex-linked growth QTL reached genome-wide significance in the present study, a suggestive sex-linked QTL was detected in the combined analysis of specific growth rate. This effect may be easier to detect closer to sexual maturity when the effects of sex on heritability of growth are largest (Crandell and Gall, 1993a).

Moving from identification of a QTL to characterization of the actual genes responsible for variation in a quantitative trait is a difficult path (Mackay, 2001). In mice, fine mapping and gene expression analyses identified *glypican-3* as the gene underlying a QTL for growth in mice (Oliver et al., 2005). A QTL influencing muscle growth in pigs has been identified as a regulatory mutation in *insulin-like growth factor 2* (Van Laere et al., 2003). Unfortunately, the extensive genetic tools and information used in these studies are currently unavailable in salmonids (Thorgaard et al., 2002). A less powerful technique is to test for associations with

candidate genes through single marker analysis or by including these genes in the linkage map. Tao et al. (2003) examined growth in Arctic charr (*Salvelinus alpinus*) using single marker analyses of single nucleotide polymorphisms in ten candidate genes, and found a significant association with a locus containing growth hormone-releasing hormone and pituitary adenylate cyclase-activating polypeptide genes. In our study, QTL influencing length and mass at 194-195 dpf were detected on OS-09, to which one of the growth hormone genes (*GH1*) is associated in the reference map (Nichols et al., 2003b). The position of *GH1* in relation to these QTL is not yet known. No QTL were associated with OS-02, the homeologous linkage group to which *GH2* is associated (Nichols et al., 2003b). The relationship between GH and growth is complex. Treatment with exogeneous GH and transgenics increases growth in salmonids (Devlin et al., 1994; Johnsson et al., 1996; Devlin et al., 1999; Johnsson et al., 1999; Devlin et al., 2001). However, associations between natural variation in growth and endogeneous GH levels do not show a clear relationship (Sumpter, 1992; Valente et al., 2003).

This is the first attempt to map QTL for growth rate in rainbow trout over multiple time points rather than using size at an instant in time (O'Malley et al., 2002); Drew et al., *in prep*). Agreement among QTL analyses of growth in rainbow trout suggests that these QTL may be shared between populations, but differences illustrate the genetic complexity of this trait. Finally, the success of mapping QTL influencing economically important traits such as those in this study suggests that more detailed analyses can be performed in the future to further elucidate the genetic architecture of variation in growth.

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Table 1: Means [± 95% confidence interval (95%CI)] for growth traits in juvenile OSU x SW doubled haploid rainbow trout and tests of differences between groups.

		Group A		Group B		
Age (dpf)[a]	**Trait**	**N**	**mean ± 95%CI**	**N**	**mean ± 95%CI**	**Significance[b]**
89	FL (mm)	58	43.2 ± 1.22	86	40.2 ± 0.78	< 0.0001
	Mass (g)		1.3 ± 0.11		1.0 ± 0.05	< 0.0001
	K		1.6 ± 0.12		1.6 ± 0.04	0.5412
126-129	FL (mm)	55	67.7 ± 1.90	86	66.2 ± 1.72	0.2403
	Mass (g)		5.4 ± 0.37		4.6 ± 0.32	0.0012
	K		1.7 ± 0.05		1.5 ± 0.04	< 0.0001
194-195	FL (mm)	52	101 ± 4.07	84	100.6 ± 3.42	0.8747
	Mass (g)		17.1 ± 1.91		15.6 ± 1.49	0.2258
	K		1.6 ± 0.08		1.5 ± 0.06	0.0202
	SGR	44	2.4 ± 0.13	66	2.5 ± 0.09	0.1989

[a]Age in days post-fertilization. [b]*P*-values from approximate t-tests assuming unequal group variances using Satterthwaite's (1946) approximation to compute degrees of freedom.

FL = fork length, K = condition factor, SGR = specific growth rate for the period from 89 to 194-195 dpf.

Table 2: Pearson correlation coefficients among raw growth traits (above diagonal) and after z-transformation (below diagonal) in juvenile rainbow trout DH progeny.

z–scores	**Raw data**						
	Fork Length1	**Mass1**	**Condition Factor1**	**Fork Length3**	**Mass3**	**Condition Factor3**	**SGR**
Fork Length1		0.779***	–0.260**	0.517***	0.492***	–0.118	0.003
Mass1	0.787***		0.332***	0.390***	0.390***	–0.096	–0.262**
Condition Factor1	–0.360***	0.238**		–0.217*	–0.178	0.052	–0.424***
Fork Length3	0.554***	0.422***	–0.254**		0.940***	–0.363***	0.735***
Mass3	0.492***	0.393***	–0.207*	0.945***		–0.102	0.727***
Condition Factor3	–0.198*	–0.152	0.094	–0.370***	–0.125		–0.095
SGR	0.058	–0.184	–0.390***	0.750***	0.758***	–0.087	

Correlation coefficients significantly greater than zero are denoted as follows: ***p < 0.0001, **p < 0.01, *p < 0.05.
SGR = Specific growth rate between time points 1 and 3.

Table 3: QTL influencing growth traits detected in separate analyses of groups A and B. QTL in bold were also detected in combined analyses of z-transformed traits.

QTL	Linkage Group	Position[a]		LOD[b]	a[c]	r^{2d}
fl89-a-1	R7b	22.7	(acgatg14)	2.69*	1.77	0.159
fl89-b-1	**R16**	**0.01**	**(aagcct06)**	**4.51****	**1.43**	**0.171**
fl89-b-2	**R17b**	**0.01**	**(agcaag315a)**	**5.8****	**–1.63**	**0.232**
mass89-a-1	OS-27	1.5	(aagcct256o)	3.29*	0.145	0.154
mass89-a-2	R7c	47.2	(acgaga03)	4.31**	–0.173	0.209
mass89-b-1	OS-03	5.9	(actacc389a)	4.06**	0.107	0.177
mass89-b-2	R16	0.01	(aagcct06)	3.29*	0.090	0.139
mass89-b-3	R17b	34.8	(agcact07)	3.5*	–0.092	0.149
fl194-b-1	R7c	49.2		5.18**	–7.62	0.193
fl194-b-2	OS-09	10.3	(agccag03)	2.95*	5.41	0.102
fl194-b-3	R17b	19.3	(agcagt23)	3.36**	–5.70	0.118
mass194-b-2	OS-09	12.3		5.39**	3.43	0.181
mass194-b-3	R17b	50.8		3.41*	–2.71	0.111
mass194-b-4	OS-07	0.01	(agcagc03)	4.7**	2.83	0.149
mass194-b-1	**R7c**	**47.2**	**(acgaga03)**	**6.91****	**–3.64**	**0.234**
sgr-b-1	OS-14	64.4		2.84*	0.236	0.256
sgr-a-1	OS-12	2.0		4.48**	0.278	0.262

[a]Position is the distance of the QTL from the top of the linkage group. If the position coincided with a marker, the marker name is reported in parentheses. [b]LOD is Log of Odds that calculated

additive effects are significantly different from zero. Significance thresholds were calculated using permutations with 1000 replications (*$p < 0.05$, **$p < 0.01$). [c]Additive effect or effect of substituting an OSU allele for a SW allele at the QTL. [d]r^2 is the proportion of the phenotypic variance explained by the QTL using background markers as cofactors.

Table 4: QTL detected in combined analyses of z-transformed growth traits. QTL in bold were also detected in separate analyses within each group.

QTL	Linkage Group	Position		LOD	a	r2
fl89z-1	**R16**	**0.01**	**(aagcct06)**	**2.86***	**0.279**	**0.083**
fl89z-2	**R17b**	**0.01**	**(agcaag315a)**	**4.00****	**–0.333**	**0.123**
k89z-1	OS-10	53.4		4.77*	–0.484	0.223
mass194z-1	OS-14	100.4		2.91*	0.510	0.094
mass194z-2	**R7c**	**49.2**		**4.83****	**–0.359**	**0.126**
k194z-1	OS-14	84.4	(actacc270a)	3.21*	0.473	0.085
k194z-2	R7b	22.7	(acgatg14)	3.46**	–0.321	0.099

[a]Position is the distance of the QTL from the top of the linkage group. [b]LOD is Log of Odds that calculated additive effects are significantly different from zero. Significance thresholds were calculated using permutations with 1000 replications (*$p < 0.05$, **$p < 0.01$). [c]Additive effect or effect of substituting an OSU allele for a SW allele at the QTL. [d]r^2 is the proportion of the phenotypic variance explained by the QTL using background markers as cofactors.

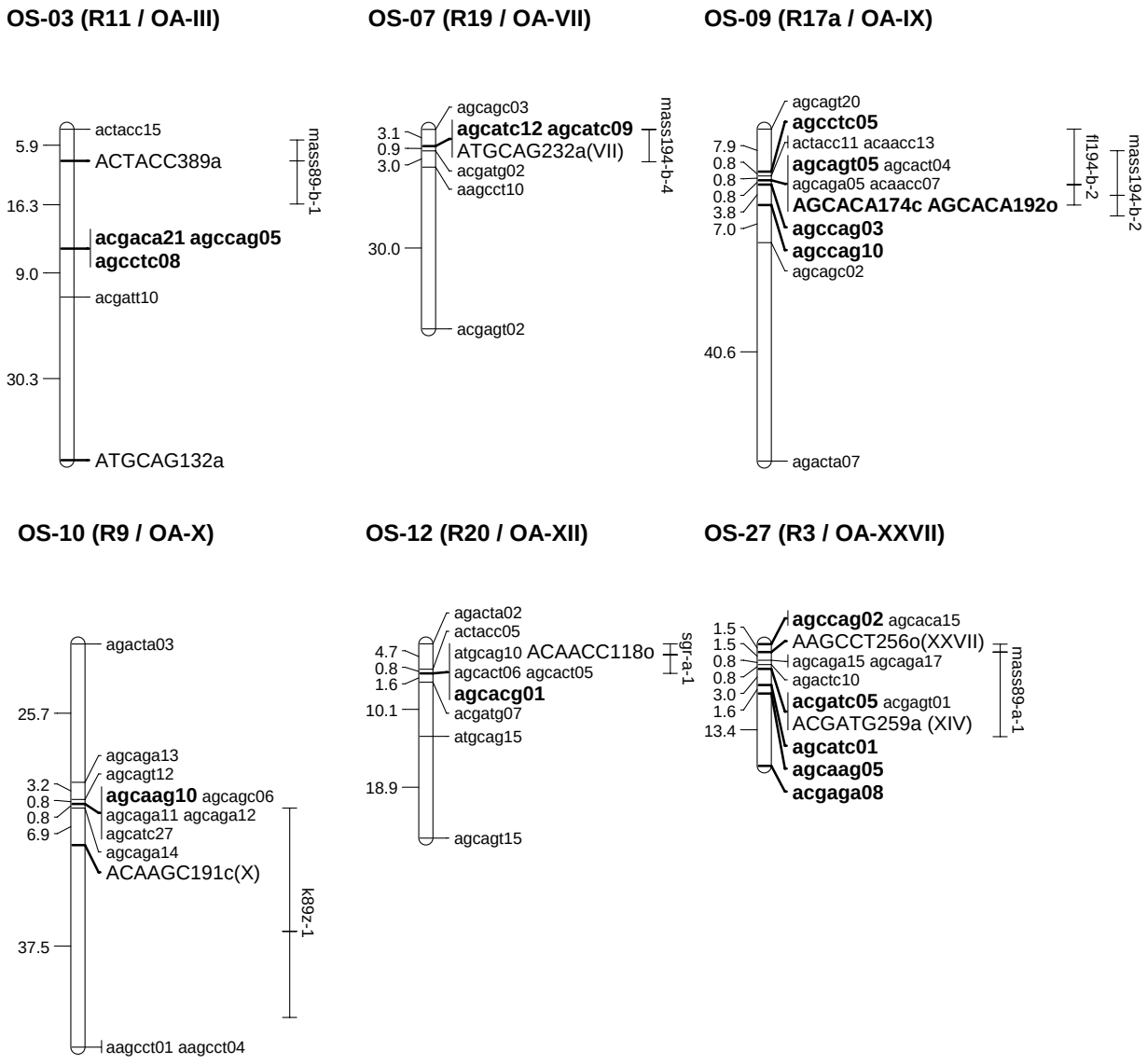
Table 5: Comparison of growth-related QTL among 5 independent studies of rainbow trout.

[a]Syntenies among linkage groups from three rainbow trout linkage maps [Nichols = Nichols et al. (2003), Robison = Robison et al. (2001), and Sakamoto = Sakamoto et al. (2000)]. Synteny between the Nichols and Sakamoto maps was determined by Nichols et al. (2003). Synteny between the Nichols and Robison maps was determined in the present study. Questionable syntenies are in parentheses. [b]Linkage groups for the present study are named with "OS" to identify the OSU x SW cross and a linkage group number in reference to linkage groups on the reference map (Nichols et al. 2003). Linkage groups for which reference synteny could not be determined were named according to Robison et al. 2001. [XY]Sex determining linkage group. [c]Summary of QTL reported by the present and four other studies [Robison = Robison et al. (2001), O'Malley = O'Malley et al. (2002), Martyniuk = Martyniuk et al. (2003), Drew = Drew et al. (*in prep*)]. Trait abbreviations: len = embryonic length, wt = embryonic mass, tth = time to hatch, m = body mass, cort = stress cortisol levels, FL = fork length, K = condition factor. Significance levels of QTL are indicated as significant (no label), [s]suggestive, and [?]marginal.

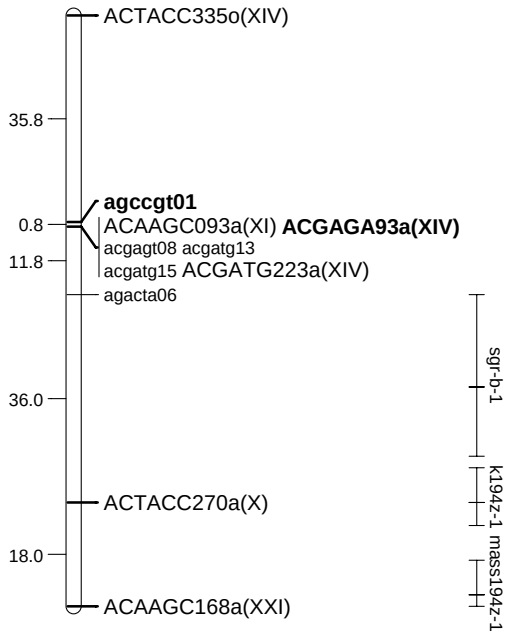
	Map syntenies[a]			QTL[c]					
Linkage Group[b]	**Nichols**	**Robison**	**Sakamoto**	**this study**	**Robison**	**O'Malley**	**Martyniuk**	**Perry**	**Drew**
OS-01	OA-I^{XY}	R21	18	sgr^{s}				FL	
OS-03	OA-III	R11	A	m	wt	m$^{?}$			
OS-06	OA-VI	R1	S			m^{s}			
OS-07	OA-VII	R19	R	m					
OS-08	OA-VIII	R13	J,N		len, tth	m^{s}	m		
OS-09	OA-IX	R17a	Oi	m, FL			m		
OS-10	OA-X	R9	H	K	tth	m^{s}			
OS-11	OA-XI	R22	E,P	K^{s}		m^{s}			
OS-12	OA-XII	R20	Fi	sgr					
OS-13	OA-XIII	R10	N			m^{s}	m		
OS-14	OA-XIV	R2	D	m, K, sgr					
OS-16	OA-XVI		Fii,5			m$^{?}$	m		
OS-21	OA-XXI	R15	B,D			m$^{?}$	m		
OS-22	OA-XXII	R7a	C			m			

OS-23	OA-XXIII	R6	Q		tth, len, wt			cort
	OA-XXIV		G			m	K, m	
OS-27	OA-XXVII	R3	15	m		m		cort
	OA-XXXI		5			m?	m	m
R7b		R7b		FL, K				
R16		R16		m, FL				
R17b	(OA-IV, XXV?)	R17b		m, FL				
R7c	(OA-XIII?)	R7c		m, FL				

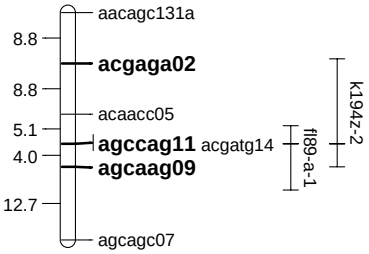
Figure 1: OSU x SW (OS) linkage groups. Only those linkage groups with significant growth trait QTL detected in separate group analyses and combined analyses are shown. Synteny to the OA reference map (Nichols et al. 2003) and the development rate map (Robison et al. 2001) are indicated in parentheses. Markers conferring synteny to the reference map are in all caps. Markers conferring synteny to the development rate map are in bold. Numbers to the left of the linkage groups are intermarker distances in cM. QTL position and 1-LOD intervals are depicted to the left of each linkage group.



OS-14 (R2 / OA-XIV)



R7b



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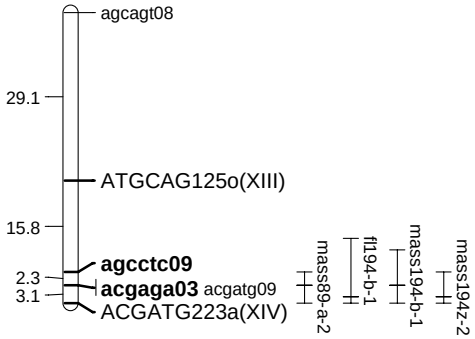
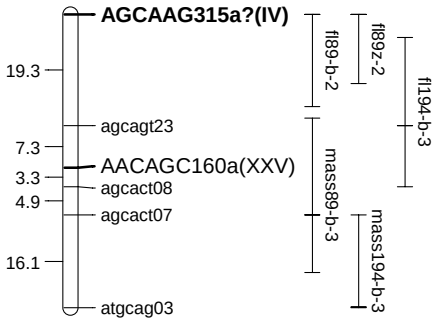
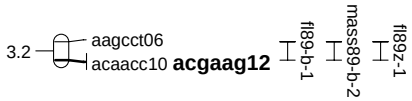


R17b (OA-IV?/OA-XXV?)



R16



CHAPTER FOUR

OVERALL CONCLUSION

These studies are a first step toward greater understanding of traits of economic and evolutionary importance in aquaculture. QTL underlying stress responsiveness and growth were successfully identified but are likely an underestimate of the number of genes influencing these traits. Analyses with larger sample sizes are more likely to detect QTL with low effect (Beavis, 1998) and epistasis between QTL (Perry et al., 2003; Carlborg et al., 2004; Carlborg et al., 2005; Ishikawa et al., 2005), and interactions between QTL and the environment (Vieira et al., 2000). The opportunities for future research are staggeringly great.

There was considerable agreement of linkage groups associated with growth in studies of embryos (Robison et al., 2001), juveniles (Drew et al. *in prep*), and adults (O'Malley et al., 2002; Martyniuk et al., 2003). This suggests that the same QTL may be influencing variation in widely different populations, and that different approaches to QTL detection (doubled haploid compared to outbred) yield similar results. However, as the locations of these QTL in relation to each other are still unknown due to incomplete synteny, additional research is encouraged. Identification of QTL that are not shared among the studies suggests that there is also considerable variation in genetic control of growth among populations.

QTL analysis of traits associated with domestication can provide useful tools for the investigation of various aspects of the process of domestication. Traits, such as stress responsiveness and aggression, that show differences between wild and captive-bred populations are also known to be variable among wild populations and among captive stocks (Iwama et al., 1992; Lahti et al., 2001). Captive populations are often at different stages in the domestication process. Captive populations range from full life-cycle, captive rearing (aquaculture) to sea

ranching or hatchery rearing, where fish are bred and reared for the early part of their life cycle in captivity and are released to spend the remaining part in the wild, often migrating to the ocean. Of twenty-four studies comparing captive and wild populations for a variety of traits, seven involved aquaculture populations, sixteen examined hatchery or sea ranched populations, and one included both farmed and hatchery stocks. Of these same twenty-four studies, ten reported using captive stocks that were selected for fast growth, four were presumed free of selection, and ten did not report selection history. In a comparison of farmed, sea ranched, and wild masu salmon (*Oncorhynchus masou*), farmed salmon were more likely to feed in the presence of chemical alarm signals than wild fish; sea ranched fish were intermediate (Yamamoto and Reinhardt, 2003). This suggests that many of the studies examining the effects of domestication using heavily cultured fishes may not be directly applicable to noncommercial hatchery populations (Brannon et al., 2004). However, characterization of QTL underlying these traits could eventually allow the examination of frequencies of QTL alleles (or associated markers) and patterns among populations at different levels of domestication. This is a far leap from the research presented here, but this study represents a step in that direction.

The task of identifying the genes responsible for quantitative trait variation can be a daunting one, requiring intensive genetic research and considerable investment of time and money (Mackay, 2001). Genomic sequences corresponding to regions of a genetic map that are associated with a QTL are quite large, often containing thousands of genes. Identification of the actual polymorphism responsible for trait variation requires fine mapping with higher numbers of recombinations, such as near-isoallelic lines (Eshed and Zamir, 1995), and extensive knowledge of the DNA sequence associated with the trait. Despite the considerable difficulty, the frequency of studies identifying genes underlying quantitative variation is increasing. Recent examples

include identification of glypican-3 underlying a QTL for growth in mice (Oliver et al., 2005), insulin-like growth factor 2 underlying muscle growth in pigs (Van Laere et al., 2003), Rgs2 underlying anxiety in mice (Yalcin et al., 2004), and cortisol-binding globulin associated with cortisol levels in Meishan pigs (Ousova et al., 2004).

Many of these tools are still unavailable to the research of rainbow trout and other salmonids (Thorgaard et al., 2002). This is unfortunate because rainbow trout is one of the most intensively studied fish species and has a wide range of research applications including, but not limited to, carcinogenesis, toxicology, immunology, disease, nutrition, genomic evolution, and ecology (Thorgaard et al., 2002). Also, clonal lines and doubled haploid mapping populations provide powerful tools with which to investigate of quantitative traits of economic importance to aquaculture and as model species of relevance to humans and other species. It is hoped that increasing interest, investment and advancing technologies will allow research in rainbow trout to reach its full potential.

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