Genomic Consequences of Hybridization between Rainbow and Cutthroat Trout

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Abstract

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Introgressive hybridization creates novel gene combinations that may generate important evolutionary novelty and thus contribute to biological complexity and diversification. On the other hand, hybridization with introduced species can threaten native species, such as cutthroat trout (*Oncorhynchus clarkii*) following the introduction of rainbow trout (*O. mykiss*). While rainbow trout introgression in cutthroat trout is well documented, neither the evolutionary consequences nor conservation implications are well understood.

Hybridization between rainbow and cutthroat trout occurs in the context of substantial chromosomal rearrangement, as well incompletely re-diploidized genomes. Rainbow and cutthroat trout are descended from an autopolyploid ancestor, and extensive chromosome arm rearrangements have occurred between the species following their divergence from the last common ancestor. Evidence for incomplete re-diploidization includes the occasional formation of multivalents and duplicated loci occasionally exhibit a mixture of disomic and tetrasomic inheritance. Thus, transmission genetics may be complicated by recombination between homeologs.

Here, I evaluated the phenotypic and genetic consequences of introgression between rainbow trout and Yellowstone cutthroat trout (*O. clarkii bouvieri*) to provide insights into genome processes that may help

explain how introgression affects hybrid genome evolution. The overall aim of the first part of this dissertation (Chapters 1 and 2) was to evaluate phenotypic variation and gene expression among parental species and hybrids to gain insight into the genetic basis of hybrid and parental morphologies. We constructed seven line crosses: both parental species, both reciprocal F1 hybrids, first-generation backcrosses, and F2 hybrids. In **Chapter 1**, we aimed to assess the role of introgression on growth (length and weight gain), morphology, and developmental instability among these seven crosses. Growth was related to the proportion of rainbow trout genome contained within crosses. Rainbow and cutthroat trout were morphologically divergent: rainbow trout were generally robust whereas cutthroat trout were typically more slender and their hybrids tended to be morphologically intermediate, although backcrosses were morphologically more similar to their backcrossing parental species. These differences in growth and body morphology may be maintained, in part, through the regulation of muscle growth-related genes. Therefore, in **Chapter 2**, we aimed to characterize the expression of muscle growth-related genes and to describe relationships between gene expression and growth patterns among parental species and hybrids to gain insight into the underlying genetic basis of the difference in their body shapes. Our findings suggest that rainbow and cutthroat trout exhibit differences in muscle growth regulation, that transcriptional networks may be modified by hybridization, and that hybridization disrupts intrinsic relationships between gene expression and growth patterns that may be functionally important for phenotypic adaptations.

The overall aim of the second part of this dissertation (Chapters 3 and 4) was to assess the genetic consequences of introgression to determine how the genomic architecture of hybrids affects allelic inheritance, and thus their subsequent evolution. In **Chapter 3**, we generated a genetic linkage map for rainbow-Yellowstone cutthroat trout hybrids to evaluate genome process that may influence introgression genome evolution in hybrid populations. Our results suggest that few genomic incompatibilities exist between rainbow and cutthroat trout, allowing their to genomes introgress freely, with the exception that differences in chromosome arrangement between the species may act as barriers to introgression and enable large portions of non-recombined chromosomes to persist within admixed populations. In **Chapter 4**, we aimed to determine the effect of incomplete re-diploidization on transmission genetics in hybrids, compared to pure species. We used the parental gametic phase from existing genetic linkage maps to identify the homeologs that recombine, to characterize this recombination, and to verify meiotic models of residual tetrasomic inheritance in autotetraploids. Recombination between homeologs occurred frequently in hybrids and results in the non-random segregation of alleles across extended chromosomal regions as well as extensive double-reduction in hybrid parental gametes. Taken together, the results from Chapters 3 and 4 suggested that chromosome rearrangements and recombination of homeologs could influence genome evolution in admixed populations.

The research presented in this dissertation indicated that the evolutionary fate of hybrid genomes is unpredictable. Some of our findings suggest that introgressions proceeds in a predictable fashion in admixed populations; rainbow and cutthroat genomes freely introgress, with the exception that chromosome rearrangements may suppress recombination across large chromosomal regions. However, homeologous recombination during meiosis in hybrids results in unpredictable segregation of chromosomes, and the segregation of these chromosomes may depend on the hybrid generation of each parent within an admixed population. Furthermore, phenotype and gene expression are quantitative traits, and expression of these traits may depend on hybrid genotypes across transcriptional networks that are controlled by genes distributed over the entire genome. Consequently, hybridization may alter transcriptional regulation of genes, resulting in unpredictable gene expression patterns, which, in turn, contribute to the high phenotypic variation in hybrids.

Contents

List of figures

List of tables

General Introduction

The widespread occurrence of hybridization has provided rich opportunities for investigating genome evolution, adaptation, speciation, reproductive isolation, and hybrid fitness (Barton and Hewitt 1989, Rieseberg *et al.* 2000, Burke and Arnold 2001). Hybridization is a natural evolutionary process, contributing to the diversification of plants and animals (Dowling and DeMarais 1993, Rieseberg and Wendel 1993). When populations hybridize and progeny are viable and fertile, genomes introgress and produce recombined chromosomes. Introgression breaks down linkage associations and generates novel gene combinations. These novel gene combinations may serve as a foundation for adaptive genetic variation, cause shifts in genetic traits that underlie phenotypic variation, and influence hybrid fitness (Rieseberg *et al.* 2000, Seehausen 2004). Thus, hybridization may cause substantial changes in genome structure and genotype-environment interactions with wide-ranging evolutionary implications.

Hybridization can also have significant conservation implications and has the potential to reduce biodiversity through loss of distinct gene pools and genetic homogenization (Rhymer and Simberloff 1996, Allendorf *et al.* 2001). In these cases, introgression may interfere with epistatic interactions by disrupting local adaptations and breaking down co-adapted gene complexes. For example, widespread introduction of non-native rainbow trout (*Oncorhynchus mykiss*) into cutthroat trout (*O. clarkii*) habitats and consequent introgressive hybridization has resulted in loss of native cutthroat trout throughout their range (Allendorf and Leary 1988, Behnke 1992). Native cutthroat trout populations are often replaced by hybrid swarms following rainbow trout introductions, causing concern for cutthroat trout conservation (Allendorf and Leary 1988).

Rainbow and cutthroat trout are sister species in the family Salmonidae, shared the last common ancestor approximately 3 million years ago (Wilson and Turner 2009), and are descendent from an autopolyploid event (a whole genome duplication event within a species) that occurred approximately 85 – 105 million years ago (Macqueen and Johnston 2014). The diploid salmonid ancestor is presumed to have had approximately 48 acrocentric chromosomes (Ohno 1970, Mank and Avise 2006). Re-diploidization in salmonids is incomplete (Wright *et al.* 1983, Allendorf and Thorgaard 1984). Chromosome rearrangements following the salmonid whole genome duplication event have produced different numbers of acrocentric and metacentric chromosomes among species (Phillips and Rab 2001).

Despite karyotypic differences between rainbow and cutthroat trout, Robertsonian rearrangements have maintained the same number of chromosome arms in the diploid genome (2n=104). Robertsonian rearrangements are translocations that involve centric fusion or fission between chromosome arms, causing a change in the number of chromosomes but not of chromosome arms. Similarity in chromosome arm number between rainbow and cutthroat trout could be an important factor that allows species to hybridize readily and to produce viable and fertile progeny. Nevertheless, karyotypic differences between rainbow and cutthroat trout could affect hybrid genome evolution by suppressing recombination, hindering gene flow, and generating linkage disequilibrium (Rieseberg and Linder 1999, Panithanarak *et al.* 2004, Machado *et al.* 2007, Stevison *et al.* 2011). In addition, reduced recombination between rearranged chromosome segments could prevent disruption of co-adapted gene complexes (Noor *et al.* 2001, Feder *et al.* 2003); enabling these adaptations to persist within hybrid populations, which could ultimately affect hybrid fitness.

Previous research suggests that hybridization with rainbow trout could have wide-ranging fitness effects on cutthroat trout. Mean spawn timing in hybrids was found to occur earlier in the year relative to cutthroat trout (Henderson *et al.* 2000, DeRito *et al.* 2010). Earlier spawn timing could allow for earlier emergence, providing hybrid fry with an initial size advantage that allows them to outcompete cutthroat trout fry. Morphological differences among rainbow trout, cutthroat trout, and their F1 hybrids have been associated with swimming performance, and higher sustained swimming activity of rainbow trout and hybrids might afford them a competitive advantage over cutthroat trout (Hawkins and Quinn 1996, Seiler *et al.* 2009). However, Muhlfeld *et al*. (2009) found that the reproductive success of hybrids declined as the proportion of rainbow trout admixture increased, suggesting that fitness advantages afforded by earlier spawn timing and morphology may be marginalized by reduced reproductive success. Furthermore, hybridization may disrupt local adaptations, such as migration pattern (Boyer *et al.* 2008), thermal tolerances (Ostberg and Rodriguez 2006), and metabolic traits (Rasmussen *et al.* 2012). In addition, reduced developmental stability in hybrids could suggest that hybridization disrupts co-adapted gene complexes (Leary *et al.* 1985). Although hybridization between rainbow and cutthroat trout is well documented, it is unknown how genome rearrangements affect introgression and hybrid performance. Identifying these processes could provide insight into the fate of co-adapted gene complexes and local adaptations that may ultimately affect hybrid fitness.

For this dissertation, we evaluated the genetic and phenotypic consequences of hybridization between rainbow trout and Yellowstone cutthroat trout (*O. c. bouvieri*). Hybridization occurs in the context of substantial chromosomal, genetic, and morphological differences between these species (Loudenslager and Thorgaard 1979, Thorgaard 1983, Leary *et al.* 1987, Behnke 1992, Seiler and Keeley 2007, Wilson and Turner 2009). These differences, along with high fertility of hybrids and the pervasive nature of hybridization, provides an excellent opportunity to investigate genome processes that affect introgression.

This dissertation is partitioned into two primary aims. The first aim is to evaluate the role of hybridization in causing differences in growth patterns and morphology among parental and hybrid crosses (introgressed and non-introgressed). Here, we characterized growth and morphology at two temperatures, and assessed developmental instability (Chapter 1). Next, we characterized expression patterns of muscle growth-related genes and described relationships between gene expression and growth patterns (Chapter 2). The second aim is to evaluate the genomic consequences of recombination in hybrids to determine whether there are genomic barriers to introgression and to assess homeologous recombination. To accomplish this aim, we developed a genetic linkage for F1 hybrids for comparison to existing rainbow trout maps, determined if introgression suppresses recombination, and estimated the prevalence of segregation distortion in the F1 hybrid map (Chapter 3). Next, we used the genetic linkage map for F1 hybrids developed in Chapter 3 to identify the homeologous chromosomes that recombine in salmonids, determine the effect of incomplete re-diploidization on transmission genetics in hybrids, and empirically verify models of residual tetrasomic inheritance in autotetraploids (Chapter 4).

Together, these four studies contribute to a growing body of research on genome evolution and the evolutionary fate of hybrids. Our findings indicate that the evolutionary fate of hybrid genomes is unpredictable. Although our findings demonstrate that chromosome rearrangements suppress recombination, which implies that the extent of introgression in some genome regions may be predictable, high phenotypic variation in the hybrids and unpredictability of gene expression patterns may indicate otherwise. Our findings may also suggest that fitness differs among hybrid generations, as well as among individuals within hybrid generation.

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Chapter 1: Growth, Morphology, and Developmental Instability of Rainbow Trout, Yellowstone Cutthroat Trout, and Four Hybrid Generations[1]

Abstract

Hybridization of cutthroat trout, *Oncorhynchus clarkii*, with non-indigenous rainbow trout, *O. mykiss*, contributes to the decline of cutthroat trout subspecies throughout their native range. Introgression by rainbow trout can swamp the gene pools of cutthroat trout populations, especially if there is little selection against hybrids. We used rainbow trout, Yellowstone cutthroat trout (*O. clarkii bouvierii*), and rainbow-Yellowstone cutthroat F_1-hybrid parents to construct seven different line crosses: F_1 hybrids (both reciprocal crosses), F_2 hybrids, first generation backcrosses (both rainbow and Yellowstone), and both parental taxa. We compared growth, morphology, and developmental instability of these seven crosses, reared at two different temperatures. Growth was related to the proportion of rainbow trout genome present within crosses. Meristic traits were influenced by maternal, additive, dominant, overdominant, and (probably) epistatic genetic effects. Developmental stability, however, was not disturbed in F_1 hybrids, F_2 hybrids, and backcrosses. Backcrosses were morphologically similar to their recurrent parent. The lack of developmental instability in hybrids, suggests that there are few genetic incompatibilities preventing introgression. Our findings suggest that hybrids are not equal. That is, growth, development, character traits, and morphology differ depending on the genomic contribution from each parental species as well as the hybrid generation.

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Introduction

Anthropogenic hybridization is a conservation concern (Allendorf *et al.* 2001) because it contributes to the loss of genetic diversity, extirpating entire gene pools (Rhymer and Simberloff 1996). Introductions of non-indigenous rainbow trout (*Oncorhynchus mykiss*) into cutthroat trout (*O. clarkii*) habitat, for example, have swamped cutthroat trout populations through introgressive hybridization, forming hybrid swarms (Leary *et al.* 1995, Boyer *et al.* 2008). Introgressive hybridization is a consequence of recombination between parental genomes (i.e., crossing over and independent assortment), producing recombinant chromosomes derived from each parental taxon (Anderson 1949). Recombination within the hybrid genome may disrupt coadapted genomic associations within each parental taxon, leading to outbreeding depression (Dobzhansky 1950, Templeton 1986).

Laboratory studies investigating hybridization between rainbow trout and cutthroat trout have demonstrated differences in morphology, swimming performance, behavior, development, and meristic counts among parental species and their F_1 hybrids (Leary *et al.* 1985, Allendorf and Leary 1988, Hawkins and Quinn 1996, Hawkins and Foote 1998, Seiler and Keeley 2007). However, since F_1 hybrid genomes are not introgressed, and contain a complete chromosome complement from each species, the differences between F_1 hybrids and their parental species may not be indicative of the phenotype in post-F_1 hybrids. Indeed, fitness reductions are often not apparent until after the F_1 hybrid generation (Edmands 2007, Tymchuk *et al.* 2007). In addition, the effects of outbreeding are unpredictable (McClelland and Naish 2007).

Although introgressive hybridization is a central concern in cutthroat trout conservation, little is known about how introgression affects morphology, development, behavior, physiology, fitness and adaptive ability of cutthroat trout (Allendorf *et al.* 2004, Allendorf *et al.* 2005, Campton and Kaeding 2005). This uncertainty is particularly relevant to the legal definition of “species” under the U.S. Endangered Species

Act and recent U.S. Fish and Wildlife Service findings for westslope cutthroat trout (*O. c. lewisii*) (USFWS 2003) and Rio Grande cutthroat trout (*O. c. virginalis*) (USFWS 2002). Rio Grande cutthroat trout populations with more than 1 percent introgression are considered hybrids, whereas westslope cutthroat trout populations with as much as 20 percent introgression are considered pure. The lack of knowledge regarding the effects of introgression on cutthroat trout contributes to the inconsistency among policies.

Our goal was to understand how introgressive hybridization between rainbow trout and Yellowstone cutthroat trout (*O. c. bouvierii*) affect fitness-associated traits. Yellowstone cutthroat trout and rainbow trout were historically allopatric but extensive stocking of rainbow trout within the range of Yellowstone cutthroat has lead to their co-occurrence and subsequent introgression. We investigated growth, morphology, meristic traits, and developmental instability in rainbow trout, Yellowstone cutthroat trout, their F_1 and F_2 hybrids, and first generation backcrosses. Our novel approach, the first rainbow-cutthroat trout hybrid investigation involving known post-F_1 hybrid crosses, was designed to address two questions. First, do rainbow trout, Yellowstone cutthroat trout and hybrid crosses exhibit equal growth? Second, do morphology, meristic traits, and developmental instability differ among rainbow trout, Yellowstone cutthroat trout and hybrid crosses? We show that differences in growth among crosses are related to the proportion of the rainbow trout genome present, that developmental stability is not disturbed in F_2 hybrids or backcrosses, and that backcrosses are morphologically more similar to their recurrent parent. Although potentially confounded by the unintentional inclusion of two different rainbow trout hatchery stocks and eggs from a single female Yellowstone cutthroat trout, we believe our data have merit from a species-level perspective and argue these points in the discussion.

Materials and Methods

Gamete collection

We collected gametes in March, 2004, from five females and five males from each of the following sources: rainbow trout (henceforth Rbt), Hayspur strain, Hayspur Hatchery, ID; Yellowstone cutthroat trout (henceforth Yct), Henry's Lake gamete collection facility, Henry's Lake, ID; and Yct-Rbt F_1 hybrids, Henry's Lake gamete collection facility, Henry's Lake, ID. Yellowstone cutthroat-rainbow trout F_1 hybrids have been routinely planted into Henry's Lake for a sport fishery and are the product of crossing female Yct from Henry's Lake with male Rbt (Kamloops strain) from Hayspur Hatchery (Campbell *et al.* 2002). The purity of parental stocks (RBT, YCT, or F_1 hybrids) was confirmed by applying 12 co-dominant, species-specific molecular markers that differentiate between RBT and YCT (RAG 3', hsc 71, IGF-2, and IK (Baker *et al.* 2002), OCC-16 (Ostberg and Rodriguez 2002), and OMM-55, OCC-34, OCC-35, OCC-36, OCC-37, OCC-38, and OCC-42 (Ostberg and Rodriguez 2004)).

Cross design

The availability of fertile F_1 hybrids returning to Henry's Lake gamete collection facility enabled us to construct a factorial mating design consisting of the following seven line crosses (genotypic classes): 1) Rbt x Rbt (Rbt); 2) Yct x Yct (Yct); 3) Rbt x F1, first generation Rbt backcross (BC-Rbt); 4) Yct x F1, first generation Yct backcross (BC-Yct); 5) Rbt x Yct, F_1 hybrids with a Rbt maternal lineage (F_1-Rbt); 6) Yct x Rbt, F_1 hybrids with a Yct maternal lineage (F_1-Yct); and 7) F1 x F1, F_2 hybrids (Table 1.1). For the cross design a single clutch of Rbt and Yct eggs were each split into thirds. One third of an egg clutch were fertilized by a single Rbt male, one third fertilized by a single Yct male, and one third fertilized by a single F_1 hybrid male. Additionally, a single F_1 hybrid female was fertilized by a single F_1 hybrid male. This design was repeated five times yielding five families within a cross. Each repetition incorporated different individuals.

Fish rearing

After fertilization, each family was randomly assigned to a Heath tray and incubated at approximately 7.4 ^{o}C at Henry's Lake gamete collection facility until the eyed stage whereupon they were shipped to U. S. Geological Survey's Western Fisheries Research Center (Seattle, WA) and incubated in randomly assigned Heath trays at 11 ^{o}C.

After yolk-sac absorption, each family was transferred to a randomly assigned 275 L flow-through tank. Unfortunately, only a single Yct female produced viable eggs, and as a result, only a single Yct, BC-Yct, and F_1-Yct family survived. Additionally, survival was poor in 2 Rbt families and 2 F_2 hybrid families. As a result, the following number of families survived to yolk-sac absorption for each cross: 3 Rbt, 5 BC-Rbt, 5 F_1-Rbt, 3 F_2 hybrids, 1 F_1-Yct, 1 BC-Yct, and 1 Yct. Families were culled to equalize tank densities and fed according manufacturer recommendations (Bio-Oregon, Inc., Warrenton, OR).

Growth performance

The experiment was conducted at two different temperatures, 8 ^{o}C and 19 ^{o}C. These temperatures were chosen because they encompass a broad range tolerable to Rbt and Yct. The experiment was designed to determine how growth patterns differed among parental and hybrid crosses and how growth patterns differed across environmental conditions (8 ^{o}C and 19 ^{o}C). Thus, this experiment can provide an understanding of how introgression affects growth in different conditions. Three families per cross were selected for experimental analysis, with the exception of Yct, BC-Yct, and F_1-Yct, in which only a single family per cross was available. Fish were raised to 90 – 105 mm fork length (FL) prior to beginning the experiment, whereupon each individual fish received an intraperitoneal PIT tag, had its FL and weight recorded, and was then placed into a specific 121 L flow-through tank. The experimental design consisted of four replicate 121 L flow-through tanks per cross, each containing 60 fish. Each cross was grown separately, but families within crosses were combined. Each replicate tank consisting of Rbt, BC-Rbt, F_1-Rbt, or F_2 crosses (crosses consisting of three families) contained 20 individuals from each of three families. Replicate tanks consisting of Yct, BC-Yct, and F_1-Yct crosses (crosses consisting of a single family) contained 60 individuals from the single family. Two replicates from each cross were acclimated to 8 ^{o}C and two replicates acclimated to 19 ^{o}C over a three week period. During the acclimation period fish were fed a specific percentage of body weight depending on daily acclimation temperature. After the acclimation period, fish were grown for 57 days at either 8 ^{o}C or 19 ^{o}C and fed to satiation twice daily, 5 days per week. Food was withheld two days before the end of the experiment. After 57 days, fish were euthanized and their PIT tag number and FL and weight recorded. The fish were then preserved in a 10 % formalin (neutral buffered) solution for analysis of meristics, morphology, and developmental instability.

Relative growth analysis

Because length and weight were highly correlated at the start (Spearman's $\rho = 0.956$, $n = 1625$, $P < 0.0001$) and end (Spearman's $\rho = 0.984$, $n = 1625$, $P < 0.0001$) of the experiment, we present the results of weight gain as an index of relative growth. To compare growth among treatments and reduce the effect of size differences among individuals at the start of the experiment, we calculated the relative growth rate per day of each individual as $((M_{\text{end}} - M_{\text{start}})/(M_{\text{start}}))/ \text{ x } 78 \text{ days}^{-1}$; where M equals weight in grams. We used a mixed effects linear regression model (Zuur *et al.* 2009) with fixed effects for the temperature of the tank and the cross type, and separate random effects representing all female and male parents from whom all 7 crosses were generated, and the 24 tanks. This accounted for dependences among fish due to shared parents and tanks. The mixed effects model for relative weight gain, y, was denoted as $y_{ijklmn} = \mu_{ij} + a_k + b_l + c_m + e_{ijklmn}$, with i = temperature, j = cross, k = mother, l = father, m = tank, and n = fish within families. Common methods for fitting mixed effects models assume normality and constant variance for maternal (a_k), paternal (b_l), and tank effects (c_m), as well as residual (fish-to-fish) variation (e_{ijklmn}). As such, the data were log transformed prior to model fitting. We fitted the model for the log transformed relative weight gain using the *lmer* function of the *lme4* package in R (http://lme4.r-forge.r-project.org). Within each temperature, the difference in mean relative weight gain between each cross type was judged as significantly different from zero at $\alpha = .05$, after conducting multiple pairwise comparisons using the R function glht(multcomp).

Meristic and fluctuating asymmetry analysis

To examine meristic differences and fluctuating asymmetry among crosses, we counted six paired meristic traits on both sides of each fish: rays in the pectoral fins (PEC), rays in the pelvic fins (PEL), branchiostegal rays (BR), mandibular pores (MP), gill rakers on the lower first gill arch (GRL), and gill rakers on the upper first gill arch (GRU). Counts were made twice to eliminate counting errors; if the first and second counts differed, a third count was made. We used a mixed effects model to investigate meristic differences among the seven cross-types with a fixed effect for the cross type and random effects for the maternal and paternal effects, while accounting for dependences among the fish due to shared parents. The meristic model for each character was denoted as $y_{jkln} = \mu_j + a_k + b_l + e_{jkln}$, where j = cross, k = mother, l = father, and n= fish within families. Multiple comparison testing was conducted as previously described above.

Individual asymmetry (d) equals $l - r$, where l is the value of a trait on the left side and r is the value of the same trait on the right side. The mean of d, if it is significantly different from zero, is a measure of directional asymmetry. To compare fluctuating asymmetry among genotypes, we used the unsigned asymmetries $| d |$ of the meristic traits. We also used the sum of standardized unsigned asymmetries as a composite measure of fluctuating asymmetry. This is Leung *et al.*'s (2000) CFA2. We used t-tests to identify directional asymmetry, and examined histograms and distributional statistics to identify antisymmetry and skew. As an additional measure of developmental instability, we examined each fish for phenodeviants. A phenodeviant is an abnormal trait expression indicative of developmental instability rather than a polymorphism (Graham *et al.* 1993). We used loglinear analysis of a three-way contingency table: seven genotypes (Rbt, Yct, F_1-Rbt, F_1-Yct, F_2, BC-Rbt), or BC-Yct) by two temperatures (8° or 19° C) by two phenodeviant categories (present or not present).

Morphometric landmark analysis

We photographed the left sides of 1446 fish, and used tpsDIG2 (Rohlf 2008) to digitize 13 landmark coordinates (Figure 1.1). These 26 *x*- and *y*- coordinates describe the entire configuration of landmarks; it is the raw data on which shape analysis occurs. A Procrustes generalized least squares superimposition (CoordGen, Integrated Morphometrics Programs, (Zelditch *et al.* 2004)) removed all differences among the 1446 landmark configurations that were not shape differences (Rohlf 1990, Bookstein 1991).

Procrustes superimposition does this by translating all landmark configurations to a common location, by scaling them to unit centroid size, and by rotating each configuration to minimize the sum of the squared deviations from a reference configuration. Our reference configuration was the average shape (consensus) of all individuals over all combinations of Genotype and Temperature.

The new Procrustes coordinates were converted into a smaller set of 22 shape variables containing the partial warp scores, coefficients indicating an individual's position, relative to the reference, along partial warps (Zelditch *et al.* 2004). The partial warps are the components of the non-uniform (non-affine) deformation. This shape space has 4 fewer dimensions than the 26 landmark coordinates; the four dimensions that are lost represent position in two-dimensions (2), orientation (1), and size (1). The partial warp scores can be used directly in statistical analyses, whereas the 26 variable coordinates cannot.

To explore differences in shape among Genotypes and Temperature, we did two canonical variates analyses (one for each temperature). We used thin-plate splines to interpret shape changes as deformations of the reference grid implied by regressing shape on the canonical variate axis scores. We also summed the variances of the 22 partial warps (Zelditch *et al.* 2004) to estimate shape variance within each combination of Genotype and Temperature.

Results

After 78 days following PIT tagging, 802 and 823 fish survived the 19 °C and 8 °C treatment temperatures, respectively. Most Cross-Temperature groups had low mortality levels (0-7 individuals), but one group (BC-Yct at 19 °C) experienced 19 mortalities over the course of the experiment.

Relative growth

All genotypic classes had higher relative weight gain at 19 °C than they did at 8 °C (Figure 1.2). Significant differences in growth were observed among crosses at 19 °C but not at 8 °C. At 19 °C, the Rbt growth rate was significantly greater than all other crosses, with the exception of BC-Rbt. The BC-Rbt, in turn, was different from F_2 and BC-Yct. Lack of growth rate differences among crosses at 8 °C but not at 19 °C may indicate differential growth patterns among crosses depending on temperature or may simply reflect that observable differences have not yet accumulated at the temperature with lower growth rate.

Meristic traits

Pectoral and pelvic fin ray counts showed complex patterns of inheritance (Figure 1.3A and 1.3B, Table 1.2). Yellowstone cutthroat trout had fewer pelvic fin rays than Rbt, but similar numbers of pectoral fin rays. The F_1-Rbt generation had more pectoral fin rays than Rbt but not Yct, and had more pelvic rays than Yct but not Rbt. The F_1-Yct generation had numbers of pelvic fin rays intermediate to Rbt and Yct; surprisingly, numbers of pectoral fin rays were more similar to Rbt than Yct. The F_2 generation had fewer pectoral fin rays than Yct but not Rbt, and had fewer pelvic rays than Rbt but not Yct. Pectoral ray numbers were not different between backcross generations, but pelvic rays were different and resembled the parents they were backcrossed to. A maternal effect was apparent for pelvic rays, but not pectoral rays.

Branchiostegal rays counts showed a pattern of simple dominance (Figure 1.3C, Table 1.2). Rainbow trout had more branchiostegal rays than Yct. All hybrids and backcrosses had numbers similar to Rbt and different from Yct, although a maternal effect was apparent. The greatest number of branchiostegal rays was observed in the F_1-Rbt.

Mandibular pores exhibited patterns consistent with additive inheritance (Figure 1.3D, Table 1.2). Yellowstone cutthroat trout had more mandibular pores than Rbt. Both F_1 generations and the F_2 were intermediate to the parentals, while the backcrosses were between the F_1 generations and the parent they were backcrossed to. The mandibular pore counts appeared to have a strong maternal effect.

Gill raker counts on the lower arch exhibited patterns consistent with overdominance, whereas counts on the upper arch did not (Figure 1.3E and 1.3F, Table 1.2). In fact, all crosses had similar numbers of gill rakers on the upper arch. For gill rakers on the lower arch, the hybrids and backcrosses either resembled the parent with the highest gill raker count, or they surpassed it.

Fluctuating asymmetry

Five of the six meristic traits exhibited fluctuating asymmetry, a symmetrical distribution of $d = l - r$ around a mean of zero (Graham *et al.* 2003). Only branchiostegal rays exhibited significant directional asymmetry ($t = 19.04$, $df = 1469$, $P < 0.0001$). Consequently, we treat asymmetry of branchiostegal rays separately as a directionally asymmetric trait. For the remaining five traits, there was no significant antisymmetry or size scaling of asymmetry. All six meristic traits, including branchiostegal rays, exhibited leptokurtosis (sequential Bonferroni test, $P < 0.05$) and four traits (PEL, BR, GRL, GRU) exhibited minor skewness (sequential Bonferroni test, $P < 0.05$).

If hybridization disrupts genomic coadaptation, fluctuating asymmetry should be greatest in the F2, followed by the backcrosses. None of the five bilateral traits were consistent with predictions (Figure 1.3). The composite index of fluctuating asymmetry (CFA2) also produced ambiguous results. The differences in CFA2 were not consistent with our predictions.

Phenodeviants

Although the frequency of phenodeviants was greatest in the F_2 (7%), and least in the two parentals (2.5% for Yct and 4.1% for Rbt), as predicted, the presence or absence of phenodeviants was independent of genotype (Chi-square = 4.626, *df* = 6, *P* = 0.593). The overall frequency of phenodeviants was 5.2%.

Morphometrics

The first three canonical variates explained 76.4% and 86.2% of the variance attributable to differences among genotypes, at 8 °C and 19 °C respectively. At both temperatures, the first canonical variate separated genotypes based on body depth and longitudinal compression of the head and the caudal region (Figure 1.4). In general, Rbt had greater body depth between the head and the caudal peduncle than Yct, and the differences were more pronounced at 19 °C than at 8 °C. Rainbow trout also had heads and caudal peduncles that were compressed longitudinally compared to Yct. The second canonical variate reflected shearing of the posterior 50-75% of the body axis (Figure 1.4). The five hybrid classes had dorsal and adipose fins that were set back posteriorly compared to the pelvic and anal fins. Yellowstone cutthroat trout had the most anterior dorsal fins. We did not attempt to interpret more than two canonical variates.

Discussion

Our data indicate differences in growth, meristic characters, and morphology occurred among Rbt, Yct, and five different hybrid crosses. These differences are associated with the proportion of the genome contributed by Rbt and Yct to each specific cross, direction of hybridization, and degree of introgression. We did not, however, detect consistent differences in developmental instability among crosses.

Two limitations in the experiment potentially hinder our ability to draw inferences from the results. First, two different Rbt stocks contributed to the crosses (Hayspur and Kamloops) and, second, only one Yct female parent produced viable eggs. Neither of these negates the relevance of our study when inferences are drawn from a species-level perspective, and our findings add to the understanding of how hybridization and introgression affects differences between Rbt and Yct.

The inclusion of two different Rbt stocks within the parental backgrounds was not known until after the experiment was completed. The Rbt parent source was from the Hayspur stock at Hayspur hatchery, ID, whereas the Rbt stock contained within the F_1 hybrid parents was the Kamloops stock, also from Hayspur hatchery. Consequently, the following crosses were composed of the following Rbt stocks: Rbt (100% Hayspur), BC-Rbt (50% Hayspur, 25% Kamloops), BC-Yct (25% Kamloops); F_1 hybrid (50% Hayspur), and F_2 hybrid (50% Kamloops). The Hayspur stock was founded from the McCloud River stock, CA, (coastal Rbt lineage), but included later additions from Mt. Lassen hatchery, CA, (also coastal lineage), and native Rbt from Big Wood River, ID (inland Rbt lineage) (Williams *et al.* 1996). Thus, the origin is coastal Rbt, but it might also include an inland Rbt contribution as well. Although the name "Kamloops" suggests the inland lineage of Rbt (Behnke 1992), the origin of the Kamloops stock at Hayspur hatchery is unclear. It apparently, however, includes a coastal Rbt component, because the Kamloops stock at Hayspur hatchery have allele frequency patterns characteristic of coastal Rbt rather than inland Rbt at the *LDH-B2** and *sSOD-1** loci (R. F. Leary, Montana Fish, Wildlife and Parks, personal communication). Allele frequency variation at the *LDH-B2** and *sSOD-1** loci reliably differentiate the coastal Rbt lineage from the inland Rbt lineage (Utter *et al.* 1980). Thus, the Kamloops and Hayspur stocks at Hayspur hatchery appear to share coastal Rbt origin.

Differences in development (Robison *et al.* 1999), growth (Tymchuk and Devlin 2005, Tymchuk *et al.* 2007) and morphology (Keeley *et al.* 2007) have been observed among some Rbt stocks, which if present between the Kamloops and Hayspur stocks would confound our results. However, to the best of our knowledge, similar studies between the stocks in question do not exist. However, at Hayspur hatchery noteworthy differences between the two stocks have not been observed (B. Dredge, Idaho Fish and Game, personal communication). In addition, mitochondrial DNA sequence variation suggests that *O. mykiss* and *O. clarki* diverged from a common ancestor approximately 3 million years ago (Wilson and Turner 2009). Thus, based on evolutionary history, one might expect greater observable differences between Rbt and Yct than between Rbt stocks. By focusing on a species perspective which assumes that differences between Rbt and Yct are indeed greater than those between the Kamloops and Hayspur stocks of Rbt from Hayspur hatchery, then we may still draw general inferences from the results.

Both limitations have the potential of affecting the genetic diversity within crosses, which can affect the variation of character traits within crosses, and ultimately the quality of the results. Thus, it may be expected that crosses derived from the single Yct female (Yct, F_1-Yct, and BC-Yct) should have reduced genetic diversity and character trait variation compared to their corresponding crosses consisting of three families (that is Rbt, F_1-Rbt, and BC-Rbt), and that the cross containing two Rbt stocks and the Yct stock (BC-Rbt) should have the highest genetic diversity and character trait variation. If this were the case, then there should be a pattern to the variances among crosses within Figures 1.2 – 1.5. That is, Yct, F_1-Yct, and BC-Yct should have consistently lower variance than Rbt, F_1-Rbt, and BC-Rbt, respectively, and BC-Rbt should have the highest variance. Upon inspection of Figures 1.2 – 1.5, no pattern is observed in the variances among crosses and neither expectation is supported. This suggests that increasing the genetic diversity in Yct (adding more Yct females) may not necessarily change our results. Additionally, despite the possibility of reduced variation stemming from the single female Yct, our findings for meristic counts and morphology in Yct and F_1-Yct hybrids are very similar to other published studies (Leary *et al.* 1985, Seiler and Keeley 2007), demonstrating that our results are in line with previously published data involving Rbt, Yct, and F_1 hybrids.

Our findings indicate that the proportions of the Rbt and Yct genome contributed to each cross played a major role in the observed growth rates. For example, at 19 °C Rbt grew larger during the experiment than BC-Rbt ($p = 0.09$), although marginally significant, which (in turn) grew larger than F1-Rbt ($p = 0.06$)

and F_2 hybrids ($p = 0.02$). Similar inter-specific hybridization studies do not exist for comparison, but intra-specific studies have demonstrated a similar relationship between growth rate and proportion of the parental genome among backcrosses and F_2 hybrids between different strains of Rbt (Tymchuk and Devlin 2005, Tymchuk *et al.* 2007) and coho salmon, *O. kisutch* (Tymchuk *et al.* 2006). Thus our results are compatible with other observations. We recognize that different hatchery stocks perform (grow) differently in common garden experiments which may not be indicative of true species differences. However, the results indicate that hybridization and introgression altered growth patterns in our study, and within our environmental conditions growth was associated with the proportion of Rbt and Yct genome present. We recommend (1) that future rainbow-cutthroat hybrid studies follow growth patterns from time of hatching to 1 – 2 years later, with periodic sampling, in order to determine how growth patterns change over time, and (2) that growth studies incorporate a line cross analysis in order to model genetic effects (additive and/or dominance interactions or epistatic effects) that may help explain mechanistic growth relationships among hybrids and parent species.

The environmental condition (temperature) may also have played a role in growth patterns. That is, the absence of significant growth rate differences among crosses at 8 °C but not at 19 °C may indicate that parental species and hybrids respond differently depending on temperature. For example, in a comparative study, Bear *et al*. (2007) identified that rainbow trout had a higher ultimate upper incipient lethal temperature than westslope cutthroat (24.3 °C and 19.6 °C, respectively), providing a potential mechanism for growth differences. However, Bear *et al*. (2007) also observed similar growth rates across a broad temperature range and similar optimum growth temperature for both species. Alternatively, significant differences in growth rate observed at the higher temperature within our study may not have accumulated yet at the lower temperature simply because growth was much slower.

The variation in meristic traits observed in our study is in general agreement with Leary *et al*. (1985) for the parental and F_1 generations (although they did not examine F_2 and backcross generations). All hybrid crosses exhibited a diverse, complex mixture of likely additive, dominance, overdominance, maternal, and (probably) epistatic genetic effects. Moreover, meristic traits appeared to be differentially effected by recombination between the Rbt and Yct genomes. For example, F_2 hybrids demonstrated the highest gill raker counts and lowest pectoral ray counts, indicating that recombination may increase or depress meristic character trait counts depending on the trait. Additionally, genomic contribution of the parental taxa also appears to play a role in meristic traits, which is demonstrated by the additive genetic effects acting on mandibular pore counts. The large amount of variation among crosses and the complexity of genetic effects observed suggest that no single model describes how meristic traits respond to introgression. Since there is no direct comparison for F_2 and backcross generations with Leary *et al*. (1985), it is difficult to conclude how our design limitations may have affected meristic traits. However, most individual meristic traits in our study exhibit a pattern among crosses (Figure 1.3), suggesting consistency within our results. We recommend repeating the experiment with more families and a single Yct and Rbt stock to validate our results.

Though F_2 and F_1-Yct had greater composite fluctuating asymmetry than F_1-Rbt hybrids, none of the hybrids had greater composite fluctuating asymmetry than the parental generations. This result stands in contrast to those of Leary *et al*. (1985), who found greater asymmetry for the same meristic traits in F_1 hybrids. The difference between the two studies may be a result of the strains used; Leary *et al*. (1985) used a hatchery strain of Yct from McBride Lake, Wyoming, and a hatchery strain of Rbt from Willow Creek Reservoir, Montana, or due to our experimental design limitations. The effect of two Rbt strains in our experiment might be expected to cause high levels of fluctuating asymmetry in the BC-Rbt since this was the only cross with recombination among three different genomes (two Rbt genomes and a Yct genome). However, BC-Rbt were not different from Rbt and Yct nor were F_2 hybrids, which exhibit the greatest potential for recombination between the Rbt and Yct genomes, suggesting that inclusion of two Rbt strains did not increase fluctuating asymmetry. We conclude that hybridization between Rbt and Yct did not disturb development within our study. Further experiments incorporating larger family sizes, backcrosses, and F_2 hybrids are warranted to determine how recombination affects fluctuating asymmetry in introgressed trout.

Our morphometric analysis of shape agreed with that of Seiler and Keeley (2007), who hybridized Hayspur Rbt and Yct from the Henry's Lake, but only through the F_1 generation. Rainbow trout and Yct were differentiated by body depth, head size, and caudal peduncle length. Studies on wild rainbow-cutthroat hybrids suggest that morphological variation appears to be influenced by the level of hybridization and introgression (Kennedy *et al.* 2009, Seiler *et al.* 2009). Indeed, our results using known crosses demonstrate that morphological variation is apparent among different hybrid crosses. Interestingly, although body depth, head size and caudal length was intermediate in the hybrids compared to Rbt and Yct, the dorsal and adipose fins in all our hybrid crosses were set back posteriorly with respect to the other fins. The similarity in fin positions among hybrid crosses suggests that the use of a single Yct female and inclusion of two Rbt strains may not have affected our results. The mechanistic processes involved and consequences of the dorsal and adipose fin positions in our hybrids with respect to body morphology are unknown and warrant further investigations.

Much debate has focused on acceptable levels and potential consequences of Rbt introgression within wild cutthroat trout populations, primarily within westlsope cutthroat trout (Allendorf *et al.* 2004, Allendorf *et al.* 2005, Campton and Kaeding 2005). The debate has centered on the fact that there is a limited amount of scientific data describing how introgression affects morphology, behavior, ecology, and life history of cutthroat trout. After considering the available scientific data, USFWS (2003) concluded that westlsope cutthroat trout populations may contain as much as 20 percent Rbt admixture while phenotypically conforming to the scientific, taxonomic description of pure westslope cutthroat trout. Recently, Muhlfeld *et al*. (2009) reported that fitness in westslope cutthroat trout declined rapidly with increasing Rbt admixture. They observed that fitness declined by nearly 50 percent with only 20 percent Rbt admixture, suggesting that low levels of Rbt introgression may adversely affect westslope cutthroat trout populations. Although our study clearly demonstrates that growth and morphology are effected by 25 percent admixture (first generation backcrossing), it does not add directly to the 20 percent threshold debate since none of our introgressed crosses were admixed less than 20 percent.

Our study does, however, indirectly add to the 20 percent threshold debate. For example, we observed that first generation backcrosses were intermediate to their parent types (pure species and F_1) on CV1 (Figure 1.5), consistent with additive genetic variance playing a role in body depths and longitudinal compression of the head and caudal regions. With additive genetic variation a second generation backcross (12.5 percent admixture) would be morphologically intermediate to a first generation backcross and pure species for these traits rather than morphologically identical to a pure species. Admittedly, the BC-Yct in our study was represented by a single family. However, the same trends were observed within the BC-Rbt (3 families) indicating consistency of our data regardless of backcrossing species. That is, introgression has a similar effect regardless of backcrossing direction. Furthermore, the distortion observed in the posterior region in all hybrid crosses on CV2 compared to the parent types also indicate significant morphological differences between hybrids and parents types which could also be reflected in additional backcross generations. Additional experiments that would provide salient data for the 20 percent threshold debate include repeating our experiment with more families and second generation backcrosses, conducting morphological and meristic comparisons among pure species and introgressed populations that differ in percent admixture (including populations that are less than 20 percent admixed), and comparing morphology and hybrid indices among individuals within hybridized populations.

Introgressive hybridization with introduced rainbow trout is a conservation concern for indigenous cutthroat trout (Allendorf and Leary 1988). However, scientific information is needed to provide resource managers with guidance and tools for managing introgressed populations. Although the literature is ripe with genetic surveys that provide metrics of introgressive hybridization within populations, there is a dearth of information describing the effects of introgression on fitness-related traits such as growth, development, physiology, behavior, and morphology. Our findings indicate that hybrids are not equal, introgression affects traits differentially, and growth and development differ depending on the genomic contribution from each parent species as well as the hybrid generation. Our findings also provide starting points for future studies.

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Tables

Table 1.1. Factorial mating design used to construct seven different test crosses. The factorial mating design was repeated five times using different sires and dams each time. A dashed line indicates a cross that was not constructed.

	Rbt_{Sire}	F_{1Sire}	Yct_{Sire}
Rbt_{Dam}	Rbt	BC-Rbt	F_1-Rbt
F_{1Dam}	-	F_2	-
Yct_{Dam}	F_1-Yct	BC-Yct	Yct

Table 1.2. Associated *p*-values for pairwise comparison between crosses for each meristic trait. PEC = rays in the pectoral fins; PEL = rays in the pelvic fins; BR = branchiostegal rays; MP = mandibular pores; GRL = gill rakers on the lower first gill arch; and GRU = gill rakers on the upper first gill arch. Bold values are judged as being significantly different from zero at $\alpha = .05$, after correcting for multiple comparisons.

PEC PEL	Rbt	BC-Rbt	F1-Rbt	F2	F1-Yct	BC-Yct	Yct
Rbt		0.93	**<0.01**	0.42	0.92	0.70	0.42
BC-Rbt	0.83		**<0.01**	**<0.01**	0.75	0.89	0.85
F1-Rbt	0.81	0.09		**<0.01**	**<0.01**	0.20	0.06
F2	**<0.01**	**<0.01**	**<0.01**		0.91	**<0.01**	**<0.01**
F1-Yct	**<0.01**	0.28	**<0.01**	**<0.01**		0.20	0.07
BC-Yct	**<0.01**	**<0.01**	**<0.01**	0.95	**<0.01**		0.99
Yct	**<0.01**	**<0.01**	**<0.01**	0.99	**<0.01**	0.95	

BR MP	Rbt	BC-Rbt	F1-Rbt	F2	F1-Yct	BC-Yct	Yct
Rbt		0.83	0.11	0.97	0.99	0.97	**<0.001**
BC-Rbt	0.002		0.75	**<0.001**	0.92	**<0.001**	**<0.001**
F1-Rbt	**<0.001**	**<0.001**		**0.005**	0.22	**0.008**	**<0.001**
F2	**<0.001**	**<0.001**	0.81		0.95	0.99	**<0.001**
F1-Yct	**<0.001**	**<0.001**	0.69	0.99		0.96	**<0.001**
BC-Yct	**<0.001**	**<0.001**	0.10	0.60	0.23		**<0.001**
Yct	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	

GRL GRU	Rbt	BC-Rbt	F1-Rbt	F2	F1-Yct	BC-Yct	Yct
Rbt		0.16	0.41	**<0.01**	**<0.01**	0.19	0.83
BC-Rbt	0.51		0.99	**<0.01**	0.99	0.99	0.95
F1-Rbt	0.99	0.82		0.24	0.99	0.99	0.89
F2	0.10	0.43	0.19		0.46	**<0.01**	0.11
F1-Yct	0.99	0.99	0.99	0.82		0.99	0.97
BC-Yct	0.89	0.99	0.94	0.99	0.55		0.90
Yct	0.99	0.99	0.99	0.72	0.99	0.23	

Figures

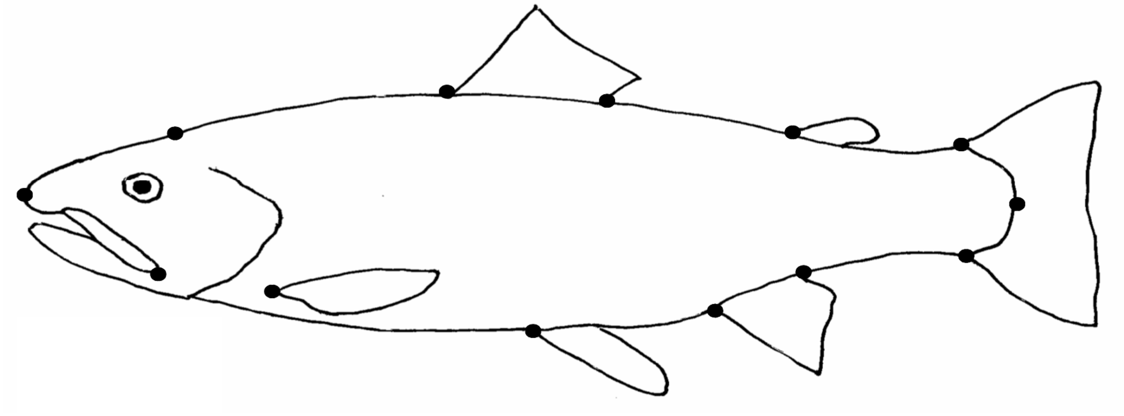

Figure 1.1. Locations of landmarks used to analyze morphometric differences among rainbow trout, Yellowstone cutthroat trout, and five hybrid crosses.

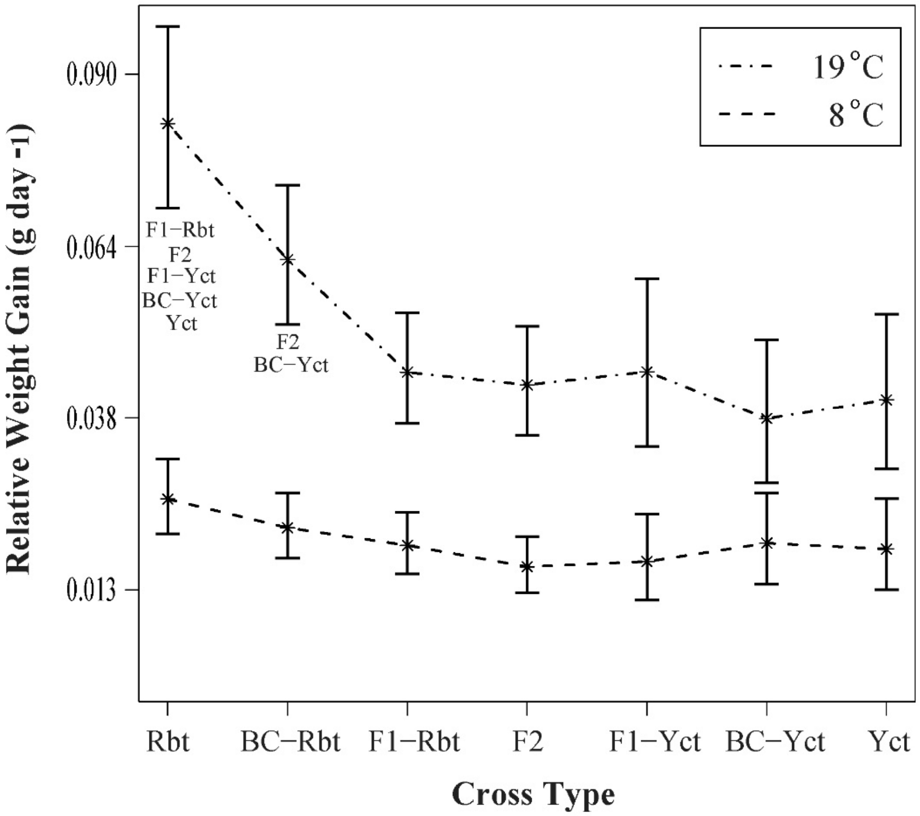

Figure 1.2. Relative weight gain per day (g d^{-1}) for rainbow trout, Yellowstone cutthroat trout, and five hybrid crosses reared separately at two different temperatures and 95% confidence intervals. Labels below estimates at 19 °C indicate crosses that were significantly different after correcting for multiple pairwise comparisons.

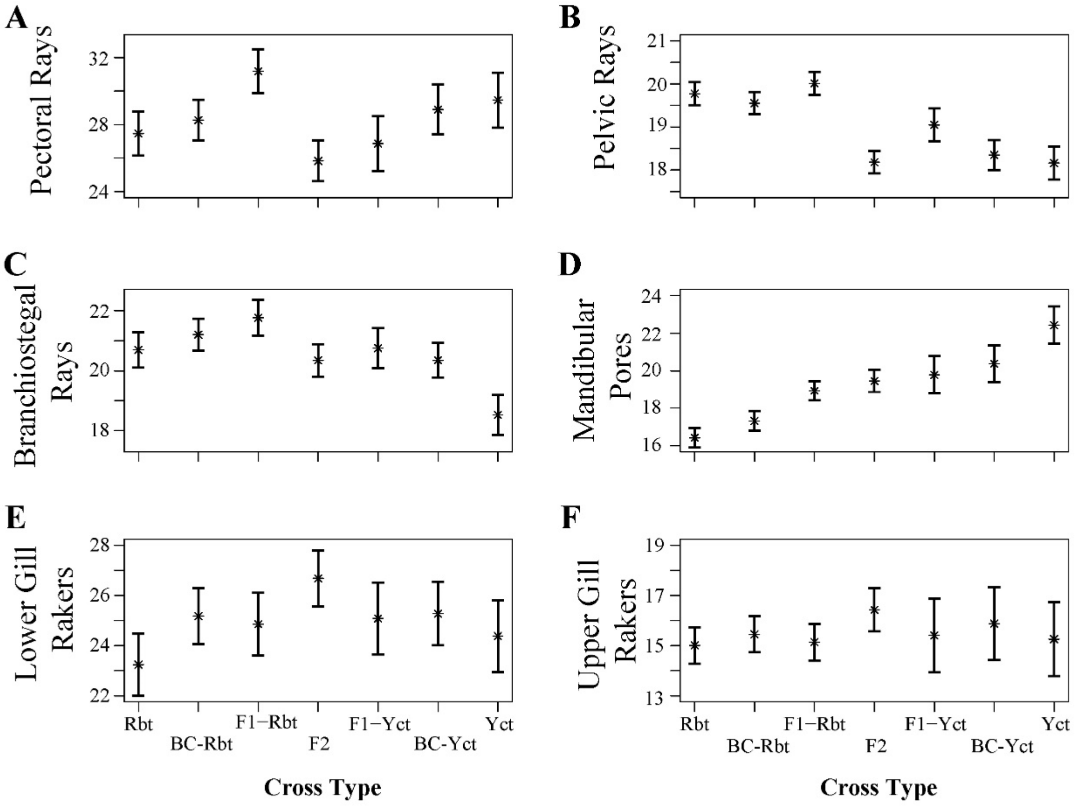

Figure 1.3. Trait means and 95 % confidence intervals for six meristic traits of rainbow trout, Yellowstone cutthroat trout, and five hybrid crosses. The Y-axis represents the count for each meristic trait. A = rays in the pectoral fins; B = rays in the pelvic fins; C = branchiostegal rays; D = mandibular pores; E = gill rakers on the lower first gill arch; and F = gill rakers on the upper first gill arch.

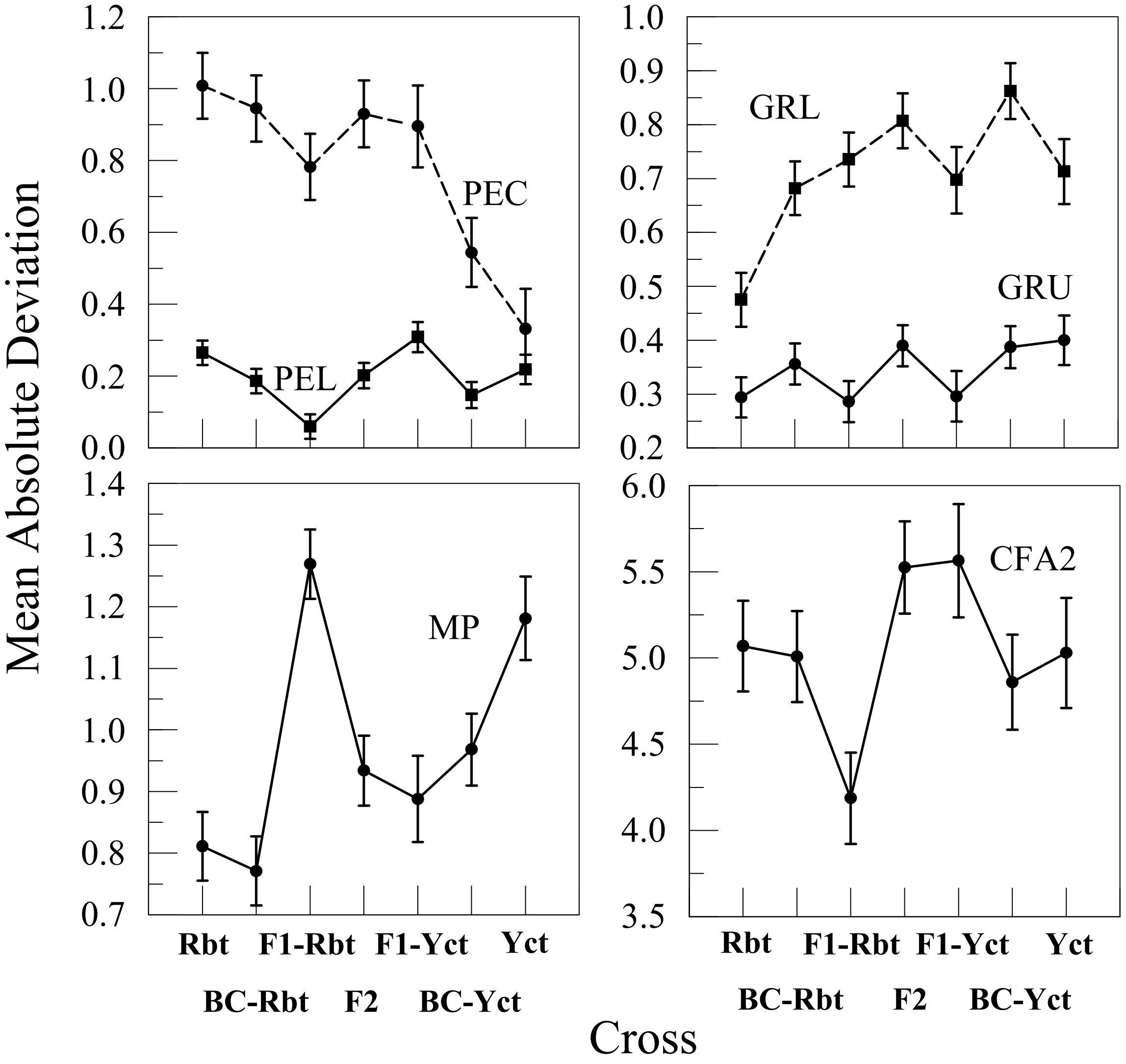

Figure 1.4. Mean absolute deviations |*d*| and standard errors for fluctuating asymmetry of five meristic traits and composite fluctuating asymmetry (CFA2) for those same five meristic traits. PEC = rays in the pectoral fins; PEL = rays in the pelvic fins; MP = mandibular pores; GRL = gill rakers on the lower first gill arch; and GRU = gill rakers on the upper first gill arch.

Figure1.5A.

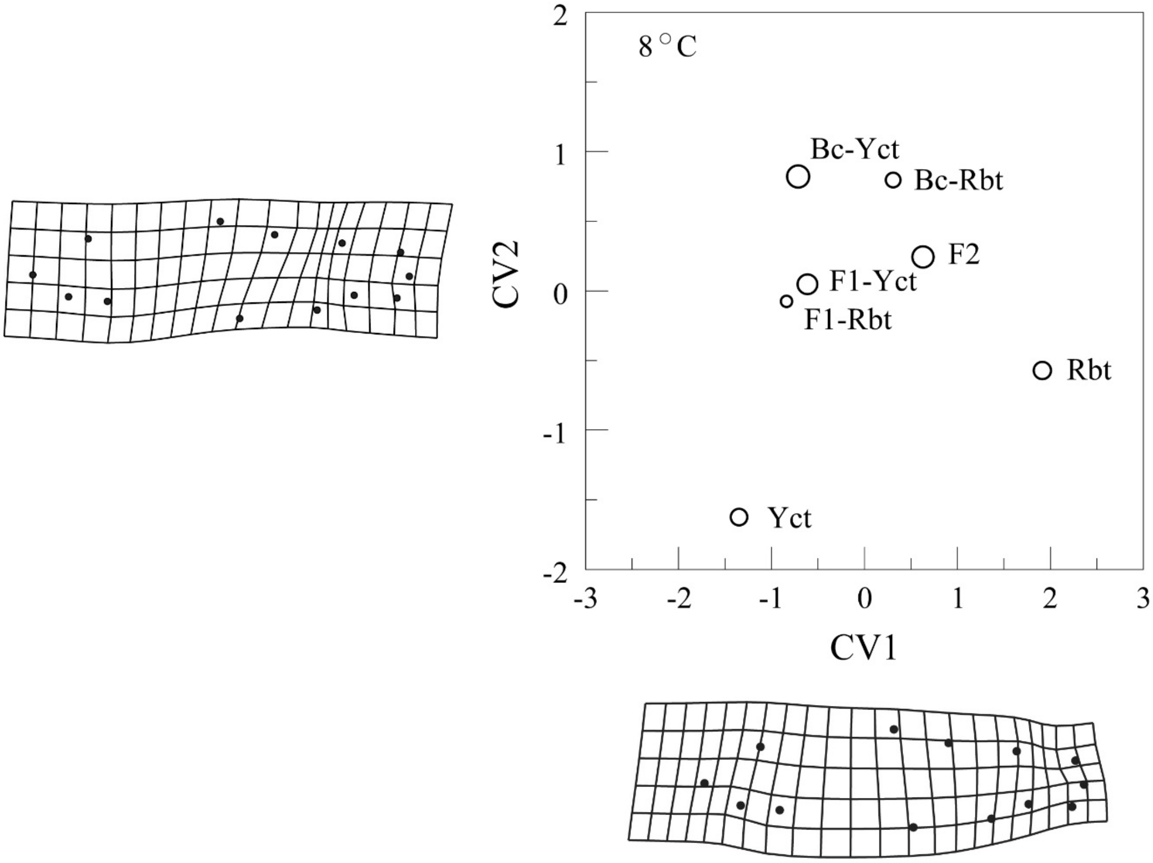

Figure 1.5. Canonical variates analysis on 22 shape variables (partial warp scores) at 8 °C (Figure 1.5A) and 19 °C (Figure 1.5B). At 8 °C the variation was 42.3% on axis 1 and 19.5% on axis 2. At 19 °C the variation was 61.0 % on axis 1 and 14.7% on axis 2. Each point shows the group mean on that axis. The size of the point is proportional to the within group variance. Thin-plate splines on each axis show the deformation of the reference grid implied by regressing shape on the canonical variate axis scores for that axis.

Figure 1.5B.

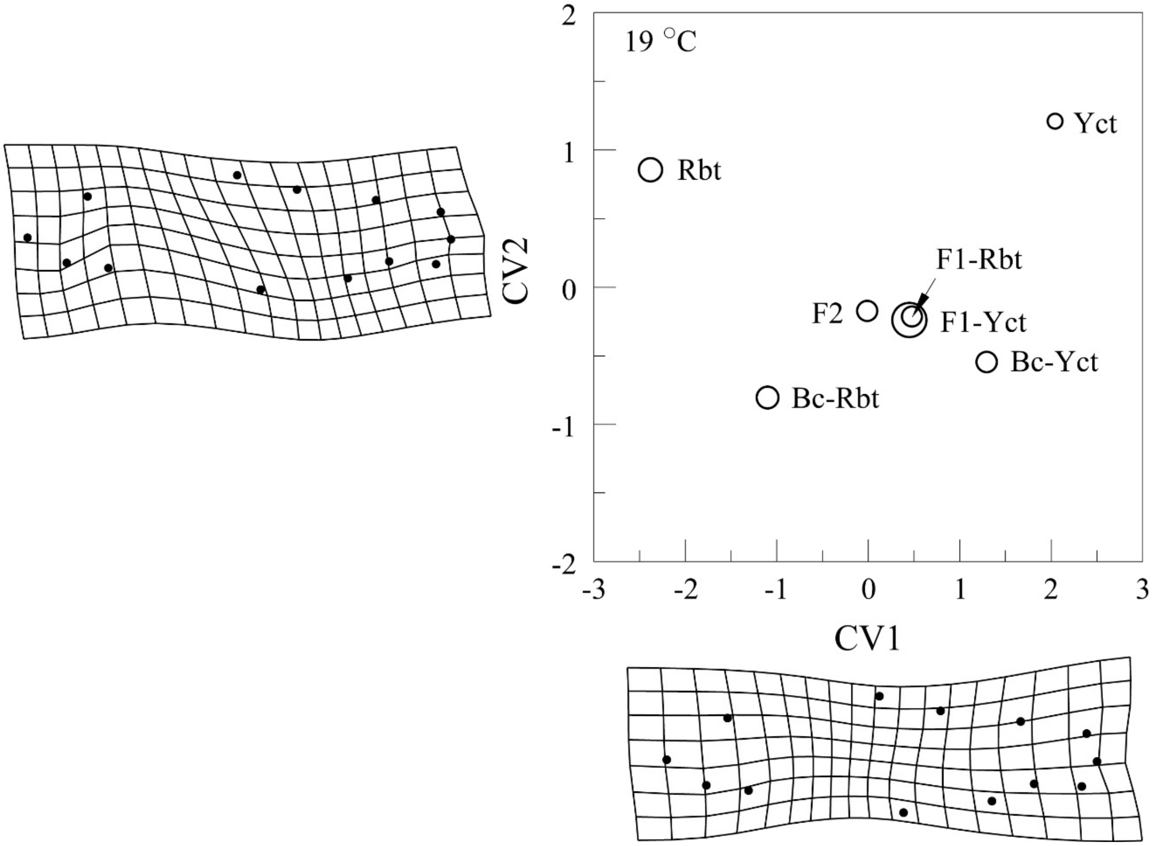

Figure 1.5 (continued). Canonical variates analysis on 22 shape variables (partial warp scores) at 8 °C (Figure 1.5A) and 19 °C (Figure 1.5B). At 8 °C the variation was 42.3% on axis 1 and 19.5% on axis 2. At 19 °C the variation was 61.0 % on axis 1 and 14.7% on axis 2. Each point shows the group mean on that axis. The size of the point is proportional to the within group variance. Thin-plate splines on each axis show the deformation of the reference grid implied by regressing shape on the canonical variate axis scores for that axis.

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Chapter 2: Hybridization between Yellowstone cutthroat trout and rainbow trout alters the expression of muscle growth-related genes and their relationships with growth patterns

Abstract

Hybridization creates novel gene combinations that may generate important evolutionary novelty, but may also reduce existing adaptation by interrupting inherent biological processes, such as genotype-environment interactions. Hybridization often causes substantial change in patterns of gene expression, which, in turn, may cause phenotypic change. Rainbow trout (*Oncorhynchus mykiss*) and cutthroat trout (*O. clarkii*) produce viable hybrids in the wild, and introgressive hybridization with introduced rainbow trout is a major conservation concern for native cutthroat trout. The two species differ in body shape, which is likely an evolutionary adaptation to their native environments, and their hybrids tend to show intermediate morphology. The characterization of gene expression patterns may provide insights on the genetic basis of hybrid and parental morphologies, as well as on the ecological performance of hybrids in the wild. Here, we evaluated the expression of eight growth-related genes (MSTN-1a, MSTN-1b, MyoD1a, MyoD1b, MRF-4, IGF-1, IGF-2, and CAST-L) and the relationship of these genes with growth traits (length, weight, and condition factor) in six line crosses: both parental species, both reciprocal F1 hybrids, and both first-generation backcrosses (F1 x rainbow trout and F1 x cutthroat trout). Six of these genes were differentially expressed among rainbow, cutthroat, and their hybrids. Transcript abundance of several genes was significantly correlated with growth traits across the parent species, but not across hybrids. Comparison of transcript abundance between backcrosses and their backcrossing parent species indicated that genotype as well as genomic background affected gene expression. Our findings suggest that rainbow and cutthroat trout exhibit differences in muscle growth regulation, that transcriptional networks may be modified by hybridization, and that hybridization disrupts intrinsic relationships between gene expression and growth patterns that may be functionally important for phenotypic adaptations.

Introduction

Hybridization creates a diversity of novel gene combinations that can have profound evolutionary consequences, including acting as a foundation for adaptive genetic variation, causing shifts in genetic traits that underlie phenotypic variation, and influencing hybrid fitness (Rieseberg *et al.* 2000, Seehausen 2004). Novel gene combinations may influence these evolutionary outcomes by disrupting transcriptional networks, which, in turn, may affect functional and regulatory mechanisms that control transcription, translation, and/or phenotype (Rockman and Kruglyak 2006, Baack and Rieseberg 2007, Landry *et al.* 2007). Such disruption could occur if parental species have evolved different transcriptional regulation mechanisms. When divergent regulatory elements are combined in a hybrid background, transcription may become disrupted (Landry *et al.* 2007), producing extreme expression phenotypes that lie outside the range found in parent populations (Brem and Kruglyak 2005). These extreme phenotypes may provide diversity for rapid adaptation, or alternatively, be maladaptive (Rieseberg *et al.* 1999, Seehausen 2004). Novel gene combinations also affect the mode of transcription regulation inheritance. Hybrids display additive, dominant, and nonadditive (over- and underdominance) modes of inheritance for transcription regulation (Gibson *et al.* 2004, Roberge *et al.* 2008, Renaut *et al.* 2009). However, the same gene may show different modes of inheritance for transcription regulation within and between hybrid generations (Renaut *et al.* 2009), indicating that inheritance patterns in hybrids are unpredictable (Bougas *et al.* 2010). Thus, hybridization may cause substantial changes in gene expression with wide-ranging evolutionary implications.

Hybridization also has the potential to reduce biodiversity through loss of distinct gene pools and genetic homogenization (Rhymer and Simberloff 1996). For example, widespread introduction of non-native rainbow trout (*Oncorhynchus mykiss*) into cutthroat trout (*O. clarkii*) habitats and consequent introgressive hybridization has resulted in loss of native cutthroat trout throughout their range (Allendorf and Leary 1988, Behnke 1992). Rainbow and cutthroat trout are sister species and shared a common ancestor approximately 3 million years ago (Wilson and Turner 2009). Because rainbow-cutthroat hybrids are viable and fertile, native cutthroat trout populations are often replaced by hybrid swarms following rainbow trout introductions, causing concern for cutthroat trout conservation (Allendorf and Leary 1988).

Rainbow and cutthroat trout are morphologically divergent: rainbow trout are generally robust whereas cutthroat trout are typically more slender, and their hybrids tend to be morphologically intermediate (Hawkins and Quinn 1996, Seiler and Keeley 2007, Ostberg *et al.* 2011). These morphological differences have been associated with swimming performance, and higher sustained swimming activity of rainbow trout and hybrids might afford them a competitive advantage over cutthroat trout (Hawkins and Quinn 1996, Seiler and Keeley 2009). Because muscle comprises the bulk of body mass and muscle growth represents change in length, weight, and morphology (Mommsen and Moon 2001), the identification of mechanisms contributing to muscle growth patterns could provide insight into the underlying genetic basis of the difference between rainbow and cutthroat trout body shapes and its manifestation in their hybrids.

Muscle growth and development, myogenesis, is a highly integrated and complex process requiring a suite of gene products (Johnston *et al.* 2011). The myogenesis program involves specification of stem cells into myoblasts followed by the proliferation and terminal differentiation of myoblasts. Terminally differentiated myoblasts have two fates: they can either fuse with other myoblasts and form myotubes or fuse with existing myotubes, resulting in an increase in cell number (hyperplasia), or they can be absorbed into muscle fibers, resulting in an increase in cell size (hypertrophy). In vertebrates with indeterminate growth, such as salmonids, both pre- and post-natal muscle growth occurs through hyperplasia and hypertrophy. In addition, muscle growth is influenced by the countering effects of protein synthesis and degradation, and, ultimately, the expression of genes that regulate these processes (Mommsen and Moon 2001). Therefore, differences in body morphology between rainbow and cutthroat trout could be maintained, in part, through the regulation of muscle growth-related genes.

Our objectives were to characterize expression patterns of muscle growth-related genes and describe relationships between gene expression and growth patterns (as inferred by length, weight, and condition factor) among rainbow trout, cutthroat trout, and their hybrids. We produced F1 and backcross hybrids, in addition to parental crosses, using rainbow trout (Rbt) and Yellowstone cutthroat trout (*O. c. bouvieri*) (Yct), because body morphology has been well characterized in these species and their hybrids (Seiler and Keeley 2007, Ostberg *et al.* 2011). We targeted eight muscle growth-related genes: two myostatin orthologs, MSTN-1a and MSTN-1b, both of which appear to negatively regulate muscle growth (Roberts *et al.* 2004); three myogenic regulatory factors, the paralogs MyoD1a and MyoD1b (Macqueen and Johnston 2008), and MRF-4, which are transcription factors that act at different stages within the myogenesis program (Watabe 2001, Wang *et al.* 2008); two insulin-like growth factors , IGF-1 and IGF-2, which stimulate protein synthesis within muscle as well as cellular proliferation and differentiation within the myogenesis program (Fuentes *et al.* 2013); and calpastatin, CAST-L (long isoform), which plays a role in muscle protein turnover by inhibiting the calpain proteases that degrade muscle proteins (Goll et al. 2003). We posit that the differences in body shape among Rbt and Yct is a consequence of evolutionary differences in muscle growth regulation between the species. Therefore, we expected to find differential expression of growth related-genes among Rbt, Yct, and their hybrids, and that these differences in expression would be associated with growth patterns.

Materials and Methods

Experimental cross design and sample collection

We generated six experimental crosses to evaluate the expression of growth-related genes and growth patterns among rainbow, cutthroat, and their hybrids. The six crosses consisted of: Rbt, first generation Rbt backcross (bc-Rbt; Rbt female x F1 male), reciprocal F1 hybrids (F1-Rbt; Rbt female x Yct male, and F1-Yct; Yct female x Rbt male), first generation Yct backcross (bc-Yct; Yct female x F1 male), and Yct. The F1 male parents used to generate the backcrosses were Rbt female x Yct male. Gametes were collected from male and female Rbt (Hayspur stock), Hayspur Hatchery, Idaho Department of Fish and Game (IDFG), male and female Yct from Henry's Lake Fish Hatchery and Fish Management Station (IDFG), and male F1 Rbt-Yct broodstock developed from the Rbt and Yct stocks described above at Western Fisheries Research Center, US Geological Survey, Seattle, WA. Fin tissue was collected from each parent. Because fertilization success varied among crosses (possibly due to due to reduced egg quality in Yct compared to Rbt), three families of Rbt, bc-Rbt, and F1-Rbt, and two families of Yct, bc-Yct, and F1-Yct developed.

After fertilization, each family was incubated in a randomly assigned Heath tray at 9 ^{o}C and, at 55 days post-fertilization, transferred to a randomly assigned 275-L flow-through tank. Fish were culled at 93 days post-fertilization in order to equalize the number of fish across tanks (85 fish per tank). At this time, and during the course of the experiment, we applied the same feed ration to each tank following Westers' (2001) recommended feeding regime. At 119 days post-fertilization, each family within a cross was differentially marked with a specific fin clip, so that families could be combined within cross and be easily identified during sampling. Families within cross were combined into randomly assigned 275-L flow through tanks and acclimated to 16 ^{o}C.

At 145, 234, and 327 days post-fertilization we sampled white muscle from nine fish per tank and recorded the weight (g) and fork length (mm) of each fish. Food was withheld 48 hours prior to each sampling day. Sampling was equalized among families within cross; three individuals per Rbt, bc-Rbt, and F1-Rbt family, and four and five individuals per Yct, bc-Yct, and F1-Yct family were sampled per time point. Muscle tissue was flash-frozen at -80 ^{o}C until RNA extraction. Fish were handled and treated according the guidelines of the University of Washington's Institutional Animal Care and Use Committee (protocol# 4153-02).

RNA extraction and cDNA synthesis

Total RNA was isolated from the nine individuals sampled per cross per time point (145, 234, and 327 days post-fertilization) using RNeasy kits (Qiagen) following the manufacture's recommendations with in-column DNase treatment. RNA was eluted in 50 µl of RNase-free water, quantified, and stored at -80 ^{o}C. cDNA synthesis was performed using 1 µg of RNA with 1X RQ1 Buffer (Promega), 1 unit RNasin (Promega), 0.25 units DNase I (Promega), and incubation at 37 ^{o}C for 30 minutes followed by 70 ^{o}C for 5 minutes. Next, 125 ng each of oligo (dt)15 and random hexamer primers (Promega) were added and annealed at 70 ^{o}C for 5 minutes, followed by 2 minutes on ice. Finally, 1mM dNTP (Promega), 1.25X RT buffer (Promega), 1 unit RNasin (Promega), and 100 units of M-MLV reverse transcriptase (Promega) were added and incubated at 42 ^{o}C for 60 minutes, followed by 70 ^{o}C for 15 minutes. All cDNA was stored at -20 ^{o}C.

TaqMan assay design

Partial gene sequences for muscle growth-related genes were isolated from each parental fish (Rbt, Yct, and F1 hybrids) that was used to generate the experimental crosses in order to design Taqman assays at gene sequences conserved between parental species. Designing Taqman assays at the conserved gene regions eliminated inter-specific sequence variation as a source for differential estimates of gene expression. DNA from each parent was extracted from fin tissues using DNeasy kits (Qiagen). We designed sequencing primers for eight muscle growth-related genes (target genes) and one reference gene (β-actin) using sequences obtained from GenBank (Table1) and Primer3 v.0.4.0 (http://frodo.wi.mit.edu/). Each parent was sequenced with each primer set. PCR amplifications were performed in 20 µl reaction volumes, consisting of 15 ng genomic DNA, 1X NH4 Reaction Buffer (Bioline), 1.5 – 2.5 mM $MgCl_2$, 200 µM each dNTP (Bioline), 150 nM of each primer, and 0.5 units Taq (Bioline). Cycling conditions consisted of 95 ^{o}C for 2 minutes, followed by 35 cycles of 95 ^{o}C for 15 seconds, anneal for 45 seconds (58 ^{o} – 68 ^{o}C, Table 2.1), and 72^{o}C for 1 minute. PCR products were sequenced using a 3730xl DNA Analyzer (Applied Biosystems Inc) and sequences were edited and aligned using SEQUENCHER v 4.10.1 (Gene Codes Corporation).

Gene regions conserved between Rbt and Yct parents were identified and TaqMan assays within these conserved regions were designed using Primer Express v 2.0.0 (Applied Biosystems Inc) (Table 2.2). Primer and probe sequences were BLASTed against the GenBank Rbt nucleotide collection to ensure homology to a single gene in the duplicated salmonid genome. Probe sequences had a minimum of three nucleotide mismatches with non-target Rbt genome sequences; with the exception of MSTN-1b in which the probe had two nucleotide mismatches with MSTN-1a (both the forward and reverse MSTN-1b primers also had two base mismatches with MSTN-1a).

Real-time PCR and data analysis

Quantitative PCRs (qPCR) were performed using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc), applying standard cycling conditions consisting of 50 °C for 2 minutes, 95 °C at 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. Reactions were performed in duplicate in 12 µl volumes containing 1X ABI Universal PCR Master Mix (Applied Biosystems Inc), 900 nM forward primer, 900 nM reverse primers, 200 nM probe, and 5 µl total cDNA. Fluorescent threshold for the determination of threshold cycle (C_T) values was set manually in the exponential phase of amplification. Internal controls were included with each run and consisted of no cDNA amplification controls (no reverse transcriptase or RNA added in reverse transcription reactions) and no template controls (water in place of cDNA template in qPCR).

The suitability of three potential reference genes, β-actin, acidic ribosomal phosphoprotein P0 (ARP), and 18s ribosomal RNA (18s) was assessed using BestKeeper (Pfaffl *et al.* 2004). Across the 162 sample data set, C_T values were highly correlated between reference genes (ARP/β-actin, r = 0.77; ARP/18s, r = 0.80; and β-actin /18s, r = 0.73). We considered ARP and β-actin as the most stable reference genes.

Data were normalized by calculating ΔC_T, the difference in C_T values between the target gene and the geometric mean for the reference genes. As transcript abundances have a negative logarithmic relationship with ΔC_T values, we estimated gene expression by applying $1/\mathrm{Log}(\Delta C_T)$. This method allowed the same transcript abundance estimate to be used for statistical testing within and among time points.

Genotyping backcrosses

We genotyped backcrossed individuals to evaluate how genotype affected gene expression. Backcrosses were genotyped at three genes only (IGF-2, MyoD1b, and CAST-L) because the sequence analysis in the parents revealed inter-specific single nucleotide polymorphisms within exons at these genes. Genotyping was accomplished by using primers in Table 2.1 and direct sequencing of PCR products (CAST-L, SNP at base pair 31, accession # KC609003), restriction enzyme digest of amplicon (MyoD1b/*Bsa*JI, SNP at base pair 319, accession # KC609011), or by applying the methods of Baker *et al*. (2002) (IGF-2, SNP at base pair 280, accession # KC609007). Genotyped bc-Rbt were categorized as homozygous for the Rbt allele (bc-Rbt$_{(RR)}$) or heterozygous for Rbt and Yct alleles (bc-Rbt$_{(RY)}$). Genotyped bc-Yct were categorized as homozygous for the Yct allele (bc-Yct$_{(YY)}$) or heterozygous for Rbt and Yct alleles (bc-Yct$_{(RY)}$).

Statistical analyses

We used weight, length, and condition factor, a weight-length relationship that describes relative body shape, as growth traits to compare differences in growth patterns among crosses. Condition factor was used as a proxy for body shape and was calculated as $K = \frac{W}{L^3} \times 100{,}000$; where K = condition factor, W = weight in grams, and L = fork length in millimeters for each cross within time point. ANOVA was used to test for differences in weight, length, and condition factor among crosses within each time point, and Tukey's post-hoc procedure was used to identify pairwise differences.

We applied the non-parametric Kruskal-Wallis test using *kruskal.test* in R v3.1.0 (R Development Core Team, www.R-project.org), followed by Dunn's post-hoc procedure to identify differences in transcript abundance among crosses within time point and within cross among time points. A discriminant function analysis was used to summarize differences in transcript abundance across the eight genes among the crosses using the MASS package in R (Venables and Ripley 2002). Transcript abundance was standardized across all crosses within genes (i.e. was centered to a mean of zero and a variance of one) prior to discriminant function analysis. The contribution of each gene to the separation among crosses was evaluated by calculating the Spearman rank correlation coefficient (r_s) between transcript abundance and canonical variates using *cor.test* in R.

Spearman's rank correlation was also used to test for associations between transcript abundance and trait (weight, length, condition factor, and the percent Yct genome within individuals) across parent species and across all hybrids at each time point. The percent Yct genome was defined as 0% in Rbt, 25% in bc-Rbt, 50% in both F1 hybrid crosses, 75% in bc-Yct, and 100% in Yct. We examined whether associations were conserved between parental species and hybrids by comparing the parental and hybrid correlation coefficients. For each transcript abundance and trait correlation that was significant in parents, hybrids, or both, we tested for a difference between the parental and hybrid r_s using the equation $Z = (z_p - z_h)/(\sigma_{zp} - \sigma_{zh})$ (Zar 1999); where Z is the normal deviate, z_p and z_h is the normalized transformation of parental and hybrid r_s values, respectively, and σ is the standard error. One-tailed critical values were applied to each test.

Differences in transcript abundance among genotyped individuals were identified by applying the Mann-Whitney test using R v3.1.0. We tested whether expression differed between homozygous and heterozygous genotypes within backcrosses (for example, between bc-Rbt$_{(RR)}$ and bc-Rbt$_{(RY)}$ genotypes) and whether gene expression in heterozygous and homozygous genotypes within backcross differed from the backcrossing parent (for example, between Rbt and bc-Rbt$_{(RR)}$, and between Rbt and bc-Rbt$_{(RY)}$). Testing was performed only at the time points where expression differed significantly between Rbt and Yct.

The false discovery rate (B-Y FDR) procedure (Benjamini and Yekutieli 2001) was applied to correct for multiple tests and test results were considered as significant only when P exceeded the B-Y FDR adjusted critical value, α, for each series of multiple tests.

Results

Weight, length, and condition factor among crosses

Weight and length were highly correlated across individuals ($r = 0.947$, $P < 0.001$), but neither length nor weight differed significantly among crosses within each time point (Table 2.3). Condition factor differed among crosses at each time point (145 days, $F_{5, 48} = 8.955$, $P < 0.01$; 234 days, $F_{5, 48} = 21.532$, $P < 0.001$; 327 days, $F_{5, 48} = 14.125$, $P < 0.001$). Rbt had higher condition factor than Yct within each time point. Condition factor differed between bc-Rbt and bc-Yct at 234 and 327 days post-fertilization, but neither backcross differed from their respective backcrossing parent. F1-Rbt had significantly higher condition factor than F1-Yct at 145 days, but not at other time points.

Differences in gene expression among crosses

More than 70% of the variation in transcript abundance across the eight genes was explained by the first and second canonical variates (CV) (Figure 2.1). The parental species were separated at 234 and 327 days, and marginally separated at 127 days. On the first canonical variate (CV1), hybrids were intermediate between parental species at 234 days, but appeared to be outside the range of parental species at other time points. On CV2, hybrids were intermediate between parental species at the earliest time point, but were outside the range of parental species at later time points. At 145 days post-fertilization, IGF-2 and MRF-4 contributed the most to the separation among crosses on CV1 and MyoD1b contributed the most to the separation on CV2 (Table 2.4). At 234 days, separation among crosses on CV1 was mainly influenced by CAST-L and the separation on CV2 was mostly influenced by IGF-2. At 327 days, MSTN-1a and MSTN-1b contributed the most to the separation on CV1 and the separation on CV2 was mostly influenced by CAST-L.

Six genes were differentially expressed among crosses following the B-Y FDR adjustment procedure for 24 simultaneous tests (critical value $\alpha = 0.013$) (Figure 2.2). Parental species differed significantly in transcript abundance for IGF-2, MyoD1b, and CAST-L at 234 days and for MRF-4 at 327 days. Yellowstone cutthroat trout produced more transcripts than Rbt, with the exception of MRF-4 at 327 days. Genes that were differentially expressed between parental species tended to be expressed in hybrids at levels that were intermediate to the parental species, with the exception of IGF-2 in bc-Yct, MyoD1b in F1-Yct, and MRF-4 in bc-Rbt. We found no instances in which hybrids had transcript levels that differed from both parental species. Transcript abundance was not different between F1 hybrid crosses, but the expression of one gene in each F1 hybrid cross was different from one parental species. Transcript abundance differed between backcrosses for MRF-4 at 145 days, and each backcross and their backcrossing parental species differentially expressed at least one gene.

Difference in transcript abundance within cross among time points

Each cross differentially expressed at least one gene among time points, with the exception of F1-Yct, following the B-Y FDR adjustment procedure for 48 simultaneous tests (critical value $\alpha = 0.011$) (Additional File 1). Transcript abundance varied the most in Yct; MyoD1b and CAST-L expression peaked at 234 days and MSTN-1a and MSTN-1b expression peaked at 327 days. Rainbow trout differentially expressed MyoD1b only, and expression peaked earlier than in Yct. F1-Rbt differentially expressed MyoD1b only, and peak expression overlapped with both parental crosses. In each backcross, gene expression timing differed from their backcrossing parental species for at least one gene. Although MRF-4 expression levels in each parental species was not different across time points, transcript levels were higher in bc-Yct at 127 days and in bc-Rbt at 324 days. In addition, CAST-L transcript level peaked earlier in bc-Yct than in Yct.

Transcript abundance-trait correlations

Across parent species, 14 transcript abundance-trait correlations were significant following the B-Y FDR adjustment for 192 simultaneous tests ($\alpha = 0.0086$) (Table 2.5). These correlations were found at two time points, but were not maintained across these time points. Transcript abundance at four genes was significantly correlated with length. Three of these correlations were positive, indicating that higher expression was associated with greater length, and one was negative. Three out four significant correlations between transcript abundance and condition factor were negative. Five out of six significant correlations between transcript abundance and percent Yct genome were positive.

Across hybrids, three transcript abundance-trait correlations were significant following the B-Y FDR adjustment. These three correlations involved the percent Yct genome only, and all were positive. The correlation that involved MRF-4 was maintained across two time points.

Sixteen parent-hybrid comparisons were evaluated for a difference in correlation coefficients between parental species and hybrid (Table 2.5). Transcript abundance-trait correlations differed significantly between parents and hybrids for nine of these comparisons, following the B-Y FDR adjustment for 16 simultaneous tests (α = 0.0148). In eight of the nine comparisons that differed significantly, transcript abundance and trait were correlated across parents but not across hybrids, suggesting these correlations that occurred in the parents were not conserved in their hybrids. The traits involved in these correlations across parental species were percent Yct genome, condition factor, and length at 234 and 327 days. In one of the nine comparisons that differed significantly, transcript abundance and trait were correlated across hybrids but not across parental species.

Comparison of transcript abundance among genotyped backcrosses and parental species

We found no difference in transcript abundance between homozygous and heterozygous genotypes within backcross (i.e. between bc-Rbt$_{(RR)}$ and bc-Rbt$_{(RY)}$ and between bc-Yct$_{(YY)}$ and bc-Yct$_{(RY)}$) at the three genes that were genotyped in the backcrosses (B-Y FDR adjusted α = 0.0143, 18 simultaneous tests) (Figure 2.3). However, occasionally, transcript abundance was different between the backcrossing parent and heterozygous backcrosses, and between the backcrossing parent and homozygous backcrosses. Yellowstone cutthroat trout produced more CAST-L transcripts than bc-Yct$_{(RY)}$ at 234 days (P = 0.004) and bc-Rbt$_{(RR)}$ produced more IGF-2 transcripts than Rbt at 234 days (P = 0.011).

Discussion

Our study indicates that muscle growth-related genes were differentially expressed among Rbt, Yct, and their hybrids. Temporal trends in gene expression within crosses contributed to the differences among crosses, suggesting that the timing of gene expression differs among Rbt, Yct, and their hybrids during juvenile development. Consequently, different genes contributed to the variation among crosses over time. The expression of several genes was significantly correlated with growth traits across parent species, but these correlations were not observed across hybrids, which could suggest that hybridization disturbed these relationships. Comparisons of transcript abundance among genotyped backcrosses and parent species revealed that genotype affected gene expression. Taken together, these results suggest that hybridization between Rbt and Yct disrupts gene regulation, thereby disturbing intrinsic relationships between gene expression and growth patterns that have evolved in each species.

Growth patterns among crosses

Growth patterns among Rbt, Yct, and hybrids appeared to be related to maternal effects and cross. The difference in condition factor between reciprocal F1 crosses at 145 days post-fertilization, but not at later time points (Table 2.3), suggested maternal effects on growth in the youngest fish, as may be expected. Maternal effects influence traits expressed during embryo and juvenile development (Wolf and Wade 2009). These effects are mediated through the provisioning of maternally derived resources, such as yolk amount, hormones, nutrients, and mRNA (Mousseau and Fox 1998, Green 2008, Wolf and Wade 2009). As a result, egg size can have a large influence on progeny size. Maternal effects on body size are commonly observed in young fish, but these effects appear to decline as fish age (Heath *et al.* 1999, McClelland *et al.* 2005). Decreasing difference in condition factor between the reciprocal F1 hybrids could suggest that maternal provisioning could have a larger effect on growth than endogenous processes during early life history stages.

Condition factor, but not length and weight, differed among crosses and appeared to be related to the proportion of Yct genome contained within cross. The differences in condition factor were consistent with relative body shapes expected for Rbt, Yct, and hybrid crosses (Seiler and Keeley 2007, Ostberg *et al.* 2011). Considering that muscle comprises the bulk of body mass in fish (Mommsen and Moon 2001), the difference in condition factor (this study) and morphology (Seiler and Keeley 2007, Ostberg *et al.* 2011) suggest that muscle growth differs among Rbt, Yct, and hybrids.

Differential expression of muscle growth-related genes

Differential patterns of gene expression between Rbt and Yct could indicate inherent differences in regulatory pathways associated with myogenesis during juvenile development, providing a possible mechanism for their body shape differences. Modulation of gene expression is recognized as an important mechanism of phenotypic change (Oleksiak *et al.* 2002, Fay and Wittkopp 2008, Gibson 2008); therefore, variation in expression of muscle growth-related genes may be expected between Rbt and Yct.

Below, we provide possible explanations of how muscle growth differed between parental species based on the functional roles of the genes investigated that were differentially expressed.

The elevated and coincident expression of IGF-2, MyoD1b, and CAST-L in Yct at 234 days could suggest that Yct had a higher rate of myogenesis at this time point, relative to Rbt. IGFs stimulate hypertrophic growth by activating transcriptional signaling pathways that regulate muscle fiber mass and size (reviewed in Johnston *et al*. 2011, and Fuentes *et al*. 2013). Specifically, muscle-derived IGF-2 mediates a signaling pathway that up-regulates MyoD expression (Wilson and Rotwein 2006). MyoD specifies stem cells to enter the myogenic program, and stimulates the expression of genes downstream in the myogenic program that are required to initiate differentiation (Wilson and Rotwein 2006). Although increased level of MyoD1b expression could indicate that Yct committed more cells to the myogenic program at 234 days, it does not imply that myoblasts differentiated and were incorporated into muscle. Differentiation involves myogenin, MRF-4, and genes of the MEF-2 family, and we found no difference in expression of MRF-4 between Yct and Rbt. However, the level of expression among these genes involved with differentiation have been found to differ across developmental stages in Rbt (Johansen and Overturf 2005). Therefore, it is possible that myogenin and/or MEF-2 family genes may have been expressed at higher levels in Yct, allowing myoblasts to differentiate and migrate through the muscle, and ultimately promoting muscle growth. Similarly, the increase in expression of CAST-L in Yct could be explained by a higher rate of muscle growth. Muscle growth is associated with decreased rates of muscle protein turnover and the functional product of CAST-L blocks the activity of calpain proteases that are involved with this turnover (Goll *et al.* 2003). In addition, CAST-L expression in Rbt was found to be higher in faster growing strains compared to a slower growing strain (Salem *et al.* 2005). Thus, higher expression of IGF-2, MyoD1b, and CAST-L in Yct relative to Rbt could indicate a functional relationship among these genes that might be expected during periods of increased muscle growth.

Higher expression of MSTN-1a and MSTN-1b in Yct at 327 days could suggest that muscle growth declines earlier in Yct, than Rbt. Although expression of both myostatins was not different between parental species at 327 days, higher expression was correlated with the Yct genome at this time point. Reduction of myostatin expression has been associated with faster growth (Roberts *et al.* 2004, Montserrat *et al.* 2007, Huang *et al.* 2012) and muscle mass has been found to increase in the absence of functional myostatin (Grobet *et al.* 1997, McPherron *et al.* 1997, Lee *et al.* 2009). The timing of myostatin expression during development in Rbt has been well characterized (Johansen and Overturf 2005, Garikipati *et al.* 2006). Peak myostatin expression in Rbt occurs in 140 gram and larger fish (Johansen and Overturf 2005), which is a similar size of our Rbt at 327 days. However, we did not find a temporal trend in myostatin expression in Rbt. In contrast, Yct at 327 days expressed both myostatins at higher levels than earlier time points, indicating that myostatin expression levels rise earlier in juvenile Yct development than in Rbt. Myostatin negatively regulates muscle growth by down-regulating muscle-specific transcription factors involved with proliferation and differentiation (Thomas *et al.* 2000, Langley *et al.* 2002, Amali *et al.* 2004, Lee *et al.* 2009). Consequently, Johansen and Overturf (2005) suggested that increased expression of myostatin could attenuate the growth promoting effects of these muscle-specific transcription factors, and thereby restrict muscle growth. We did not find a decrease in expression of muscle-specific transcription factors (Myod1a, MyoD1b, and MRF-4) in response to increased myostatin expression in Yct. However, expression of MRF-4 differed between species at 327 days and lower levels of MRF-4 expression were associated with the Yct genome, suggesting that Yct expressed fewer MRF-4 transcripts, in general. Our data suggest that the relatively slender body shape of Yct is due in part by early declines in muscle growth caused by myostatin expression.

Rainbow trout and Yct exhibited differential patterns in the timing of gene expression. Temporal differences in expression may suggest that these muscle growth-related genes affect different life stages during juvenile development between the species. Furthermore, the timing of gene expression in hybrids was often different from parental species, as well as among hybrids, suggesting that genes may affect hybrids and parental species at different life stages. In salmonids, hyperplasia and hypertrophy have been reported to make different contributions to muscle growth between populations that differ in growth rate (Valente *et al.* 1998, Valente *et al.* 1999). Taken together with the difference in condition factor (our study) and morphology (Seiler and Keeley 2007, Ostberg *et al.* 2011), the timing of expression of muscle growth-related genes may be expected to differ among Rbt, Yct, and their hybrids.

Gene expression in hybrids was highly variable and difficult to predict based on the gene expression levels in the parental species. Overall, gene expression in hybrids was roughly intermediate between that of Rbt and Yct. However, hybrids exhibited large variation in expression among genes, as well as temporally highly variable expression. Hybrids often exhibit higher variation in gene expression than their parents (Roberge *et al.* 2008, Normandeau *et al.* 2009, Renaut *et al.* 2009, Bougas *et al.* 2010). This variation is largely presumed to be a result of divergence in elements of transcriptional pathways between parental genomes, causing functional changes in gene regulation when these elements are inherited in their hybrids (Landry *et al.* 2007, Ortiz-Barrientos *et al.* 2007). Furthermore, transcription regulation is highly polygenic (Brem and Kruglyak 2005) and demonstrates complex patterns of inheritance that contribute to gene expression variation in hybrids (Rockman and Kruglyak 2006). For example, many studies comparing gene expression in hybrids to their parental types have found that nonadditive transcription regulation inheritance is fairly commonly in hybrids (Gibson *et al.* 2004, Rottscheidt and Harr 2007, Roberge *et al.* 2008, Renaut *et al.* 2009). These nonadditive gene interactions promote high variability. Additionally, because recombination between parental genomes may break down co-adapted transcriptional pathways and create novel gene combination, gene expression may be expected to be more variable in backcross hybrids than F1 hybrids. Our data, albeit limited, support this hypothesis, which is consistent with findings in lake whitefish (*Coregonus clupeaformis*) (Renaut *et al.* 2009).

We found that transcript abundance of four genes was significantly and positively correlated with percent Yct genome across parental species and across hybrids, possibly suggesting that the expression of these genes may be associated with the level of Yct admixture. Expression of growth-related genes has been associated with admixture in salmonids (Lamaze *et al.* 2013). Furthermore, the association between gene expression and percent Yct genome was not consistent over time, indicating that relationships may be dependent on the life history stage. Consequently, high temporal variation in gene expression contributes to the difficulty in predicting gene expression in hybrids.

Relationships between gene expression and traits

The majority of significant relationships between transcript abundance and growth traits observed across the parental species were not conserved across hybrids, indicating that hybridization disturbed gene expression-growth trait relationships. Such associations between gene expression and growth traits could indicate functional impacts of hybridization between Rbt and Yct populations. These relationships, limited to length and condition factor, suggest that hybridization may alter co-adapted transcriptional networks associated with fitness-related traits. Although the relationships were correlational, rather than causal, they indicate associations between transcript abundance and growth, and suggest a mechanistic link between gene expression and phenotype. Furthermore, gene expression-growth trait relationships were not conserved across time points, indicating these relationships were dependent on size and/or age. Nevertheless, the irregularity of conserved gene expression-growth trait relationships across hybrids suggests that hybridization with Rbt could disrupt co-adapted functional genes within native Yct.

Comparison among genotyped backcrosses and parent species

Transcript abundance in backcrossed hybrids was affected by the genotype at specific genes as well as by the genomic background. Although transcript abundance did not differ significantly between genotypes within backcrosses, comparisons of transcript abundance in heterozygous and homozygous genotypes within backcross to the backcrossing parent species suggested otherwise. In one gene (CAST-L), the difference between backcross and backcrossing parent comparisons was dependent on the genotype in the backcross, suggesting a genotypic effect possibly due to transcription regulatory variation that acts locally on the gene. In another gene (IGF-2) each individual had a comparable homozygous genotype (RR) contained within one of two different genomic backgrounds (backcross or Rbt), suggesting transcription regulatory variation that acts distantly on the gene. Genotypic effects were not consistent between the bc-Rbt x Rbt and bc-Yct x Yct comparisons, possibly indicating evolutionary divergence of gene regulation mechanisms between the species. However, sample size may have limited the power to detect significant differences in these comparisons, and our conclusions are preliminary.

Conclusion

Our study provides evidence for muscle growth regulation differences between Rbt and Yct, and demonstrated that the expression of muscle growth-related genes and their relationship with growth traits is altered following hybridization. From the perspective of cutthroat trout conservation, these results suggest that transcriptional signaling pathways inherently differ between Rbt and Yct and that hybridization with non-native Rbt may alter transcriptional regulation of genes that are functionally important for muscle growth. Our results expand the current understanding of hybridization between Rbt and cutthroat trout; transcriptional networks may be modified in hybrids, resulting in unpredictable gene expression patterns, and gene expression may be dependent on hybrid genotypes across transcriptional networks. The growth-related genes herein may have application to future hybridization studies and could be used, for example, to characterize allelic expression patterns and identify relationships between these patterns and growth traits, as well as characterize relationships between gene expression and morphology within hybrid populations.

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Tables

Table 2.1. PCR primers, and anneal temperature, used to isolate partial gene sequences from parental fish.

Gene	Primer sequence
IGF-1 (68 ^{oC})	Forward: 5'-AGAACAAACTGGAACGACAGC-3'
AF063216	Reverse: 5'-TGTCCATGCTGTTTCACTGAT-3'
IGF-2 (68 ^{oC})	Forward: 5'-AGTGGGCCAATTGATCTGTC-3'
X97225	Reverse: 5'-ACGGAGACAAGGGAGATGTG-3'
MSTN-1a (68 ^{oC})	Forward: 5'-CTCTGTAGTCCGCCTTCACAT-3'
DQ136028	Reverse: 5'-TGGCCATTGTCATGATTGTT-3'
MSTN-1b (66 ^{oC})	Forward: 5'-CCGCATCCGTAATTTGATTT-3'
DQ138300	Reverse: 5'-GCTTGACAACATCTCGGCTA-3'
MyoD1a (64 ^{oC})	Forward: 5'-GGAGTTGCCGGATATTCCTT-3'
AJ618978	Reverse: 5'-GGAATCATTTGGTGCTTCGT-3'
MyoD1b (66 ^{oC})	Forward: 5'-CGCAAGACGAAGCAACTATG-3'
FJ793566	Reverse: 5'-CCACAGCAACATTCTGCAAC-3'
MRF-4 (64 ^{oC})	Forward: 5'-TGAAGGCGTCCAATAAGGAG-3'
EF450079	Reverse: 5'-TCTCTTGAGGATTTGTTTCTGC-3'
CAST-L (58 ^{oC})	Forward: 5'-TCAAGTGGCCTGACTGACTG-3'
AY937407	Reverse: 5'-CTCACAGATGCATCCATATCAAG-3'
β-actin (64 ^{oC})	Forward: 5'-CATGTTTGAGACCTTCAACACC-3'
AF254414	Reverse: 5'-TGATCTTGATCTTCATGGTGGA-3'

GenBank accession number is provided below each gene.

Table 2.2. Primer and probes used for real-time PCR.

Gene	Primer and probe sequence
IGF-1	Forward: 5'-TGTGCCTCTGTCCACGCTTT-3'
KC609005,KC609006	Reverse: 5'-AATGTACTGTGCCCCTGTCAAGTC-3'
	Probe: 6-FAM-TAGGTGTTCTTGGCATGTCTGTGTGGCG-TAMRA
IGF-2	Forward: 5'-CGCTCCCAGAACCGTGGTAT-3'
KC609007, KC609008	Reverse: 5'-TCTGTAGAGAGGTGGCCGACAC-3'
	Probe: 6-FAM-TGGAGGAGTGTTGTTTCCGTAGCTGTGACCT-TAMRA
MSTN-1a	Forward: 5'-ACACGCCATCAAGTCCCAAAT-3'
KC609015, KC609016	Reverse: 5'-CTGGTCAAGAAGTTGCTGCAAAG-3'
	Probe: 6-FAM-AGCAGAGATGTTGTCAAGCAGCTCCTGCCTAA-TAMRA
MSTN-1b	Forward: 5'-CCGCACCTTAGATAATGAATCTGATG-3'
KC609017, KC609018	Reverse: 5'-TTGGTGGTGCGCCGTTT-3'
	Probe: 6-FAM-TGAGTTTTATGGTTGCTTTCGGTCCAATGGG-TAMRA
MyoD1a	Forward: 5'-TCCTGCGGAATGCCATCAG-3'
KC609013, KC609014	Reverse: 5'-GGACTGGACGCATCCGAGTC-3'
	Probe: 6-FAM-ATTGAGTCTCTCCAAGGCCTGCTTCGTGG-TAMRA
MyoD1b	Forward: 5'-CCAGCCTGGATTGTCTTTCCA-3'
KC609011, KC609012	Reverse: 5'-CCCTCCTGGCCTGATAACACA-3'
	Probe: 6-FAM-TCGTGGAGAGAATCTCTACAGACACGTCAGCG-TAMRA
MRF4	Forward: 5'-TCGCCTGTCATCAATCGTTGA-3'
KC609009, KC609010	Reverse: 5'-GCTGACGTCGCTTATACAGGCTAC-3'
	Probe: 6-FAM-AAGAGAAACCGACTTGCAACGAAGAAGTCTCAG-TAMRA
CAST-L	Forward: 5'-AGAAAACTCACTCTTCTGTGTACAACAAT-3'
KC609003, KC609004	Reverse: 5'-GGGTAATAGTGAGTGTCATGTGTTTAGG-3' Probe: 6-FAM-ACAGACGTCCCAATCCTACATAACCCCTATATACTGT-TAMRA
β-actin	Forward: 5'-GGAGAAGCTGTGCTACGTGGC-3'
KC888023, KC888024	Reverse: 5'-CTCGTTGCCGATGGTGATGA-3'
	Probe: 6-FAM-CTGGACTTTGAGCAGGAGATGGGCACC-TAMRA
ARP	Forward: 5'-GAAAATCATCCAATTGCTGGATG-3'
Purcell et al. (2004)	Reverse: 5'-CCTCCCACGCAAGGACAGA-3'
	Probe: 6-FAM-CTATCCCAAATGTTTCATTGTCGGCGC-TAMRA
18s	Life Technologies catalogue number 4308329
(X03205)	

GenBank accession number (Rbt, Yct) or Taqman assay reference is provided below each gene.

Table 2.3. Mean length (L, mm), weight (W, g), and condition factor (K) (one standard deviation in parentheses) for each cross at each time point (days post-fertilization). Rbt = rainbow trout, bc-Rbt = first generation Rbt backcross (Rbt x F1), F1-Rbt = F1 hybrid with Rbt maternal lineage, F1-Yct = F1 hybrid with Yct maternal lineage, bc-Yct = first generation Yct backcross (Yct x F1), and Yct = Yellowstone cutthroat trout. Crosses that differed significantly (*P* < 0.05) within time points are indicated by different letters.

Days		Rbt	bc-Rbt	F1-Rbt	F1-Yct	bc-Yct	Yct
145	L	68.67 (2.84)	72.11 (2.31)	70.78 (0.70)	72.78 (1.26)	73.33 (2.42)	68.89 (2.26)
	W	4.32 (0.49)	4.78 (0.44)	4.49 (0.26)	4.29 (0.28)	4.75 (0.42)	3.54 (0.34)
	K	1.29 (0.03)*a*	1.25 (0.03)*a*	1.26 (0.05)*a*	1.10 (0.03)*b*	1.18 (0.03)*ab*	1.06 (0.01)*b*
234	L	146.22 (3.84)	138.00 (7.19)	152.56 (6.08)	153.89 (5.91)	157.11 (3.45)	157.22 (5.16)
	W	46.27 (3.28)	41.41 (6.29)	49.68 (7.79)	47.14 (6.40)	48.56 (3.78)	44.94 (4.99)
	K	1.46 (0.03)*a*	1.48 (0.03)*a*	1.31 (0.04)*b*	1.23 (0.03)*bc*	1.23 (0.03)*bc*	1.12 (0.02)*c*
327	L	212.44 (6.03)	214.56 (6.40)	219.67 (6.47)	220.89 (10.57)	218.00 (6.61)	224.78 (7.76)
	W	131.66 (10.41)	137.99 (11.30)	131.01 (9.29)	138.92 (22.91)	126.22 (10.94)	137.59 (14.50)
	K	1.35 (0.03)*a*	1.37 (0.02)*a*	1.23 (0.03)*b*	1.20 (0.02)*b*	1.20 (0.02)*b*	1.17 (0.02)*b*

Table 2.4. Correlation between transcript abundance and the first and second canonical variates at 145, 234, and 327 days post-fertilization for eight muscle growth-related genes.

Day	Gene	CV1	CV2
145	MSTN-1a	0.140	-0.081
	MSTN-1b	0.361	0.130
	IGF-1	0.122	0.306
	IGF-2	0.489	-0.337
	MyoD1a	-0.027	0.550
	MyoD1b	-0.177	0.746
	MRF-4	0.491	0.586
	CAST-L	0.164	0.360
234	MSTN-1a	-0.466	-0.277
	MSTN-1b	-0.248	-0.249
	IGF-1	-0.092	-0.167
	IGF-2	-0.192	-0.354
	MyoD1a	-0.104	-0.129
	MyoD1b	-0.411	0.063
	MRF-4	0.051	0.225
	CAST-L	-0.674	-0.223
327	MSTN-1a	-0.729	0.542
	MSTN-1b	-0.710	0.409
	IGF-1	-0.025	0.079
	IGF-2	0.027	-0.041
	MyoD1a	0.058	0.649
	MyoD1b	-0.138	0.458
	MRF-4	0.296	0.290
	CAST-L	0.144	0.781

Table 2.5. Significant Spearman rank correlation coefficients (r_s) between transcript abundance and trait (percent Yct genome, % Yct; condition factor, K; length, L; and weight, W) across parent species and across hybrids within each time point and the probability that r_s differed between parents and hybrids, after correcting for simultaneous tests.

Day	Trait	Gene	Parents (r_s)	Hybrids (r_s)	Probability that (r_s) differed
145	% Yct	MRF-4	-0.442	0.539*	<0.001*
234	% Yct	IGF-2	0.671*	0.239	0.042
	% Yct	MyoD1b	0.656*	0.132	0.021
	% Yct	MRF-4	-0.015	0.471*	0.050
	% Yct	CAST-L	0.677*	-0.210	<0.001*
	K	MyoD1b	-0.651*	0.021	0.006*
	K	CAST-L	-0.742*	-0.283	0.019
	L	IGF-1	0.754*	0.000	0.001*
	L	IGF-2	0.647*	-0.237	0.001*
	L	MyoD1b	0.726*	-0.045	0.001*
327	% Yct	MSTN-1a	0.778*	0.429	0.035
	% Yct	MSTN-1b	0.616*	0.702*	0.319
	% Yct	MRF-4	-0.828*	-0.046	<0.001*
	K	MSTN-1a	-0.686*	-0.315	0.054
	K	MRF-4	0.637*	-0.238	0.001*
	L	MyoD1a	-0.677*	-0.069	0.009*

*Significant after correcting for false discovery rate (FDR adjusted $\alpha = 0.0086$ for parent and hybrid r_s and $\alpha = 0.013$ for comparisons of r_s between parents and hybrids).

Figures

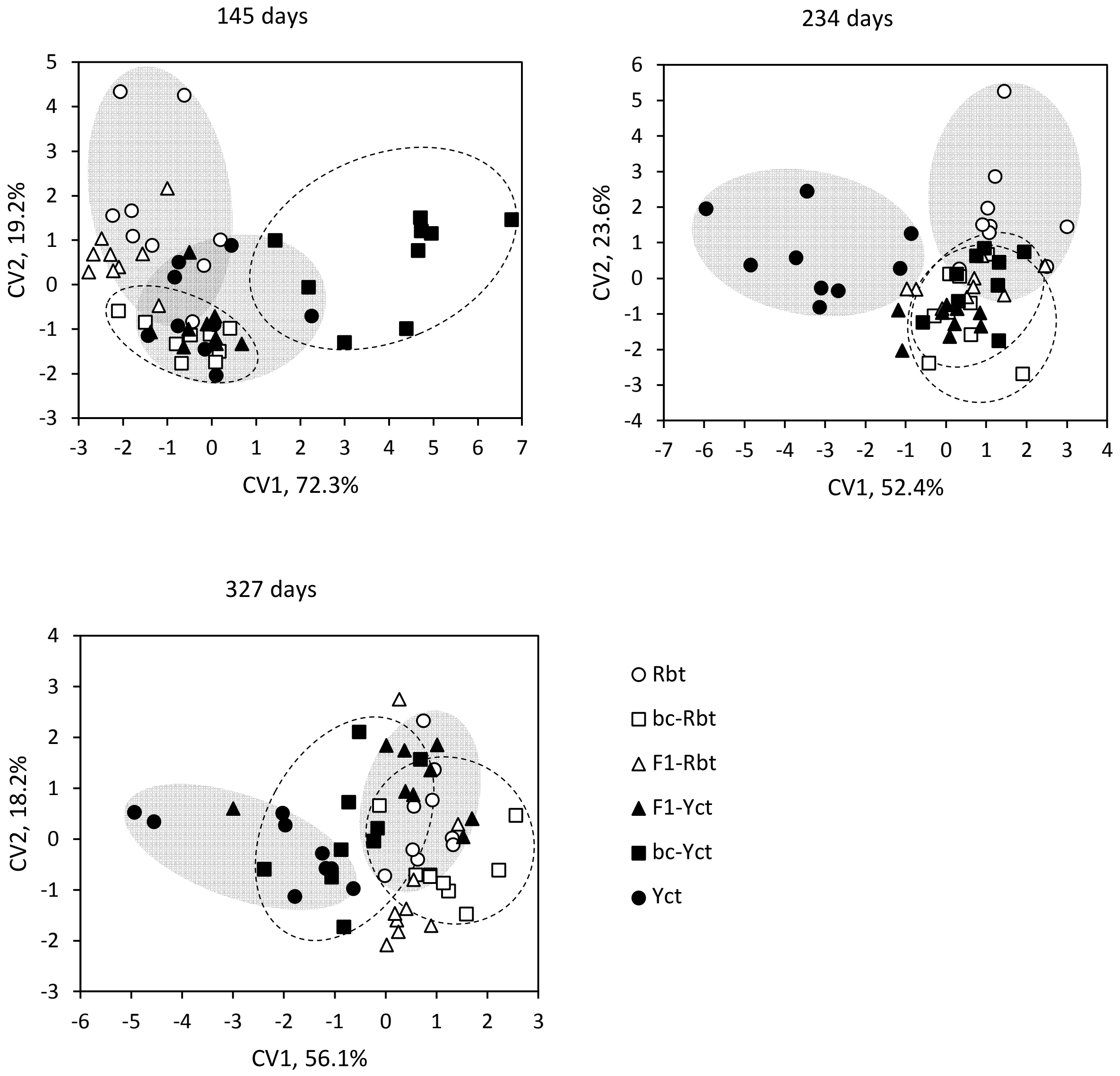

Figure 2.1. Individual canonical variate plots and the percent variation on the first and second axes at 145, 234, and 327 days post-fertilization across eight muscle growth-related genes. Grey shaded ellipses indicate the spread of Rbt and Yct individuals and stippled ellipses indicate the spread of bc-Rbt and bc-Yct individuals.

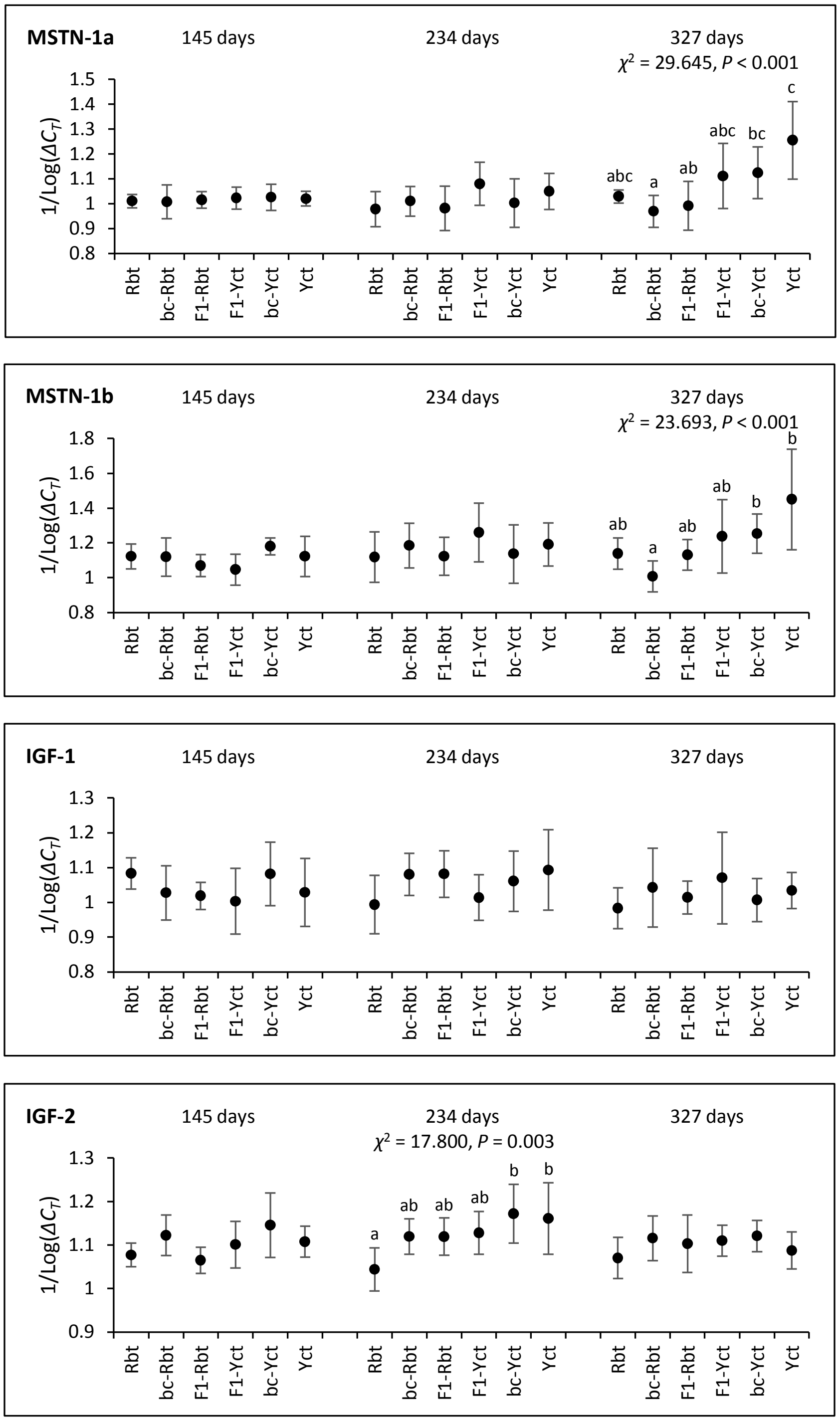

Figure 2.2. Transcript abundance (± SD) of eight muscle growth-related genes among crosses at 145, 234, and 327 days post-fertilization. Significant Kruskal-Wallis tests are indicated following B-Y FDR adjustment procedure for 24 simultaneous tests (critical value α = 0.013) and lowercase letters that differ among crosses within time point indicate a significant difference in expression between crosses.

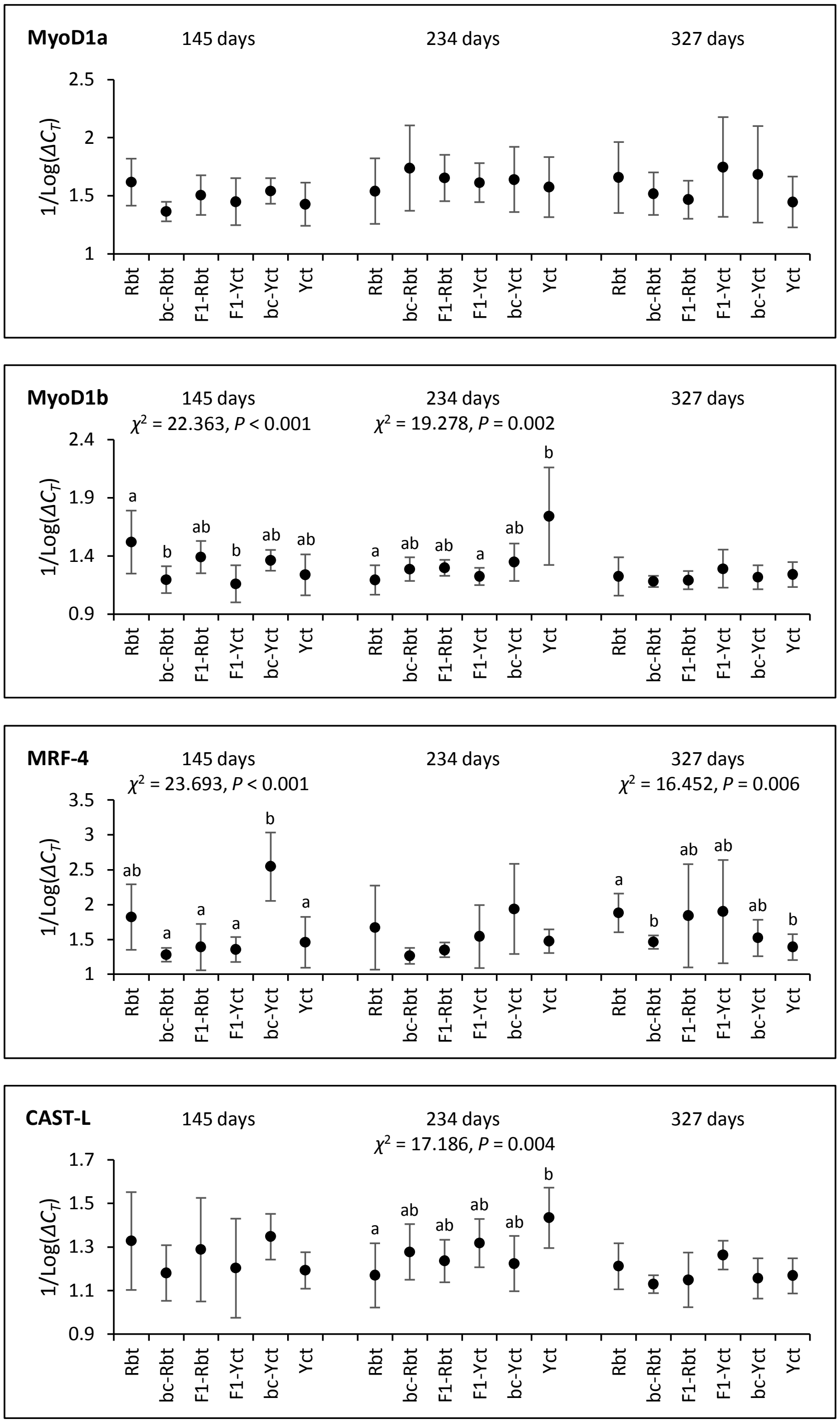

Figure 2.2 (continued). Transcript abundance (± SD) of eight muscle growth-related genes among crosses at 145, 234, and 327 days post-fertilization. Significant Kruskal-Wallis tests are indicated following B-Y FDR adjustment procedure for 24 simultaneous tests (critical value $\alpha = 0.013$) and lowercase letters that differ among crosses within time point indicate a significant difference in expression between crosses.

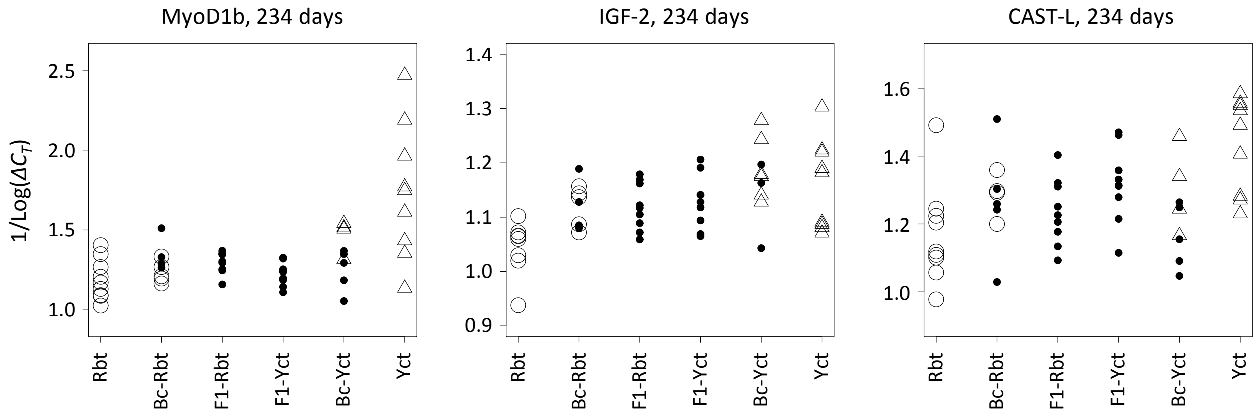

Figure 2.3. Transcript abundance of individual genotypes at three genes. Each symbol represents an individual; open circles indicate the homozygous Rbt genotype (RR), black dots indicate the heterozygous genotype (RY), and triangles indicate the homozygous Yct genotype (YY). Each gene was differentially expressed between Rbt and Yct.

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Chapter 3: Chromosome rearrangements, recombination suppression, and limited segregation distortion in hybrids between Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) and rainbow trout (*O. mykiss*)[1]

Abstract

Introgressive hybridization is an important evolutionary process that can lead to the creation of novel genome structures and thus potentially new genetic variation for selection to act upon. On the other hand, hybridization with introduced species can threaten native species, such as cutthroat trout (*Oncorhynchus clarkii*) following the introduction of rainbow trout (*O. mykiss*). Neither the evolutionary consequences nor conservation implications of rainbow trout introgression in cutthroat trout is well understood. Therefore, we generated a genetic linkage map for rainbow-Yellowstone cutthroat trout (*O. clarkii bouvieri*) hybrids to evaluate genome processes that may help explain how introgression affects hybrid genome evolution. The hybrid map closely aligned with the rainbow trout map (a cutthroat trout map does not exist), sharing all but one linkage group. This linkage group (RYHyb20) represented a fusion between an acrocentric (Omy28) and a metacentric chromosome (Omy20) in rainbow trout. Additional mapping in Yellowstone cutthroat trout indicated the two rainbow trout homologues were fused in the Yellowstone genome. Variation in the number of hybrid linkage groups (28 or 29) likely depended on a Robertsonian rearrangement polymorphism within the rainbow trout stock. Comparison between the female-merged F_1 map and a female consensus rainbow trout map revealed that introgression suppressed recombination across large genomic regions in 5 hybrid linkage groups. Two of these linkage groups (RYHyb20 and RYHyb25_29) contained confirmed chromosome rearrangements between rainbow and Yellowstone

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cutthroat trout indicating that rearrangements may suppress recombination. The frequency of allelic and genotypic segregation distortion varied among parents and families, suggesting few incompatibilities exist between rainbow and Yellowstone cutthroat trout genomes. Chromosome rearrangements suppressed recombination in the hybrids. This result supports several previous findings demonstrating that recombination suppression restricts gene flow between chromosomes that differ by arrangement. Conservation of synteny and map order between the hybrid and rainbow trout maps and minimal segregation distortion in the hybrids suggest rainbow and Yellowstone cutthroat trout genomes freely introgress across chromosomes with similar arrangement. Taken together, these results suggest that rearrangements impede introgression. Recombination suppression across rearrangements could enable large portions of non-recombined chromosomes to persist within admixed populations.

Introduction

The widespread occurrence of hybridization has been a catalyst for intensive study in evolutionary biology and has provided rich opportunities for investigating genome evolution, adaptation, speciation, reproductive isolation, and hybrid fitness (Barton and Hewitt 1989, Rieseberg *et al.* 2000, Burke and Arnold 2001). Hybridization is a natural evolutionary process, contributing to the diversification of plants and animals (Dowling and DeMarais 1993, Rieseberg and Wendel 1993). When populations hybridize and progeny are viable and fertile, genomes introgress and produce recombined chromosomes. Introgression breaks down linkage associations and generates novel gene combinations which may have fitness consequences (Burke and Arnold 2001). However, hybridization can also have significant conservation implications, leading to the formation of hybrid swarms and extinction (Rhymer and Simberloff 1996, Allendorf *et al.* 2001). In these cases, introgression may interfere with epistatic interactions by disrupting local adaptations and breaking down co-adapted gene complexes. Regardless of the consequences, understanding the genome processes that affect introgression is fundamental to understanding hybrid genome evolution.

Genetic linkage maps provide a means for investigating genome evolution and function, and have contributed to an improved understanding of hybridization and introgression (Rieseberg and Linder 1999, Fishman *et al.* 2001, Fitzpatrick *et al.* 2009). They have provided empirical evidence that chromosome rearrangements can act as barriers to gene flow by suppressing recombination between rearranged chromosomes (Rieseberg *et al.* 1995, Panithanarak *et al.* 2004, Stevison *et al.* 2011). They can be used to identify segregation distortion (loci that deviate from Mendelian inheritance patterns), which may indicate the presence of fitness-linked loci or genetic incompatibilities (Danzmann and Gharbi 2001). When applied to introgressed populations, linkage maps have identified specific genomic regions that might be important for providing adaptive fitness advantages (Buerkle and Rieseberg 2001, Fitzpatrick *et al.* 2009). Finally, they provide a framework for detecting quantitative trait loci (QTL), enabling identification of genomic regions associated with ecological, evolutionary, or physiological processes within hybrids and parental species (Bradshaw Jr. *et al.* 1995, Rieseberg and Linder 1999, Rieseberg *et al.* 2003).

Genome maps for salmonid fishes show signatures of two significant events: genome duplication and chromosome rearrangements (Danzmann *et al.* 2005, Gharbi *et al.* 2006, McClelland and Naish 2008, Rexroad *et al.* 2008). The first significant event, genome duplication, is thought to have occurred through autopolyploidy approximately 25-100 million years ago, resulting in an ancestral tetraploid genome (Allendorf and Thorgaard 1984). Rediploidization of the genome is occurring but is not complete (Allendorf and Thorgaard 1984). This residual tetraploidy has two consequences exclusive to males. First, males form multivalents between homeologues during meiosis (Wright *et al.* 1983), suppressing crossing-over between homologues and reducing recombination rates compared to females (Sakamoto *et al.* 2000). Second, residual tetraploidy results in pseudolinkage between homeologous chromosomes in males (Wright *et al.* 1983), producing statistical rather than physical linkage (Sakamoto *et al.* 2000). The second significant event, chromosome rearrangements, has generated highly variable chromosome numbers among salmonid species (Phillips and Rab 2001). Although chromosome numbers differ among species, chromosome arm numbers have remained relatively constant because rearrangements have primarily been of the Robertsonian type between acrocentric and metacentric chromosomes.

Robertsonian rearrangements are translocations that involve centric fusion or fission between chromosome arms, causing a change in chromosome number but not chromosome arm number.

Rainbow trout (*Oncorhynchus mykiss*) and cutthroat trout (*O. clarkii*) are two salmonid species that inhabit western North America. Rainbow and cutthroat trout are sister species and shared a common ancestor approximately 3 million years ago (Wilson and Turner 2009). Despite karyotypic differences between the species, Robertsonian rearrangements have maintained the same number (n=104) of diploid chromosome arms (Loudenslager and Thorgaard 1979, Thorgaard 1983). Similarity in chromosome arm number between the two species could be an important factor that enables the species to readily hybridize and produce viable and fertile progeny. In fact, where non-indigenous rainbow trout have been introduced into indigenous cutthroat trout habitats, introgressive hybridization has led to hybrid swarms and extinction of local cutthroat trout populations, and has thus become a major conservation concern (Allendorf and Leary 1988). Although introgression between rainbow and cutthroat trout is well documented, it is unknown how introgression affects the genomic architecture of hybrids and thus their subsequent evolution. For example, karyotypic differences between rainbow and cutthroat trout could affect hybrid genome evolution by suppressing recombination, hindering gene flow, and generating linkage disequilibrium (Rieseberg and Linder 1999, Panithanarak *et al.* 2004, Machado *et al.* 2007, Stevison *et al.* 2011). As a result, the ability of invading alleles to become established within a host genome could be influenced by the presence of chromosome rearrangements. In addition, reduced recombination between rearranged chromosome segments could prevent disruption of co-adapted gene complexes (Noor *et al.* 2001, Feder *et al.* 2003); enabling these adaptations to persist within hybrid populations, which could ultimately affect hybrid fitness.

Here, we present the first hybrid genetic linkage map between two introgressing salmonid species, rainbow trout and Yellowstone cutthroat trout (*O. c. bouvieri*). Rainbow trout (RBT) have 58-64 diploid chromosomes depending on the chromosome race (Thorgaard 1983) whereas Yellowstone cutthroat trout (YCT) have 64 diploid chromosomes (Loudenslager and Thorgaard 1979). We generated F_2 hybrids between RBT and YCT and developed a F_1 hybrid linkage map to investigate the genomic consequences of introgression. The objectives for constructing the F_1 hybrid linkage map were to 1) determine if linkage groups were conserved between the F_1 map and existing RBT maps, 2) determine if introgression suppressed recombination, and 3) estimate the prevalence of segregation distortion in the F_1 hybrid map. Our hybrid linkage map has application to conservation and management of indigenous cutthroat trout subspecies because it localizes species-specific markers to linkage groups and identifies genomic regions where recombination is suppressed, both of which may assist resource managers in determining accurate estimates of RBT admixture throughout the native cutthroat trout range. It will also have application for identifying QTL associated with species-specific traits. Finally, it can be used as a baseline for future comparative mapping studies in rainbow-cutthroat trout hybrids.

Materials and Methods

Mapping families

Hybrid F_1 YCT-RBT parents were generated by crossing female YCT collected from Henry's Lake Fish Hatchery and Fish Management Station, Idaho Department of Fish and Game (IDFG), with male RBT (Kamloops stock) from Hayspur Hatchery (IDFG). Mature F_1 hybrids were collected at Henry's Lake in March of 2004 and used to generate two F_2 hybrid full-sib crosses (Family 1, N = 54 mapping progeny; Family 2, N = 53 mapping progeny). Because a YCT genetic map does not exist, we also constructed a YCT cross (N = 48 mapping progeny) so that we could clarify linkage anomalies observed between the F_1 hybrid and published RBT maps (Guyomard *et al.* 2006, Rexroad *et al.* 2008). Fin tissues were sampled from the parents and we confirmed the hybrid/species status of F_1 hybrid and YCT parents by screening 12 species-specific markers that differentiate RBT and YCT (Supplementary material S3.1). Crosses were reared 10 months post-fertilization at which point the fish were euthanized and fin tissues sampled. DNA was extracted using DNeasy kits (Qiagen Inc., Valencia, CA, USA).

Mapping markers

Rainbow trout genetic linkage maps developed by Guyomard *et al.* (2006) and Rexroad *et al.* (2008) served as templates for F_1 hybrid map construction. We applied 294 microsatellite primers

(Supplementary material S3.1), spanning a large portion of each RBT linkage group and ensuring coverage across the centromere for metacentric chromosomes. Microsatellite amplification and PCR product visualization followed the methods of McClelland and Naish (2008).

We also applied 169 SNPs (Supplementary material S3.1). SNPs were interrogated using TaqMan 5' nuclease assays (Applied Biosystems Inc., Carlsbad, CA, USA) or SNPtype assays (Fluidigm Corporation, San Francisco, CA, USA). All genotyping was carried out in 96.96 Dynamic Genotyping Arrays on an EP1 Genotyping System (Fluidigm Corporation), with a pre-amplification step, following manufacturer's protocols. Genotypes were determined using the Fluidigm SNP Genotyping Analysis software (v 3.0.2), with confidence threshold set to 80%.

In addition, 14 insertion/deletion and four RFLP species-diagnostic primers (Supplementary material S3.1) were included in the map. Amplifications were performed in 20 µl reaction volumes consisting of 15 ng genomic DNA, 1X NH_4 Reaction Buffer (Bioline, Taunton, MA, USA), 1.5-2.5 mM $MgCl_2$, 200 µM each dNTP, 1.5 pmol of each primer, and 0.5 units *Taq* polymerase (Bioline, Taunton, MA, USA). PCR products were visualized on 2-4% agarose gels stained in ethidium bromide.

Linkage analysis

Linkage maps were established using LINKMFEX v2.3 software package (Danzmann and Gharbi 2001) with an LOD threshold at 3.0. Because male salmonids show less recombination across the genome than females (Sakamoto *et al.* 2000), we initially constructed parent-specific linkage maps. Hereafter, parent-specific linkage maps are referred to as Female 1 (Family 1 female parent), Male 1 (Family 1 male parent), Female 2 (Family 2 female parent), and Male 2 (Family 2 male parent). Salmonids have high crossover interference and typically have one or no crossovers per chromosome arm (Thorgaard *et al.* 1983); we therefore used the pairwise recombination fraction, theta (Θ), between adjacent markers to estimate map distances. Male and female maps were compared by generating sex-specific and sex-merged maps using LINKMFEX. The total number of linkage groups in the F_1 hybrid map was determined from sex-merged maps. Linkage maps were graphically represented using the program MAPCHART

(Voorrips 2002). Markers that were heterozygous for the same alleles in both parents were excluded from the map because these parental genotypes reduce the number of informative progeny, generate missing data, and reduce mapping power for these markers. All species-diagnostic SNPs, indels, and RFLPs were heterozygous in both of the F_1 hybrid parents, and thus these markers were not ordered within the map. However, we did assign each of these markers to a specific linkage group.

Recombination rate analyses

We estimated the average recombination ratio across the genome and across each linkage group between parents within each family and between parents of the same sex using LINKMFEX. Significant differences in genome-wide and linkage group-wide recombination rates were identified by summing G-test values and degrees of freedom across each comparison.

To determine if introgression suppressed recombination rates, we generated a consensus female RBT map from Guyomard *et al*. (2006) and Rexroad *et al*. (2008) using recombination distances between markers and compared recombination rates to the female-merged F_1 map. The consensus female RBT map was generated using only markers in common with the female-merged F_1 hybrid map (Supplementary material S3.1). We ensured that maps had the same marker order among RBT and female-merged F_1 hybrids by removing markers that differed in their rank in the order. Markers used to compare recombination rates generally covered a substantial proportion of each RBT linkage group and were not restricted to areas of low recombination (centromeric regions) or areas of high recombination (telomeric regions). Kosambi map distances from Rexroad *et al*. (2008) were converted to Θ map distances by applying the formula $\theta = 0.5(e^{4k} - 1)/(e^{4k} + 1)$ (Ott 1991), where k = Kosambi distance. Prior to generating the consensus female RBT map, we tested for significant differences in recombination distances between adjacent loci between the two female RBT maps for each linkage group. The two female RBT maps were merged into the consensus map using LINKMFEX. We then tested for significant differences in recombination rate between the female-merged F_1 map and the consensus female RBT map across linkage groups against the null hypothesis of no difference in recombination rate. Significant differences in the recombination rate were identified as described above.

Segregation distortion analyses

We tested for allelic distortion using a sliding window analysis, because loci exhibiting segregation distortion often cluster together within the genome (Fishman *et al.* 2001). We established a 25 centiMorgan (cM) interval for the sliding window, because linkage was not supported for loci greater than 25 cM in distance at the LOD = 3.0 threshold. Species-specific markers were used to specify the most likely F_1 parent chromosome phases as either YCT or RBT using LINKMFEX. Locus-specific allele frequencies were tested for deviation from 1:1 Mendelian expectations by summing G-test values and degrees of freedom within each 25 cM interval.

For testing genotypic segregation distortion, we used the most likely allele phases obtained from LINKMFEX in the sliding window analysis to assign locus-specific genotypes in the F_2 hybrid progeny as YCT homozygote (YCT/YCT), RBT homozygote (RBT/RBT), or heterozygote (YCT/RBT). Significant deviation from the expected 1:2:1 Mendelian genotypic proportions was determined for each locus by applying chi square tests followed by multiple comparison corrections using a false discovery rate (B-Y FDR) (Benjamini and Yekutieli 2001) across all loci.

Results

Hybrid linkage map

After removing markers that were heterozygous for the same alleles in both parents, 310 microsatellite loci, 72 single nucleotide polymorphisms (SNPs), and one species-specific insertion/deletion (indel) were mapped in YCT-RBT F_1 hybrids using two families (Family, 54 progeny; Family 2, 53 progeny) (Supplementary material S3.1, S3.2, and S3.3). We identified a total of 28-29 linkage groups in the sex-merged map for the hybrids (Table 3.1) and have designated hybrid linkage groups as RYHyb (rainbow-Yellowstone hybrid). Comparisons to RBT chromosomes (Guyomard *et al.* 2006, Rexroad *et al.* 2008) revealed that all linkage groups identified in the F_1 hybrids were syntenic with and had similar marker orders to specific RBT chromosomes, and therefore we defined each specific linkage group with respect to its homologous RBT chromosome, except RYHyb20 (a chromosome fusion) and RYHyb28 (RBT sex chromosome homologue) (Table 3.1).

We found evidence for a fusion between two RBT chromosomes in the hybrids. Rainbow trout chromosomes Omy20 (a metacentric chromosome in RBT) and Omy28 (an acrocentric chromosome in RBT), were fused into a single linkage group, RYHyb20, in both sexes (Figure 3.1). Several loci mapping to Omy20 and Omy28 in RBT (Guyomard *et al.* 2006, Rexroad *et al.* 2008) did not recombine in the F_1 hybrids, indicating that a major portion of RYHyb20 was inherited as a single, non-recombining block of markers. Additional mapping in a male YCT (48 progeny) indicated that the Omy20 and Omy28 homologues were fused in YCT.

Linkage groups RYHyb25 and RYHyb29 (homologues to Omy25 and Omy29, respectively, in RBT) differed in arrangement among parents. The distal mapping loci OMM1301 (on RYHyb25) and Ogo2UW/ii (on RYHyb29) were not linked in Female 1 and Male 2, indicating two independent linkage groups (Figure 3.2A). However, there was no recombination between these loci in Female 2 and Male 1, suggesting RYHyb25 and RYHyb29 were fused in these parents (noted as RYHyb25_29). The recombination estimate between OMM1301 and OMM1797 in the progeny of a male YCT (Θ = 0.46, LOD = 0.03) confirmed that the RYHyb25 and RYHyb29 homologues are not fused in YCT. We found a similar recombination estimate between the same loci in Male2 (Θ = 0.45, LOD = 0.08), but not in Male 1 (Θ = 0.0, LOD = 12.94).

Marker order was largely conserved among parent-specific and between female- and male-merged maps (Supplementary material S3.1, S3.2, and S3.3). However, we observed inconsistent marker order among parent-specific maps for RYHyb25 and RYHyb29 and, therefore, these two linkage groups could not be merged within sex. Further, marker order differed between sexes for distally mapping loci at RYHyb13p, RYHyb19, and RYHyb24. Loci with different order between sexes were removed prior to generating the sex-merged map, and removal did not appear to alter marker order within linkage groups. Marker order differences could be due to marker informativeness within families, insufficient chromosome coverage, or reduced recombination in males.

Assignment of species diagnostic markers to linkage groups

We assigned 114 diagnostic species markers (97 SNPs, 13 indels, and 4 restriction fragment length polymorphisms (RFLPs)) to specific linkage groups (Supplementary material S3.1). Specific mapping locations within linkage groups could not be determined because the F_1 parents were heterozygous at these loci. One indel, OCC-37, was polymorphic within YCT and mapped to a specific location within RYHyb20. All hybrid linkage groups contained at least one species-specific diagnostic marker. Linkage groups RYHyb21 and RYHyb11, both metacentric chromosomes, were each assigned a single diagnostic species marker indicating that only one of the two chromosome arms in both linkage groups had a species-specific marker assigned.

Homeologies

Using duplicated loci, we observed six homeologous hybrid linkage groups (RYHyb12q/RYHyb13q, RYHyb13p/RYHyb17p, RYHyb14p/RYHyb29, RYHyb15q/RYHyb21q, RYHyb10q/RYHyb19p, and RYHyb06p/RYHyb26) that have been previously identified within RBT (Phillips *et al.* 2006). We also observed one homeologous pairing that has not been observed in rainbow trout (RYHyb03 centromere region/RYHyb22q). Additionally, for 10 other duplicated loci, only one homeologue could be scored confidently. Linkage groups RYHyb13q and RYHyb19p each contained two of these loci, and RYHyb02p, RYHyb02 centromere region, RYHyb10q, RYHyb10 centromere region, RYHyb18p, and RYHyb21q each contained one of these loci.

Pseudolinkage

We found eight pseudolinkage groups exclusive to both male maps (RYHyb01/RYHyb23, RYHyb02/RYHyb03, RYHyb06/RYHyb26, RYHyb07/RYHyb18, RYHyb10/RYHyb19, RYHyb15/RYHyb21, RYHyb12/RYHyb13, and RYHyb13/RYHyb17). Pseudolinkage was represented in males by statistical linkage between markers that mapped to independent linkage groups in the two female maps. All cases of pseudolinkage were between chromosomes identified as homeologous within RBT (Phillips *et al.* 2006).

Recombination rates

Females within both families had a significantly higher recombination rate across the genome than males. The female to male recombination ratio in Family 1 and Family 2 was 6.92 (*P*<0.001, G-test) and 5.65 (*P*<0.001, G-test), respectively (Table 3.2). Females had significantly higher recombination rates across each linkage group than males within at least one family (*P* < 0.001, G-test), with the following exceptions. Female and male recombination rates were not different across RYHyb24, and the female to male recombination ratios could not be estimated across RYHyb11, RYHyb14, and RYHyb23 because male pairwise recombination values were zero for all corresponding female pairwise comparisons. The recombination rate was not different between mapping parents of the same sex across the genome.

We identified 13 linkage groups where recombination rates differed significantly between the female consensus F_1 hybrid and RBT maps (Supplementary material S3.4). We treated RYHyb20 as two separate linkage groups, noted as RYHyb20(Omy20) and RYHyb20(Omy28), because Omy20 and Omy28 are not fused in RBT. Further, RYHyb25 and RYHyb29 represent a fusion/fission polymorphism in the female maps, and so we treated these groups as independent in Female 1 and used the RBT linkage group in Guyomard *et al.* (2006) for comparison. We also treated RYHyb25_29 as a metacentric linkage group in Female 2 and used the map of Rexroad *et al*. (2008) for comparison.

We found five instances of suppressed and one instance of elevated recombination distance across large numbers of loci within several linkage groups in the female hybrid map relative to the female RBT map (Supplementary material S3.4) (Figure 3.3). All instances of recombination suppression mapped across centromeres. Linkage group RYHyb11, a metacentric chromosome in RBT, was the shortest linkage group in the female hybrid map. The recombination distance across the hybrid map relative to the RBT map (relative hybrid:RBT recombination distance) was 0.21, indicating the recombination rate was suppressed across all loci mapped in RYHyb11. Linkage group RYHyb20(Omy20) had a relative hybrid:RBT recombination distance of 0.46. The reduction in map distance appeared to be due to six non-recombining loci in the hybrid map, whereas the same six loci mapped over 40 cM to the *p*- and *q*-arms in the RBT map. In contrast, the relative hybrid:RBT recombination distance on the other arm of the hybrid map, RYHyb20(Omy28), appeared elevated across the four loci closest to the telomere in the hybrid map (2.44). However, the recombination distance appeared suppressed in the hybrid map between the two markers closest to the centromere. Recombination suppression near the centromere was likely associated with the fusion to RYHyb20(Omy20). Linkage group RYHyb25_29 had a relative hybrid:RBT recombination distance of 0.59; however, we were unable to include the majority of the q-arm in the comparison. We observed five non-recombining loci in the hybrid map, whereas the same five loci mapped over 40 cM in the RBT. In contrast, the single-armed linkage groups RYHyb25 and RYHyb29 had relative hybrid:RBT recombination distance of 0.82 and 1.12, respectively. The reduced relative map distance in RYHyb25_29 compared to RYHyb25 and RYHyb29 suggests that the fusion between the two chromosome arms caused a reduction in the recombination rate across the centromere. Linkage group RYHYb15 had a relative hybrid:RBT recombination distance of 0.66. Although the hybrid map had a reduced recombination distance across the centromere compared to the RBT map, the recombination distance was greater in the hybrid map than in the RBT map near telomeres on both chromosome arms. Linkage group RYHyb14 had a relative hybrid:RBT recombination distance of 0.59; however, we were unable to include the majority of the q-arm in the comparison.

Segregation distortion

Analyses based on a sliding window revealed that 27 of the 286 total 25 cM intervals among all parent-specific maps contained YCT allele frequencies that deviated significantly from Mendelian expectations (Supplementary material S3.2) (Table 3.2).The proportion of 25 cM intervals deviating significantly within each parent-specific map was as follows: Female 1, 9/104; Female 2, 8/107; Male 1, 4/35; and Male 2, 6/40. We found no consistent distortion in allele frequencies among all parents. However, both females showed a significant reduction in YCT allele frequencies in the same region on RHYb01p and both males showed a significant increase in YCT allele frequencies across both RYHyb09 arms (Figure 3.4). Interestingly, homeologues within pseudolinkage groups RYHyb07/RYHyb18 and RYHyb06/RYHyb27 had inverse proportions of YCT allele frequencies in Male 2. Linkage group RYHyb07 had significantly less, and homeologue RYHyb18 had significantly greater, YCT allele frequencies than expected. Similarly, RYHyb27 had significantly less, and the homeologue RYHyb06 had a trend for greater (although not significantly so), YCT allele frequencies than expected.

We found 15 linkage groups where genotypic segregation distortion occurred at $P < 0.05$ (Supplementary material S3.5). Approximately 6 % of the total loci genotyped were distorted at this significance level. After applying B-Y FDR corrections for multiple tests, the observed genotypic frequencies differed significantly from expected frequencies within only four linkage groups (Supplementary material S3.5), and represented approximately 1.6 % of the total loci genotyped. Linkage groups RYHyb09 and RYHyb18 contained loci with an excess of YCT/YCT genotypes, and RYHyb06 and RYHyb07 contained loci with an excess of RBT/RBT genotypes. Interestingly, none of the 309 loci mapped in common between the two families showed significant genotypic distortion within both families.

Discussion

The genetic linkage maps established for YCT-RBT hybrids provide novel insights into the genomic consequences of introgression between RBT and YCT. Hybrid and RBT linkage groups were syntenic and had similar marker order, suggesting that RBT and YCT share chromosome arms. In addition, hybrids and RBT shared linkage groups, with one exception where a hybrid linkage group involved a fusion between a bi-armed metacentric and a single-armed acrocentric RBT chromosome. This result was confirmed by additional mapping in YCT. Comparison between hybrid and RBT female maps indicated that introgression suppressed recombination across several large chromosome segments. Two hybrid linkage groups showing recombination suppression (RYHyb20 and RYHyb25_28) contained different chromosome arrangements between YCT and RBT. Segregation distortion was generally limited and distortion patterns varied among parents and families, suggesting that few incompatibilities exist between RBT and YCT genomes. Taken together, these results suggest that RBT and YCT genomes freely introgress, with the exception that differences in chromosome rearrangements between the species could impede introgression across large portions of specific linkage groups.

Chromosome rearrangements between RBT and YCT

The hybrid map is a product of differences in chromosome rearrangements between RBT and YCT, as well as mixed ancestry of the RBT source stock. Using data from karyotypes (Loudenslager and Thorgaard 1979, Thorgaard 1983) and the hybrid map, we suggest that RBT and YCT differ by at least five species-specific chromosome rearrangements. We first consider the fusion between Omy28 and Omy20q in YCT. The most parsimonious explanation for the fusion between the metacentric Omy20 and the acrocentric Omy28 involves two rearrangements. The first rearrangement would have occurred prior to the Omy20-Omy28 fusion as a pericentric inversion of the entire q-arm of Omy20. This inversion would have resulted in Omy20 becoming an acrocentric chromosome and a reduction in the expected 52 haploid chromosome arms (Loudenslager and Thorgaard 1979, Thorgaard 1983) to the observed 51 haploid arms in the hybrid map. The possibility that Omy20q could be inverted in YCT relative to RBT is supported in Chinook salmon (*O. tshawytscha*), as the Omy20 homologue in Chinook is an acrocentric chromosome and the segment homologous to Omy20q appears inverted compared to RBT (unpublished observations, K. Naish). The second rearrangement would have been a Robertsonian type involving centric fusion between the acrocentric Omy20 homologue and Omy28, conserving chromosome arm number. The three remaining differences may be explained by comparing acrocentric chromosome numbers between RBT (seven in the 60 chromosome race) and YCT (twelve). The seven RBT acrocentric chromosomes have been identified as such in the hybrid map, suggesting that they have an acrocentric YCT homologue. The remaining five YCT acrocentric chromosomes likely have homologs with RBT metacentric chromosomes. The third and fourth rearrangement differences probably represent centric fusion/fission events between two RBT metacentric and four of the five remaining YCT acrocentric chromosomes, which would not change the chromosome arm number. The fifth rearrangement difference may have involved a fission event within a RBT metacentric chromosome arm, generating the fifth acrocentric and a submetacentric chromosome in YCT. Fission within a RBT metacentric arm would gain one YCT chromosome arm and restore the 52 haploid chromosome arm number, countering the loss of a chromosome arm by the pericentric inversion.

The variable number of linkage groups identified in the hybrid maps (28 and 29) is likely due to mixed ancestry of the source RBT stock. Linkage analysis in YCT indicated that the Omy25 and Omy29 homologues each represent acrocentric linkage groups in YCT, but these chromosomes are known to be a Robertsonian polymorphism in RBT (Thorgaard 1983, Phillips *et al.* 2005, Phillips *et al.* 2006). The Kamloops stock at Hayspur Hatchery appears to include ancestry from inland RBT (2N = 58; Omy25 and Omy29 are fused as a metacentric chromosomes) as well as the common hatchery RBT derived from coastal California (2N = 60; Omy25 and Omy29 are acrocentric chromosomes) (R. F. Leary, Montana Fish, Wildlife and Parks, personal communication). Admixture between the two RBT stocks would generate Robertsonian metacentric (2N = 58) and acrocentric (2N = 60) polymorphs, as well as Robertsonian heterozygotes (2N = 59). Subsequent hybridization between the polymorphic Kamloops stock and YCT would produce F_1 hybrids that were Robertsonian heterozygotes (for example, Female 2 and Male 1) comprised of 28 linkage groups, containing the RBT metacentric fusion Omy25_Omy29 and the YCT acrocentric homologues to Omy25 and Omy29, as well as Robertsonian acrocentric homozygotes (for example, Female 1 and Male 2) comprised of 29 linkage groups, containing RBT acrocentric chromosomes Omy25 and Omy28 and their YCT homologues (Figure 3.2).

Recombination suppression

Conclusions based on recombination differences between the hybrid and RBT maps have limitations, as rates can vary between related species (Smukowski and Noor 2011), as well as among individuals within species (Sakamoto *et al.* 2000, Danzmann *et al.* 2005, Gharbi *et al.* 2006). Indeed, we found significant differences in pairwise recombination rates between the two RBT maps (Guyomard *et al.* 2006, Rexroad *et al.* 2008) used to construct the female consensus map across Omy5, Omy8, Omy14, Omy19, Omy20, and Omy22. Furthermore, differences in marker density among hybrid and RBT maps could also account for recombination differences by affecting map distance estimates. However, our objective was to perform a comparative analysis to identify recombination suppression broadly across the genome. Because recombination frequency appears correlated with chromosome arm number (Pardo-Manuel de Villena and Sapienza 2001) and broad-scale recombination rates tend to be conserved between closely related species (Smukowski and Noor 2011), we might expect similar recombination rates between RBT and YCT. Therefore, although absolute differences between map distances can be affected by number of markers and number of individuals mapped, the trend revealed by the comparative analyses yields interesting insight into recombination suppression in the hybrids, which could indicate the presence of chromosome rearrangements or genic incompatibilities.

Chromosome rearrangements can generate recombination suppression in heterokaryotypes (chromosomal hybrids) through unbalanced gametes which results in non-recombinants being the only viable gametes (Griffiths *et al.* 2005), or by restricting recombination between rearrangements (Borodin *et al.* 2008). Although unbalanced gametes cannot be ruled out, we consider them an unlikely cause of suppression in the F_1 hybrids. First, conservation of synteny and marker order between the hybrid and RBT maps (Guyomard *et al.* 2006, Rexroad *et al.* 2008) suggests that YCT and RBT chromosomes are highly collinear, which would facilitate normal pairing between homologues and alternate disjunction in F_1 hybrids. Second, because RBT and YCT contain the same number of chromosome arms, Robertsonian type rearrangements are considered to have played a significant role in generating the chromosome number differences between the species (Phillips *et al.* 2006). Meiotic pairing between homologues that differ by a Robertsonian rearrangement (i.e., pairing between a metacentric chromosome and the two acrocentric homologues) would produce a trivalent in the F_1 hybrids, which would not necessarily cause malsegregation and unbalanced gametes. For example, the rate of nondisjunction was not different in individuals that were heterozygous for Robertsonian rearrangements compared to homozygotes in pink salmon (*O. gorbuscha*) (Phillips and Kapuscinski 1987), house mouse (*Mus musculus domesticus*) (Britton-Davidian *et al.* 1990), and Eurasian common shrew (*Sorex araneus*) (Banaszek *et al.* 2002), suggesting this type of rearrangement produces balanced gametes.

Recombination was restricted in the F_1 hybrids across several loci spanning chromosome fusion and fission differences between RBT and YCT; the Robertsonian fusion/fission within RYHyb25_29 and

RYHyb20. This suggests that chromosome rearrangements did indeed suppress recombination. Rearrangements generate extensive linkage disequilibrium in heterokarotypic hybrids (Rieseberg *et al.* 2000) and suppress recombination across genomic regions that extend beyond rearrangements (Panithanarak *et al.* 2004, Machado *et al.* 2007, McGaugh and Noor 2012). Therefore, broad-scale recombination suppression across other linkage groups in the F_1 hybrids could indicate the presence of rearrangements. We suggest that three other metacentric hybrid linkage groups, RYHyb11, RYHyb14, and RYHyb15, contained chromosomes that differed by arrangement between RBT and YCT. These linkage groups might represent Robertsonian rearrangements because recombination was suppressed across putative centromeres which could indicate that F_1 hybrids were heterozygous for centric fusions/fissions. However, we found marker order differences within the suppressed regions in each of these three linkage groups compared to RBT linkage maps (Guyomard *et al.* 2006, Rexroad *et al.* 2008), which could possibly indicate inversions or translocations, or, alternatively, be due to reduced mapping power in regions with low recombination or the number of progeny used to construct the various maps.

Significant differences in recombination rates between several hybrid and RBT linkage groups could indicate the presence of inter-specific, genic incompatibilities. However, our broad-scale analysis hinders inference across smaller genomic scales where incompatibilities have been reported (Ting *et al.* 2000, Rieseberg 2001, Phadnis and Orr 2009). Nevertheless, we found numerous instances where two adjacent markers did not recombine in the female-merged hybrid map, but these same markers recombined in the consensus female RBT map. This might suggest that recombination between particular YCT and RBT genomic regions is maladaptive, or that the difference in adjacent recombination is an artifact of the number of offspring genotyped. Finer scale mapping across these regions that differ in recombination rate could be fruitful for identifying the presence of incompatibilities. Inter-specific incompatibilities may also be inferred from segregation distortion, as genotypes that occur less often than expected may be incompatible (Rieseberg *et al.* 2000, Danzmann and Gharbi 2001). In addition, we expected that inter-specific incompatibilities would show consistent distortion among maps. For example, consistent allelic distortion within both RYHyb01 female hybrid maps and both RYHyb09 male hybrid maps could indicate the presence of incompatibilities. Although segregation distortion was present in several other linkage groups, a lack of consistency across hybrid maps suggests these distorted loci do not reflect genic incompatibilities between RBT and YCT.

Recombination suppression within Robertsonian rearrangements

The recombination suppression pattern across the Robertsonian rearrangement RYHyb25_29 and the presumed Robertsonian rearrangement RYHyb15 differed from patterns reported between chromosome races in the house mouse (Bidau *et al.* 2001, Dumas and Britton-Davidian 2002) and common shrew (Borodin *et al.* 2008). Recombination in mice and shrews that were homozygous for Robertsonian fusions (i.e., homozygous for the metacentric polymorphism) appeared suppressed near centromeres and elevated toward telomeres in comparison to Robertsonian heterozygotes (Bidau *et al.* 2001, Borodin *et al.* 2008). However, we observed the opposite; recombination was suppressed in Robertsonian heterozygotes (i.e., hybrids) across the putative centromere and elevated toward telomeres in comparison to fusion homozygotes (i.e., the female RBT consensus map). The mechanisms causing the difference between our results and the house mouse and common shrew are unclear. Given the approximate 3 million year divergence time between RBT and YCT (Wilson and Turner 2009) and the absence of historical secondary contact, genomic differences could have evolved near the centromeres of rearranged chromosomes that would restrict crossover events in Robertsonian heterozygotes, such as para- or pericentric inversions or genic incompatibilities. Alternatively, mechanistic processes governing meiotic crossover could differ among taxa. Determining how recombination suppression in the Omy25-Omy29 Robertsonian rearrangement differs between intra-specific (RBT chromosome races) and inter-specific hybrids could indicate the efficiency of this type of rearrangement as a barrier to gene flow in salmonids.

Rearrangements protect genomic regions from recombination

Our results suggest that chromosome rearrangement is the main genomic obstacle for gene exchange between RBT and YCT. Rearrangements have been observed to reduce gene flow between several species pairs, including *Drosophila pseudoobscura* and *D. persimilis* (McGaugh and Noor 2012), *Helianthus petiolaris* and *H. annuus* (Rieseberg and Linder 1999), and *S. araneus* and *S. antinorii* (Basset *et al.* 2006, Yannic *et al.* 2009). Rearrangements protect genomic regions from recombination, enabling genes within or closely linked to the rearranged genome to differentiate between heterokaryotypes while unrestricted gene flow occurs between regions with similar composition (Noor *et al.* 2001, Rieseberg 2001). As a result, fitness related genes could accumulate within regions that are protected from recombination and diverge in the face of hybridization (Noor *et al.* 2001, Rieseberg 2001). Indeed, genes involved with reproductive isolation have mapped to chromosome rearrangements (Noor *et al.* 2001, Feder *et al.* 2003). The effectiveness of rearrangements as recombination suppressors may be dependent on how chromosomes are reorganized; rearrangements that change gene order (e.g., inversions or translocations) may be more effective in protecting the genome from being disrupted than rearrangements that do not change gene order (e.g., fusions or fissions). However, several studies indicate that Robertsonian type rearrangements restrict gene flow (Panithanarak *et al.* 2004, Basset *et al.* 2006, Yannic *et al.* 2009, Franchini *et al.* 2010), but with extreme interbreeding this type of rearrangement may be an ineffective barrier (Panithanarak *et al.* 2004).

Given the hybrid linkage map results, we predict that, within introgressed populations, inter-specific recombination will be restricted in particular genomic regions where chromosome arrangement differs between YCT and RBT. This prediction is supported by studies that have reported reduced gene flow across chromosome rearrangements within stable hybrid zones (Rieseberg and Linder 1999, Panithanarak *et al.* 2004, Yannic *et al.* 2009, Franchini *et al.* 2010). Because the majority of hybrid linkage groups appeared to have similar marker order and arrangement with RBT, few chromosomes would be expected to contain regions with restricted recombination, although suppression could potentially extend broadly across these chromosomes (Panithanarak *et al.* 2004, Machado *et al.* 2007, McGaugh and Noor 2012). Within a long standing hybrid zone between house mouse chromosome races, linkage disequilibrium persisted among loci mapping near Robertsonian rearrangements (Panithanarak *et al.* 2004), indicating that these types of rearrangements can generate and maintain linkage disequilibrium. Given that RBT-YCT introgression is recent and rearrangements produce extensive linkage disequilibrium (Rieseberg *et al.* 2000), large genomic regions that flank centric fusion/fission differences could be expected to remain intact within some YCT chromosomes. As a consequence, recombination suppression between heterokaryotypes could protect genomic regions from being disrupted and enable co-adapted gene complexes and/or local adaptations linked to the rearrangements to persist within YCT admixed with RBT.

Segregation distortion mechanisms

The low level of segregation distortion observed in the RBT-YCT hybrid maps was unexpected. Several studies have reported high levels of segregation distortion, greater than 15% of loci at $P < 0.05$, within hybrid maps across a variety of taxa, including interspecific crosses between *Mimulus guttatus* and *M. nastutus* (Fishman *et al.* 2001), *Lepomis cyanellus* and *L. megalotis* (López-Fernández and Bolnick 2007), and *Nasonia giraulti* and *N. vitripennis* (Gadau *et al.* 1999) and between intraspecific crosses of *Salvelinus alpinus* (Woram *et al.* 2004), *M. guttatus* (Hall and Willis 2005), *Ceratodon purpureus* (McDaniel *et al.* 2007), *Coregonus clupeaformis* (Rogers *et al.* 2007), and *Tigriopus californicus* (Pritchard *et al.* 2011). Divergence time estimates between parental lineages used for several of these maps ranged from 0.1-0.2 MYA to 13-16 MYA (Campbell *et al.* 1994, Bernatchez *et al.* 1999, Brunner *et al.* 2001, Near *et al.* 2005). We would expect comparable distortion levels to these studies following the estimated 3 million year divergence time between RBT and YCT. Our findings are probably not due to a lack of power for detecting distortion, because we mapped a similar number of markers per linkage group as the studies above, although the number of individuals per mapping panel was less than these studies, except Woram *et al*. (2004). Nevertheless, it is intriguing that segregation distortion was limited and few consistent distortion patterns emerged between sexes and families. The low level of distortion suggests that few incompatibilities exist between RBT and YCT, and may partially explain why these species readily form hybrid swarms.

Although segregation distortion was limited, we found a few noteworthy cases. A variety of mechanisms may cause distortion (Danzmann and Gharbi 2001, Pardo-Manuel de Villena and Sapienza 2001, Taylor and Ingvarsson 2003). Understanding the causes of distortion typically requires in-depth study, but we discuss two mechanisms. First, pseudolinkage likely caused allelic distortion within two homeologous linkage groups in Male 2 (RYHyb07/RYHyb18 and RYHyb06/RYHyb27). Pseudolinkage may be implicated when homeologous linkage groups both show allelic segregation distortion (Danzmann and Gharbi 2001), and we observed this distortion in YCT allele frequencies. Second, meiotic drive could have caused distortion. Meiosis is asymmetric in females and results in one functional haploid gamete per germ cell, compared to males where symmetric meiosis results in four functional gametes per germ cell (Pardo-Manuel de Villena and Sapienza 2001). Therefore, any process that results in non-random segregation of chromosomes during oogenesis may lead to distortion in females. Female meiotic drive is characterized by competition among centromeres for spindle fiber attachment during meiosis and oogenesis (Pardo-Manuel de Villena and Sapienza 2001). Because the centromere on one chromosome may outcompete the centromere on the homologue for deposition into the oocyte, allele frequencies in female maps may be distorted at loci mapping near centromeres (Fishman and Willis 2005). Male meiotic drive is characterized by competition between alleles during sperm development and results in differential gamete success. Genomic divergence between species could escalate competition between meiotic drive elements, causing segregation distortion in their hybrid progeny (Fishman and Saunders 2008). Consistent distortion patterns within RYHyb01 between female hybrid maps and within RYHyb09 between male hybrid maps suggest that meiotic drive could be acting within the hybrids.

Sex chromosomes

Conservation of synteny with the RBT sex chromosome, Omy1, suggests that RYHyb28 is the sex-linkage group. Indeed, sex has been established as mapping to homologs in YCT and RBT (Alfaqih *et al.* 2008). Conservation of sex-linkage groups is uncommon across several salmonid species (Woram *et al.* 2003, McClelland and Naish 2008, Davidson *et al.* 2009, Li *et al.* 2011). Two alternative mechanisms have been proposed to account for the lack of conservation among sex-linkage groups in salmonids: either the sex-determining gene is the same among species but has moved to different chromosomes in different lineages, or sex determination has evolved independently in different lineages (Davidson *et al.* 2009). Regardless of the mechanism, the lack of homology among sex chromosomes could be an important factor for restricting inter-specific hybridization, and could explain why hybridization is not commonly observed between many sympatric salmonid species and, conversely, why two species pairs identified as having homologous sex-linkage regions, YCT and RBT (Alfaqih *et al.* 2008) and Arctic charr

(*S. alpinus*) and brook charr (*S. fontinalis*) (Timusk *et al.* 2011), introgress in the wild (Hammar *et al.* 1991, Gunnell *et al.* 2008). The relationship between conservation of sex chromosomes and introgression is confounded in brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) because their sex chromosomes lack homology (Li *et al.* 2011), yet the species hybridize in the wild (Verspoor and Hammar 1991). However, brown trout and Atlantic salmon hybrids suffer reduced viability (McGowan and Davidson 1992) and introgression is rare (Verspoor and Hammar 1991). Nevertheless, homology between sex-linkage groups could possibly facilitate introgression between RBT and YCT.

Conclusions

Our results are consistent with a growing number of studies demonstrating that chromosome rearrangements reduce gene flow by suppressing recombination (Rieseberg and Linder 1999, Panithanarak *et al.* 2004, Basset *et al.* 2006, Machado *et al.* 2007, Yannic *et al.* 2009, Franchini *et al.* 2010, McGaugh and Noor 2012). Although much of the RBT and YCT genome appears porous to gene exchange, our study indicates that chromosome rearrangements between RBT and YCT act as genomic obstacles to introgression. As a consequence, chromosome arrangements could have a significant influence on the evolution of YCT-RBT hybrid genomes. For example, within admixed populations, rearrangements could protect particular YCT genomic regions from RBT introgression, enabling large segments of the YCT genome to remain intact. If fitness related genes are linked to these rearrangements, recombination suppression could preserve them from being disrupted. This could provide an adaptive advantage to hybrids that contain these fitness related genes and enable these genes to persist within admixed populations. In contrast, unobstructed gene flow between chromosomes with similar arrangement would disrupt linkage associations within each species and create new genetic variation for selection to act upon.

The genetic linkage map established herein for YCT-RBT F_1 hybrids provides an initial framework for investigating hybridization and is likely relevant to other cutthroat trout subspecies that introgress with RBT, and, therefore, may serve as a general model of genomic introgression. In addition, our study defines a set of genome-wide species markers that can be applied to conservation and management of indigenous YCT.

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Tables

Table 3.1. Hybrid linkage groups and alignment to rainbow trout chromosome (Omy) and linkage group (RT) arms (*p* and *q*).

Hybrid linkage group	Inferred linkage group structure	Omy arms	RT arms
RYHyb01	Metacentric	Omy01 (p, q)	RT6 (p, q)
RYHyb02	Metacentric	Omy02 (p, q)	RT27 (p, q)
RYHyb03	Metacentric	Omy03 (p, q)	RT31 (p, q)
RYHyb04	Metacentric	Omy04 (p, q)	RT24 (p, q)
RYHyb05	Metacentric	Omy05 (p, q)	RT8 (p, q)
RYHyb06	Metacentric	Omy06 (p, q)	RT10 (p, q)
RYHyb07	Metacentric	Omy07 (p, q)	RT12 (p, q)
RYHyb08	Metacentric	Omy08 (p, q)	RT23 (p, q)
RYHyb09	Metacentric	Omy09 (p, q)	RT21 (p, q)
RYHyb10	Metacentric	Omy10 (p, q)	RT20 (p, q)
RYHyb11	Metacentric	Omy11 (p, q)	RT19 (p, q)
RYHyb12	Metacentric	Omy12 (p, q)	RT9 (p, q)
RYHyb13	Metacentric	Omy13 (p, q)	RT2 (p, q)
RYHyb14	Metacentric	Omy14 (p, q)	RT3 (p, q)
RYHyb15	Metacentric	Omy15 (p, q)	RT7 (p, q)
RYHyb16	Metacentric	Omy16 (p, q)	RT22 (p, q)
RYHyb17	Metacentric	Omy17 (p, q)	RT29 (p, q)
RYHyb18	Metacentric	Omy18 (p, q)	RT16 (p, q)
RYHyb19	Metacentric	Omy19 (p, q)	RT14 (p, q)
RYHyb20	Metacentric	Omy20 (p, q)_Omy28(q)	RT17 (p, q)_RT13 (q)
RYHyb21	Metacentric	Omy21 (p, q)	RT15 (p, q)
RYHyb22	Metacentric	Omy22 (p, q)	RT5 (p, q)
RYHyb23	Acrocentric	Omy23 (q)	RT30 (q)
RYHyb24	Acrocentric	Omy24 (q)	RT26 (q)
RYHyb25[1]	Acrocentric	Omy25 (q)	RT4 (q)
RYHyb25_29[1]	Metacentric	Omy25 (q)_Omy29 (q)	RT4 (q)_RT25 (q)
RYHyb26	Acrocentric	Omy26 (q)	RT18 (q)
RYHyb27	Acrocentric	Omy27 (q)	RT11 (q)
RYHyb28	Acrocentric	Omysex (q)	RT1 (q)
RYHyb29[1]	Acrocentric	Omy29 (q)	RT25 (q)

[1] Female 1 and Male 2 were homozygous for the acrocentric chromosomes RYHyb25 and RYHyb29. Female 2 and Male 1 were heterozygotes for the polymorphism, comprising the fused metacentric RYHyb25_29 and the acrocentric chromosomes RYHyb25 and RYHyb29.

Table 3.2. Map distances, in centiMorgans (cM), in female and male merged hybrid maps, female to male recombination ratios (F:M), and significant allelic segregation distortion across 25 cM intervals for each linkage group (sliding window analysis).

Linkage group	Female map distance (cM)	Male map distance (cM)	Family 1 F:M ratio	Family 2 F:M ratio	Significant number of 25 cM intervals[1]
RYHyb01	89.4	3	14.25*	51.17*	↓F1(1), ↓F2(2)
RYHyb02	82.9	19.3	5.22*	35.00*	↓F2(2)
RYHyb03	88	10.8	21.64*	3.63*	↓F1(1)
RYHyb04	106.9	4.3	30.29*	11.31*	
RYHyb05	73.9	44.1 (10.8 + 33.3)[2]	-	2.78*	↓M2(2)
RYHyb06	83	10.2	-	12.25*	
RYHyb07	59	23.9	-	3.03*	↓M2(1)
RYHyb08	84.5 (21.8 + 62.7)[2]	33.8	16.89*	4.66*	↑M1(1)
RYHyb09	88.5	35.1	3.32*	3.59*	↑M1(2), ↑M2(1)
RYHyb10	89.7	3.3	25.58*	-	
RYHyb11	16.7	2.3	-	-	↑M1(1)
RYHyb12	77.4	27.5	17.37*	6.24*	↓F1(3)
RYHyb13	85.9	13.3	8.00*	-	↓F2(3)
RYHyb14	46.4 (46.4 + 0)[2]	1.4	-	-	
RYHyb15	66.5	22	6.29*	13.13*	
RYHyb16	83	39.2	6.21*	4.89*	
RYHyb17	97.4	7	2.25*	-	
RYHyb18	92.9	5.1	27.90*	3.5	↑M2(1)
RYHyb19	79.5	24	8.81*	-	↑F1(2)
RYHyb20	79.2	11.7	7.81*	7.58*	
RYHyb21	58	15.8	8.01*	23.30*	
RYHyb22	67.8	56.3	2.30*	1.78*	↑F1(2)
RYHyb23	64.6	0	-	-	
RYHyb24	41.8	22.6	-	0.67	
RYHyb25	32.1	31.5	1.21*	0.79	
RYHyb25_29	76.1 (54.8 + 21.3)[2]	46.9 (8.6 + 38.3)[2]	1.91*	1.54*	
RYHyb26	44.2	13.3	0.67	22.40*	↑M2(1)
RYHyb27	39.4	1.8	29.60*	-	↓F2(1)
RYHyb28	56.2	7.9	9.90*	6.75*	
RYHyb29	44.6	40.3	3.60*	1.60*	
Genome wide[3]	2019.1	518.35	6.92*	5.65*	

Dashed line (-) indicates that the recombination rate in the male parent was zero and therefore undefined.

*Significant G-tests at $P < 0.001$ following corrections for multiple tests.

[1] Arrows indicate significantly greater (↑) or less (↓) YCT allele frequencies than expected within each parent–specific map (F1 = Female 1, F2 = Female 2, M1 = Male 1, and M2 = Male 2). The number in parentheses indicates the number of significant 25 cM intervals within a parent map.

[2] Map distance is the product of the two groups in parentheses.

[3] The genome wide map distance was calculated using the average map distance across RYHyb25 + RYHyb29 and RYHyb25_29 within each sex (i.e., $((RYHyb25 + RYHyb29) + RYHyb25\_29)/2$).

Figures

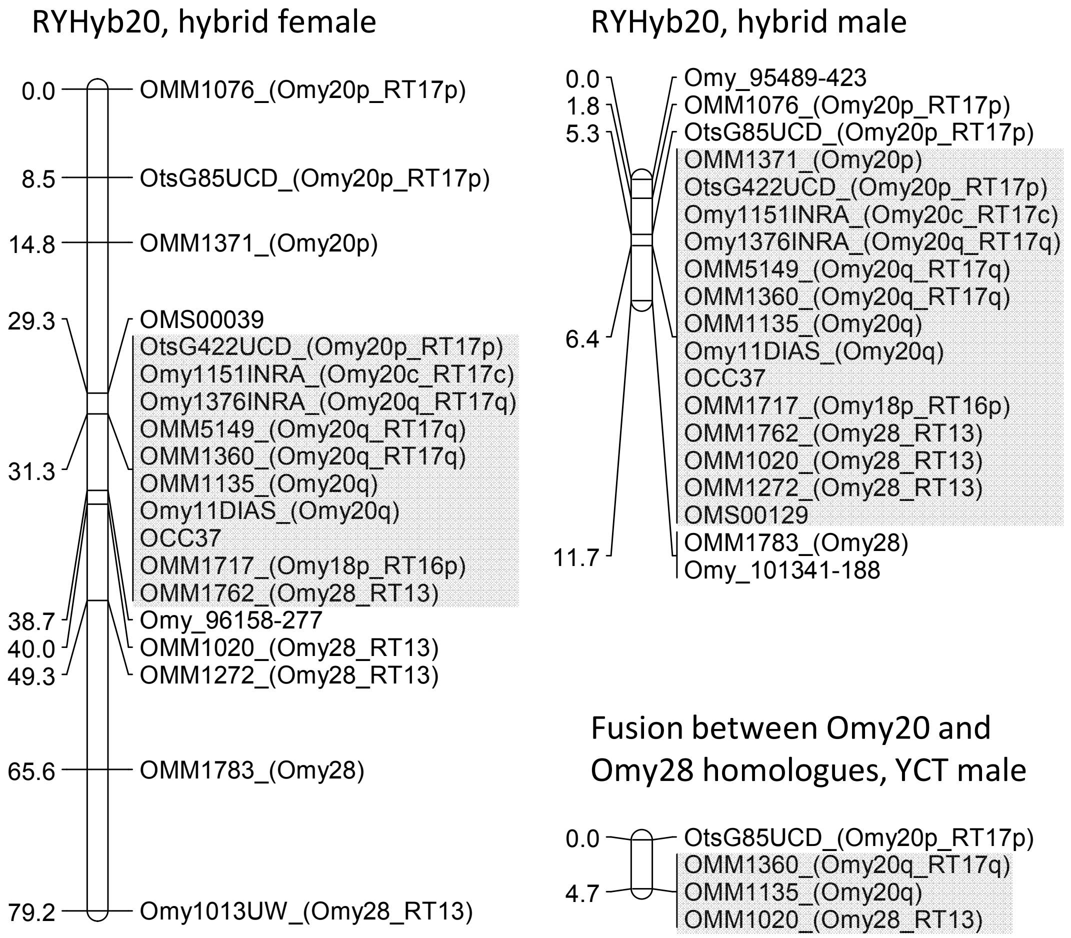

Figure 3.1. Hybrid linkage group RYHyb20 showing the fusion between rainbow trout chromosomes Omy20 and Omy28. Female- and male-merged hybrid maps are shown. The grey shaded area represents a block of non-recombining loci spanning the *p*-arm, centromere, and *q*-arm of Omy20 and the acrocentric Omy28. The fusion between the Omy20 and Omy28 homologues in a male Yellowstone cutthroat trout (YCT) map, identified by applying four loci in common to the hybrid and rainbow trout maps, is also shown. The rainbow trout chromosome (Omy), linkage group (RT), and chromosome arm (*p*, *q*) or centromere (c) location for each locus mapped in rainbow trout by Guyomard *et al*. (2006) and Rexroad *et al*. (2008) is indicated. Map distances are in centiMorgans.

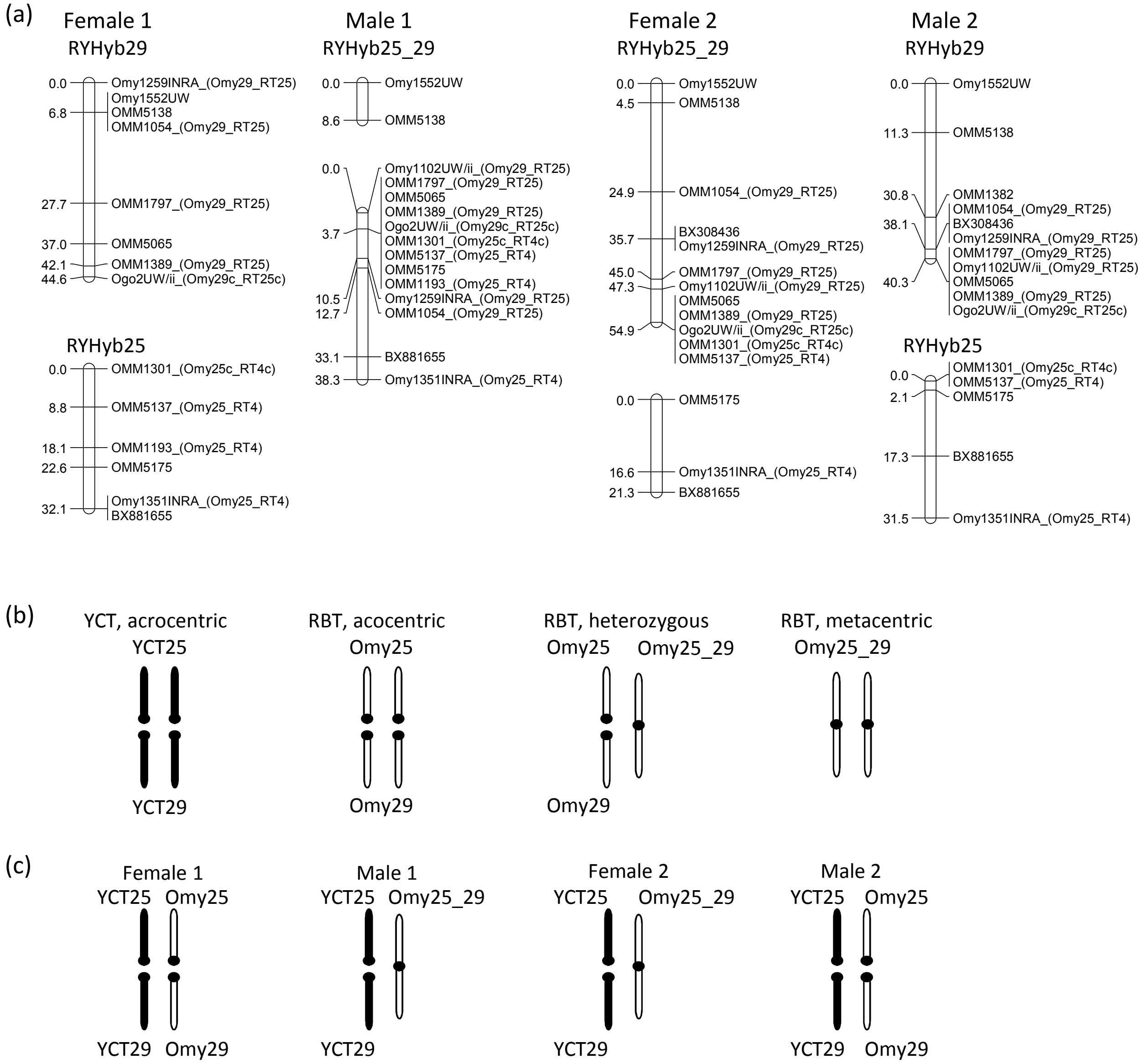

Figure 3.2. Robertsonian polymorphism between RYHyb25 and RYHyb29. (a) Linkage groups RYHyb25 and RYHyb29 represent acrocentric chromosomes in Female 1 and Male 2 while RYHyb25_29, a metacentric chromosome, represents a fusion between the two acrocentric chromosomes in Male 1 and Female 2. Chromosome (Omy), linkage group (RT), and putative centromere (c) location for each locus mapped in Guyomard *et al*. (2006) is indicated. Map distances are in centiMorgans. (b) Possible diploid chromosome constitutions for the Omy25 and Omy29 Robertsonian polymorphism in the rainbow trout (RBT) parent stock (acrocentric, 2N = 60; heterozygous, 2N = 59; or metacentric 2N = 58) and the Yellowstone cutthroat trout (YCT) homologues (YCT25 and YCT29). RBT chromosomes are in white and YCT chromosomes are in black. (c) Inferred diploid chromosomal constitutions for F_1 hybrid parents. YCT crossed by RBT with either the acrocentric or heterozygous polymorphism would yield RYHyb25 and RYHyb29 in Female 1 and Male 2, and YCT crossed by RBT with either the metacentric or heterozygous polymorphism would yield RYHyb25_29 in Male 1 and Female 2.

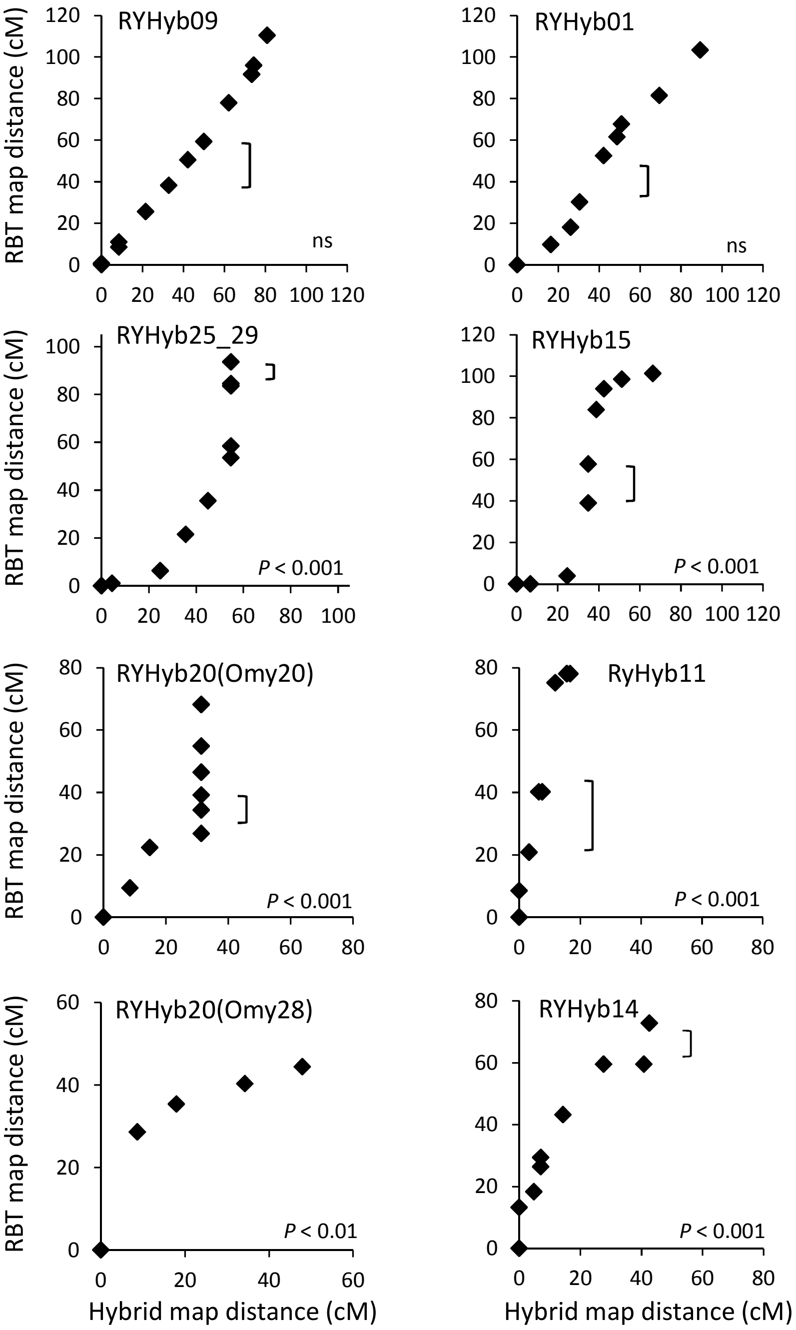

Figure 3.3. Map distance comparison between the female-merged F_1 hybrid map and the female consensus RBT map. Selected linkage groups show map distance comparisons in centiMorgans (cM) across the same markers in the F_1 hybrid map (X-axis) and the rainbow trout (RBT) map (Y-axis). Black diamonds indicate mapped markers (*p* to *q* orientation) and brackets indicate putative centromere locations for metacentric linkage groups identified in Guyomard *et al*. (2006). For acrocentric linkage groups, the marker closest to the centromere is plotted at zero. Significant differences in recombination (*P*-values) between the female-merged F_1 and consensus RBT female maps across linkage groups are indicated (ns, not significant). See Supplementary material S3.4 for comparisons across all linkage groups.

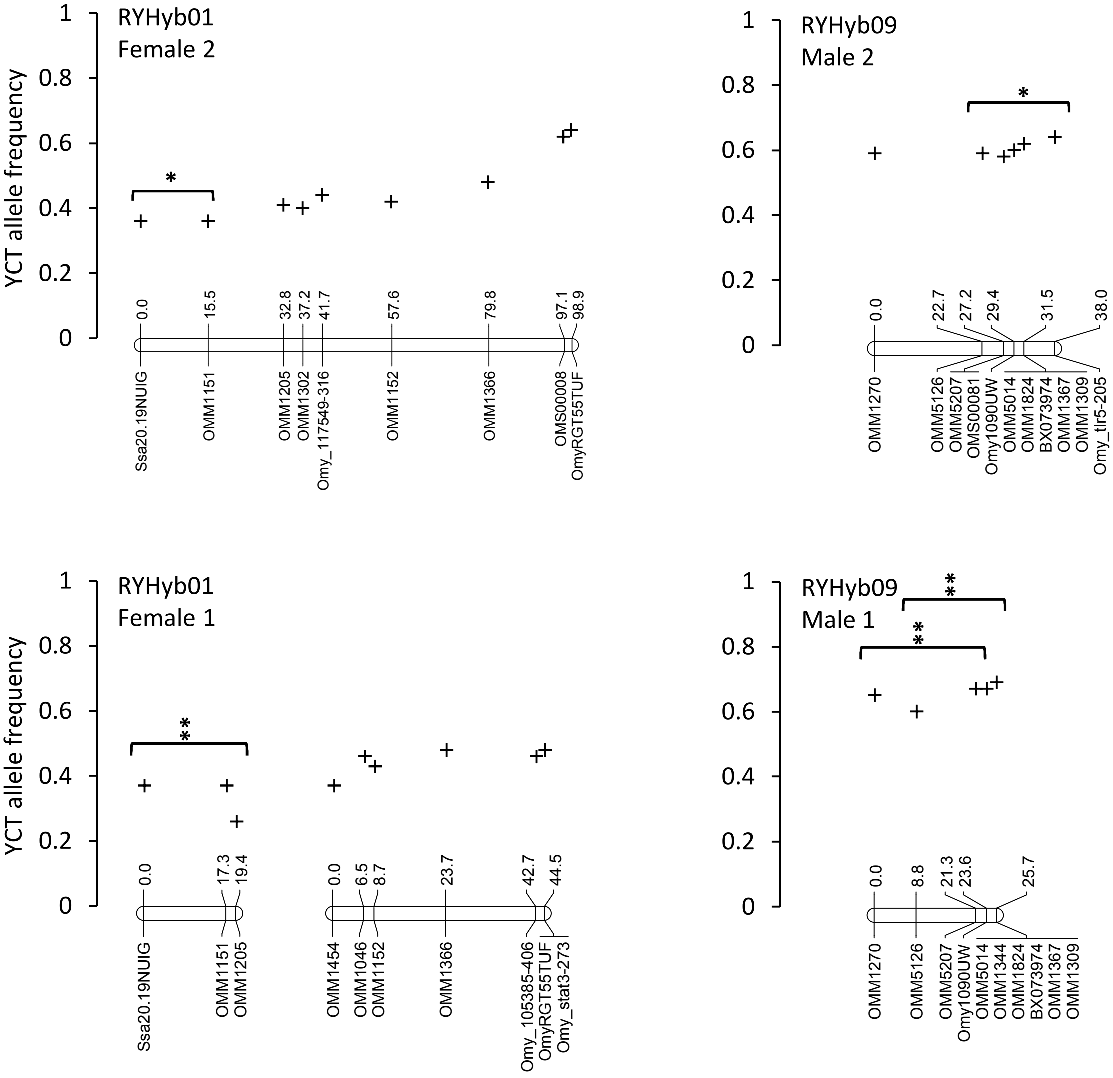

Figure 3.4. Yellowstone cutthroat trout allele frequencies plotted against linkage maps. Selected linkage groups show RYHyb01 in Females 1 and 2 and RYHyb09 in Males 1 and 2. A 25 centiMorgan sliding window was used to identify blocks of loci with Yellowstone cutthroat trout (YCT) allele frequencies that deviated significantly from expected frequencies. Linkage maps are in *p* to *q* orientation. * indicates $P < 0.05$ and ** indicates $P < 0.01$. See Supplementary material S3.2 for YCT allele frequencies within each mapping parent for all linkage groups.

Supplementary Material

S3.1: This file contains numbered worksheets that provide information on mapping loci and linkage maps in Excel file format. The worksheets includes mapping loci and references (worksheet 1), parent-specific linkage maps (worksheets 2-5), female- and male-merged linkage maps (worksheets 6 and 7), sex-merged linkage map (worksheet 8), species diagnostic markers localized to hybrid linkage groups (worksheet 9), and F_1 and RBT consensus maps used to investigate recombination suppression.

S3.2: This PDF file includes figures representing parent-specific F_1 hybrid linkage maps and inferred Yellowstone cutthroat trout (YCT) allele frequencies for each locus.

S3.3: This PDF file includes figures representing female-, male-, and sex-merged F_1 hybrid linkage maps.

S3.4: This PDF file includes figures that compare map distances, in centiMorgans, across the same markers in the female-merged F_1 hybrid linkage map (X-axis) and the female consensus rainbow trout map (Y-axis) for each linkage group.

S3.5: This PDF file includes figures representing inferred genotypic frequency distributions for Yellowstone cutthroat trout homozygotes, rainbow trout homozygotes, and heterozygotes at each locus in Family 1, Family 2, and loci scored in both families combined across each hybrid linkage group.

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Chapter 4: Empirical testing of models of inheritance in the autotetraploid salmonid fishes reveals that incomplete re-diploidization affects allelic inheritance across eight homeologous chromosome arm pairs

Abstract

Diploidization following whole genome duplication involves chromosome rearrangement and loss of duplicated genes. This genome restructuring provides meiotic stability that allows disomic inheritance to be fully or partially restored. Evidence for whole genome duplication in the autotetraploid salmonid fishes includes multivalent formations and undifferentiated distal duplicated loci that occasionally exhibit a mixture of disomic and tetrasomic inheritance. Such duplicated loci have allowed identification of homeologous chromosome arm pairs. Eight of these homeologous arm pairs, involving at least one metacentric chromosome, are conserved in *Oncorhynchus* and have been implicated as likely candidates for homeologous recombination. Here, we used the parental gametic phase from existing genetic linkage maps, both within and between species, to identify the homeologs that recombine, to characterize this recombination, and to verify meiotic models of residual tetrasomic inheritance in autotetraploids. Recombination between homeologs was limited to the eight homeologous chromosome arm pairs conserved in *Oncorhynchus*. We found that recombination between homeologs occurred in males and females, and that recombination was restricted to the distal regions of chromosome arms, verifying the meiotic models for residual tetrasomic inheritance. Homeologous recombination resulted in extensive pseudolinkage in males, affecting the inheritance of alleles across broad chromosomal regions. Pseudolinkage was also found in a female, although the effect was not as extensive. We used our results from an inter-specific hybrid cross to extend the meiotic model for tetrasomy by including double-reduction, multiple crossovers within a homeologous arm pair, and hexavalent formation.

Introduction

Whole genome duplication (WGD) is an important and widespread feature of eukaryote evolution (Amores *et al.* 1998, Jaillon *et al.* 2004, Dehal and Boore 2005, Soltis *et al.* 2007, Van de Peer *et al.* 2009). Polyploidization may allow evolutionary innovations (Ohno 1970), which, in turn, may promote biological complexity, novel adaptations, and diversification (Kassahn *et al.* 2009, Van de Peer *et al.* 2009, Ramsey 2011, Storz *et al.* 2013, Glasauer and Neuhauss 2014, Rensing 2014). Indeed, WGD may have been instrumental in the evolution of several species-rich lineages, including teleost fishes (Jaillon *et al.* 2004) and flowering plants (Jiao *et al.* 2011). Polyploids that are in the process of transitioning to a diploid state (mesopolyploids) present a unique window into the genome restructuring process that may follow a WGD (Mayfield-Jones *et al.* 2013). Studies on mesopolyploids can therefore contribute to a clearer understanding of the evolutionary process that restores genome stability, as well as provide insight into the evolutionary fate of duplicated regions.

Diploidization may restructure the polyploid genome, and thus restore disomic inheritance (Hufton and Panopoulou 2009). This restructuring involves the process of fractionation (loss of one duplicated gene copy) and genome rearrangement, such as chromosome fusions, fissions and pericentric inversions (Langham *et al.* 2004, Adams and Wendel 2005, Hufton and Panopoulou 2009, Schubert and Lysak 2011). These processes can erode similarity among duplicated chromosomes and restore meiotic stability, causing a change from multivalent chromosome pairing (meiotic association of more than two chromosomes resulting in their synapsis in prophase I) and polysomic inheritance to bivalent pairing and disomic inheritance (Mayfield-Jones *et al.* 2013). Diploidization rates are expected to differ between allopolyploids (formed by combination of genomes between species) and autopolyploids (formed by doubling of a genome within species) (Ramsey and Schemske 2002). Allopolyploid chromosomes predominantly form bivalents because the ancestral homeologous chromosomes are sufficiently differentiated; therefore, disomic inheritance can be achieved relatively quickly. In contrast, homeologous chromosomes are initially undifferentiated in autopolyploids. Multivalent pairings may persist until fractionation and rearrangement have caused homeologs to accumulate a sufficient level of divergence to allow bivalent pairings and disomic inheritance. Despite fractionation and rearrangement, some duplicated genes may be preferentially retained long after WGD events (Scannell *et al.* 2007, Berthelot *et al.* 2014), and the number of duplicated genes retained may differ among chromosomes and across regions within a chromosome (Liti and Louis 2005, Brieuc *et al.* 2014, Kodama *et al.* 2014). Furthermore, polysomic inheritance at some duplicated parts of the genome may be retained in mesopolyploids (Danzmann and Bogart 1982, Soltis and Soltis 1993, Allendorf and Danzmann 1997). Uneven distribution of duplicated markers could indicate evolutionary important regions in mesopolyploids, or conversely, evolutionary dead ends.

Fishes of the family Salmonidae evolved from an autopolyploid ancestor that underwent a WGD approximately 85 – 105 million years ago (Macqueen and Johnston 2014). Thus, salmonid fishes are ancestrally autotetraploid. It is presumed that the diploid ancestor of salmonids possessed 48 acrocentric chromosomes (Ohno 1970, Mank and Avise 2006). The genome duplication in salmonids is a fourth round (4R) in a series of WGD events. Two rounds of WGD (1R and 2R) occurred early in the vertebrate lineage (Dehal and Boore 2005) and a third round (3R) WGD occurred early in the teleost lineage (Amores *et al.* 1998, Jaillon *et al.* 2004) approximately 225 – 330 million years ago (Hurley *et al.* 2007, Near *et al.* 2012). Chromosome rearrangements following the salmonid WGD have produced different numbers of acrocentric and metacentric chromosomes among species and even populations (Thorgaard 1983, Ostberg and Thorgaard 1999, Phillips and Rab 2001). In the subfamily Salmoninae, rearrangement has primarily occurred through Robertsonian type centric fusions. This type of fusion has resulted in the relatively similar chromosome arm number (NF = 98-104) across most species, with the exception of Atlantic salmon (*Salmo salar*). Tandem fusions are thought to have contributed to the lower number of chromosome arms in Atlantic salmon (NF= 54-58) (Phillips and Rab 2001, Phillips *et al.* 2009).

Re-diploidization in salmonids is incomplete. Multivalent pairings occasionally form during meiosis (Wright *et al.* 1983, Allendorf and Thorgaard 1984). Residual tetrasomic inheritance (Table 4.1), in which some distal duplicated loci may exhibit disomic and tetrasomic inheritance while proximal loci exhibit strictly disomic inheritance, is occasionally observed (Wright *et al.* 1983, Allendorf and Danzmann 1997).

Both events appear to occur mostly in males (Wright *et al.* 1983, Allendorf and Danzmann 1997, Danzmann *et al.* 2005). Multivalent formation and tetrasomic inheritance at some distal loci suggests diploidization has been achieved quicker in some regions of the salmonid genome than in other regions.

The meiotic model for residual tetrasomic inheritance in salmonids provides a mechanism for the differential rate of diploidization along chromosome arms in the salmonid genome. This model postulates that when multivalents form in prophase I, homologs pair first and may recombine in regions proximal to the centromere; subsequently, homeologs randomly pair and recombine in distal regions (Figure 4.1) (Wright *et al.* 1983, Allendorf and Thorgaard 1984, Allendorf and Danzmann 1997). These events are followed by the independent assortment of parental homeologs to opposite poles of the meiotic cell through alternate disjunction (Table 4.1) during anaphase I. Alternatively, parental homeologs may assort together, through adjacent disjunction (Table 4.1). Alternate disjunction is presumed to be more common than adjacent disjunction in salmonids (Wright *et al.* 1983). Thus, the meiotic for residual tetrasomic inheritance provides an explanation for the observed divergence of homeologs in chromosome arm regions proximal to the centromere, where strictly disomic inheritance occurs (differentiated loci), and the sharing of alleles in distal regions through tetrasomic inheritance (undifferentiated loci). Such a model would allow residual tetrasomy to persist.

Homeologous recombination can result in two intriguing phenomena. The first is pseudolinkage where an excess of non-parental haplotypes is observed between two differentiated loci that are not physically linked on the same chromosome (Table 4.1, Figure 4.1). (Herein, we define non-parental haplotypes as the haplotypes between chromosomes that differ from the original maternal and paternal haploid chromosomes (Table 4.1)). The second is double-reduction, the segregation of both sister chromatids into the same gamete (Table 4.1, Figure 4.1). Pseudolinkage and double-reduction in salmonids is thought to be limited to males (Wright *et al.* 1983, Allendorf and Danzmann 1997), although pseudolinkage has been suspected to occur in females (Danzmann *et al.* 2005). Both phenomena require the same sequence of events in prophase I that are required for residual tetrasomic inheritance; multivalent formation and recombination between homeologs (Figure4.1). Pseudolinkage, however, requires non-random pairing of homeologs in prophase I, after homologs have paired and recombined (Allendorf and Thorgaard 1984). Non-random pairing is presumed to occur more frequently when homeologs from one duplicated haploid genome have greater sequence similarity than between two divergent haploid genomes (Table 4.1). This situation occurs, for example, when recombining genomes are from two diverse sources (Wright *et al.* 1983), such as in F1 hybrids between species descended from a WGD. Pseudolinkage also requires alternate disjunction of non-randomly paired homeologs in anaphase I. This mode of disjunction allows an excess of non-parental haplotypes between homeologs to segregate into the gametes formed in meiosis II. Consequently, pseudolinkage has been commonly observed in interspecific hybrids (May *et al.* 1980, Wright *et al.* 1983, Johnson *et al.* 1987). Double-reduction does not require a specific pairing configuration between homeologs in prophase I; however, it does require a mode of disjunction in anaphase I that allows the homeologs that recombine to migrate to the same pole in the dividing cell (Figure 4.1). In the case of one recombination event between the centromere and the locus under study, double-reduction can only occur with adjacent disjunction in anaphase I. Following the disjunction in anaphase I, the recombined sister chromatids assort independently and may segregate into the same gamete in meiosis II, resulting in a double-reduction. It is apparent that residual tetrasomic inheritance, pseudolinkage, and double-reduction could impact genetic variation, and thus evolution in salmonids, particularly in systems where intra- and interspecific hybridization is common.

Comparative genome mapping has revealed that eight metacentric chromosomes are conserved among rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*O. tshawytscha*), and coho salmon (*O. kisutch*) (Naish *et al.* 2013, Phillips *et al.* 2013, Brieuc *et al.* 2014, Kodama *et al.* 2014). These metacentric chromosomes are likely ancestral to the *Oncorhynchus* lineage (Naish *et al.* 2013). Each of these eight metacentric chromosomes has a known homeolog (Table 4.2), and these eight pairs have retained higher numbers of distal duplicated loci relative to other chromosome arms (Danzmann *et al.* 2008, Brieuc *et al.* 2014, Kodama *et al.* 2014). The retention of distal duplicated loci and conservation of the homeologous pairs across *Oncorhynchus* has led to the hypothesis that these eight metacentric chromosomes are involved with ongoing homeologous pairings in *Oncorhynchus* (Kodama *et al.* 2014). Several of these homeologous chromosome arm pairs are also conserved with Atlantic salmon, even though only one of the ancestral *Oncorhynchus* metacentric chromosomes is shared between the two genera (Danzmann *et al.* 2008, Phillips *et al.* 2013, Brieuc *et al.* 2014, Kodama *et al.* 2014). Knowledge of parental gametic phase in genetic linkage mapping would allow the identification of pseudolinkage between two loci on physically unlinked chromosomes, indicate homeologous chromosome arm pairs that recombined, and provide empirical support for this hypothesis.

A large number of genetic linkage maps have been developed in salmonids, providing opportunity to examine models of inheritance for homeologous chromosome arm pairs. These maps have been created over a broad range of parental genomes (Woram *et al.* 2004, Danzmann *et al.* 2005, Timusk *et al.* 2011, Naish *et al.* 2013, Ostberg *et al.* 2013), allowing models of inheritance to be studied across different levels of genome divergence. Furthermore, salmonids exhibit sex-specific differences in recombination; male recombination rate has been shown to be lower in centric regions but higher in telomeres than in females (Rexroad *et al.* 2008, Lien *et al.* 2011, Kodama *et al.* 2014), allowing models of inheritance to be studied between sexes with divergent recombination. Linkage maps developed with knowledge of F0 grandparental genotypes, for example the genome map for rainbow trout and Yellowstone cutthroat trout hybrids (*O. clarkii bouvieri*) (Ostberg *et al.* 2013), can be used to identify homeologs that recombine in the F1. The original F0 haplotypes can be attributed to the female or male grandparent in the F2 offspring. Therefore, parental or non-parental haplotypes created during recombination in the F1 can be accurately identified. Such information is required to verify pseudolinkage, which in turn indicates homeologous recombination.

Our overall aim was to empirically verify models of inheritance in autotetraploids by identifying the homeologous chromosomes that recombine in salmonids, and tracking their segregation in offspring following recombination. We used data from several existing salmonid genetic linkage maps representing crosses both within and between species to test models across different levels of parental divergence, as well as between sexes. The first objective was to identify the extent of homeologous recombination across the genome, to determine if recombination is restricted to the eight homeologous chromosome arm pairs as proposed by Kodama *et al*. (2014), and to determine if homeologous recombination always involves a metacentric chromosome. The second objective was to identify the extent and distribution of disomic versus tetrasomic inheritance across single chromosome arms involved in homeologous pairing. The third objective was to compare the frequency of homeologous recombination across different levels of parental divergences and between sexes to determine whether the models apply mainly to males. We then use our findings in hybrids to expand the models of tetrasomic inheritance by 1) incorporating double-reduction into the model, 2) contrasting the incidence of alternate versus adjacent disjunction, 3) examining the effects of multiple recombination events within a homeologous arm pair, and 4) examining more complex arrangements by adding a hexavalent.

Methods

Genetic linkage maps

Genotype data from existing linkage maps for five salmonid species (rainbow trout, Chinook salmon, Coho salmon, Atlantic salmon, Arctic charr (*Salvelinus alpinus*), and brook charr (*S. fontinalis*)) and one interspecific F1 hybrid (rainbow trout-Yellowstone cutthroat trout) were used to assess the frequency of disomic and tetrasomic inheritance, and the fate of homeologous recombination (Table 4.3). Linkage maps allow recombination events that occurred in F1 parents during meiosis I to be tracked by examining F2 progeny genotypes. The genotype data included microsatellite, single nucleotide polymorphism, and allozyme loci. In total, 22 linkage maps were represented (11 male and female maps) and each map was developed from full-sib families. The linkage maps in our study comprised three levels of genome divergence in the F1 parent being mapped; the parent was not an F1 hybrid (F0 grandparents were from the same population), the parent was an intraspecific F1 hybrid (F0 grandparents were from different source populations), and the parent was an interspecific F1 hybrid (F0 grandparents were different species) (Table 4.3).

To accomplish our objectives, we required F1 parent-specific linkage maps. Such maps were not provided by the Arctic and brook charr linkage map studies (Woram *et al.* 2004, Danzmann *et al.* 2005, Timusk *et al.* 2011), necessitating their construction. In the published rainbow trout maps (Danzmann *et* *al.* 2005, Danzmann *et al.* 2008), several duplicated loci had allele pairs that mapped to different chromosomes between the male and female parent, causing chromosomes to merge into single linkage groups. Because this merger of linkage groups could hinder our objectives, these duplicated loci were removed from the data set and linkage maps were reconstructed for individual chromosomes. However, duplicated loci were used for further analysis. The published rainbow-cutthroat F1 hybrid map was updated in the current study by the addition of five duplicated microsatellite locus pairs (Supplementary material S4.1). Linkage maps were generated using LINKMFEX v2.3 (Danzmann and Gharbi 2001) with an LOD threshold of 3.0.

Linkage groups in rainbow trout, Chinook salmon, and Atlantic salmon have been anchored to specific chromosomes (Phillips *et al.* 2006, Phillips *et al.* 2009, Phillips *et al.* 2013). Metacentric chromosome arms in these species and the rainbow-cutthroat F1 hybrids are assigned as p and q. The q arm of several acrocentric chromosomes in Atlantic salmon consist of two ancestral arms, separated by a block of repetitive DNA (Phillips *et al.* 2009), assigned as qa and qb. Linkage groups in Arctic and brook charr have not been correlated with specific chromosomes, and the assignment of chromosomes as metacentric and acrocentric is tentative.

Identification of haplotype phase in the mapping progeny

We reconstructed the most likely parental gametic phase by identifying the haplotype phase across all progeny chromosomes. This step permitted the observation of the recombination patterns that occurred in meiosis I in each parent, as well as the subsequent segregation of homologs and homeologs into gametes. The parental phase prior to meiosis I was established for each of the original F0 maternal and F0 paternal haploid chromosomes for each F1 parent using F2 progeny genotypes. Gamete haplotypes were determined using the GENOVECT-batch application in the LINKMFEX v2.3 package (Danzmann and Gharbi 2001). Species-specific markers (114 total) that were polymorphic in rainbow-cutthroat F2 progeny were used to identify the original Yellowstone cutthroat trout (Y) and rainbow trout (R) haploid phase across all chromosomes for each F1 parent (Ostberg *et al.* 2013). The Chinook salmon grandparents were genotyped at a subset of loci, allowing the phase of the F0 maternal and paternal haploid chromosomes prior to meiosis I to be identified across four chromosomes in the F1 female parent and across 15 chromosomes in the F1 male parent. These data on chromosome phase allowed identification of parental gametic phase across all F2 progeny chromosomes in rainbow-cutthroat hybrids and across a subset of F2 progeny chromosomes in Chinook salmon. Grandparents were not genotyped at the remaining Chinook salmon chromosomes and all rainbow trout, Atlantic salmon, Arctic charr, and brook charr chromosomes, and the phase of the F0 maternal and paternal haploid chromosomes prior to meiosis I could not be identified. Consequently, the most likely phase prior to meiosis I was inferred across each F1 parent chromosome by comparing the composite of genotypes across each haploid chromosome in the F2 progeny using GENOVECT-batch. The two composite genotypes that occurred most frequently across each F2 haploid chromosome were assumed to represent the F1 parental phase prior to meiosis I. This procedure permitted the parental gametic phase to be inferred across F2 progeny chromosomes, using GENOVECT-batch.

Identifying the homeologs that recombine

To determine the effect of homeologous recombination on the inheritance of alleles across chromosomes, it was necessary to identify instances where homeologs recombined. We tested for non-random segregation of alleles between loci within homeologous chromosome pairs by inferring the parental gametic phases from the progeny haplotype phase. In the progeny of the parent being mapped, four haplotypes (two parental and two non-parental) are expected to occur at equal frequency (0.25) between loci on homeologous chromosome arms that exhibit disomic inheritance. Fisher's exact test of independence with a false discovery rate (B-Y FDR) procedure (Benjamini and Yekutieli 2001) was used to identify segregation patterns that deviated from the expected ratio of 1:1:1:1. Tests were performed using R v3.1.0 (R Development Core Team, www.R-project.org). We calculated the proportion of non-parental haplotypes, f_{NP}, for each pair of loci tested. We assumed the two allele combinations that occurred at the highest frequency represented non-parental haplotypes when the parental phases was unknown. Loci with allelic segregation distortion at $P < 0.05$ were excluded, since these alleles could confound the identification of recombination between homeologs.

Segregation across eight Oncorhynchus homeologous chromosome pairs. We determined if there was evidence of ongoing recombination between the eight *Oncorhynchus* homeologous chromosome pairs with a high number of duplicated markers, as hypothesized by Kodama *et al*. (2014). This goal was achieved testing for non-random segregation between loci across each of the chromosome pairs using progeny haplotype phase for male parents. We also examined whether these relationships could be extended to Atlantic salmon and the two charr, by performing these same tests in the equivalent homologous arms in these three species. Testing was limited to male gametes because males have a higher frequency of homeologous recombination (Morrison 1970, May *et al.* 1980, Wright *et al.* 1983, Johnson *et al.* 1987, Allendorf and Danzmann 1997). One locus per map position was used for these tests.

Extent of non-random segregation across Oncorhynchus homeologous chromosome pairs in males relative to females. We also determined the extent of non-random segregation between loci in the male parent, relative to the recombination distance in the female parent linkage map, because salmonids exhibit sex-specific differences in recombination rate. To achieve this goal, we first calibrated the markers in the male parent to the female parent linkage map, using one marker per female map position and markers in common to both parents. We then tested for non-random segregation between loci across *Oncorhynchus* chromosome pairs using progeny haplotype phase for male and female parents. Loci at some female map positions were not in common to the male parent, although tests were performed in the female parent using these loci. Loci with allelic segregation distortion at $P < 0.05$ were included to facilitate the comparative analysis between sexes.

Gametic phase disequilibrium between homeologs

We tested for gametic phase disequilibrium to identify statistical linkage of loci between homeologous chromosomes. This was accomplished calculating the correlation coefficient, R, between alleles at loci on homeologous chromosomes in the F1 parental gametes, using the progeny haplotype phase and Excel. The correlation coefficient is commonly used as a measure of gametic phase disequilibrium between biallelic markers (Campton 1987). For linkage maps with known parental gametic phase, a positive R

value indicates an excess of association between parental haplotypes and a negative *R* value indicates an excess of association between non-parental haplotypes. We used the absolute value of *R* for the linkage maps with unknown parental gametic phase. Significant deviations in gametic phase disequilibrium were identified across all duplicated locus pairs and across each homeologous chromosome pair using the chi-square statistic $\chi^2 = R^2 N$ (Weir 1996), where *N* = the number of gametes in the sample, and summing chi-square values and degrees of freedom across each series of tests followed by a false discovery rate (B-Y FDR) procedure (Benjamini and Yekutieli 2001).

Patterns of inheritance in duplicated loci with tetrasomic inheritance

We determined the frequency of disomic and tetrasomic inheritance in duplicated loci that segregated tetrasomically, and identified their map position, across single chromosome arms involved in homeologous pairing. In the rainbow-cutthroat F1 hybrid parents, four duplicated loci (OMM1200, OMM1218, OMM1258 and OMM1274) each segregated six allelic combinations and/or double-reduction into parental gametes; patterns consistent with homeologous recombination. To determine the frequency of disomic and tetrasomic inheritance in these duplicated loci, it was necessary to assign the segregating alleles to each of the duplicated loci. This goal was achieved by first using existing rainbow trout linkage maps (Guyomard *et al.* 2006, Danzmann *et al.* 2008, Rexroad *et al.* 2008) to position the four duplicated loci within the homeologous chromosome arms RYHyb12q and RYHyb13q (Table 4.2) in the rainbow-cutthroat F1 hybrid linkage map, relative to the positions of the disomically inherited markers mapped in the F1 hybrids. The putative centromere region in these two F1 hybrid linkage groups was inferred from homologous markers mapped near the centromere in rainbow trout. Placing the centromere would provide information on the portion of the chromosome arm that was exhibiting tetrasomic inheritance, since duplicated markers might be inherited disomically. Second, we assumed that the four duplicated loci segregated disomically when one crossover occurred between homologous chromosome arms. Salmonids have high crossover interference (Table 4.1) during meiosis and typically have less than 3% multiple crossovers per chromosome arm (Thorgaard *et al.* 1983, Guyomard *et al.* 2006, Brieuc *et al.* 2014). The original F0 maternal and paternal phases for all disomically inherited loci in each of the homeologous arms were used to identify the frequency of crossover events between homologous arms.

We enumerated F2 progeny haplotypes that reflected with one crossover between homologs in the parents, and then recorded the parental gametic phase that was distal to the crossover between homologs, relative to the centromere. Third, we used the enumerated progeny haplotypes with one crossover between homologs to assign each of the four alleles at a duplicated locus to the most likely locus pair (that is, the two homologous loci). Assignments were based on the frequency that the allele co-segregated with the parental gametic phase that was distal to the crossover between homologs. Following the assignment of alleles to each of the duplicated loci, we determined the frequency of disomic and tetrasomic inheritance and mapped these loci in the rainbow-cutthroat F1 chromosome arms RYHyb12q and RYHyb13q using LINKMFEX.

Double-reduction

To incorporate double-reduction into the meiotic model for tetrasomic inheritance, it was necessary to identify and quantify the frequency of double-reduction in parental gametes. This was achieved by surveying rainbow-cutthroat F2 progeny genotypes across duplicated loci. Double-reduction at a duplicated locus was identified by the absence of a parental allele in the F2 genotyped progeny and inferred only when all four alleles in a progeny could be unambiguously assigned to each parent. We assumed that all loci distal to a double-reduction also had double-reduction.

Incidence of alternate and adjacent disjunction

We determined if alternate disjunction occurred more frequently than adjacent disjunction in anaphase I during gamete formation, as proposed by Wright *et al*. (1983). This goal was achieved by first identifying gametes that resulted from bivalent and tetravalent formation in prophase I, using duplicated loci in the homeologous chromosome pair RYHyb12 and RYHyb13 that segregated six allelic combinations into rainbow-cutthroat F1 female gametes. We considered that tetravalent formation occurred when duplicated loci segregated tetrasomically and bivalent formation occurred when duplicated loci segregated disomically. Second, we used the original F0 maternal and paternal phase across the putative centromere region prior to meiosis I for female F1 parents, to identify F2 progeny haplotypes with intact parental and non-parental centromeres between the homeologs. Alternate disjunction from tetravalent formations during anaphase I was inferred when the F2 progeny haplotypes between centromeres of homeologs were not in the same phase as the F0 maternal and paternal chromosomes (non-parental centromere pairs). Adjacent disjunction from tetravalent formations was inferred when the F2 progeny haplotypes between centromeres of homeologs were in the same phase as the original maternal and paternal chromosomes (parental centromere pairs). The mode of disjunction was not inferred for bivalents. Third, we used Fisher's exact test of independence to test for a difference in frequency of parental and non-parental centromere pairs originating from bivalent and tetravalent formations.

Segregation of alleles within a hexavalent

We determined how a hexavalent formation in prophase I, a chromosome pairing arrangement that is more complex than tetravalent formation, affects the inheritance of alleles across chromosomes. This goal was accomplished by testing for non-random segregation of alleles across three chromosomes that can form a hexavalent (RYHyb12, RYHyb13, and RYHyb17), using rainbow-cutthroat F2 progeny haplotype phase for male and female parents. Each arm of the metacentric chromosome RYHyb13 forms a homeologous pairing with two non-homeologous metacentric chromosomes, RYHyb12 and RYHyb17 (Table 4.2). First, we tested for non-random segregation between loci across the non-homeologs RYHyb12 and RYHyb17. Second, we tested for non-random segregation across the tetravalent using a set of three disomically inherited loci (Omy1004UW on RYHyb12, OMM3006 on RYHyb13, and OMM1162 on RYHyb17). In the F2 progeny of the F1 parent being mapped, eight haplotypes (two parental and six non-parental) are expected to occur at equal frequency (0.125) across three disomically inherited loci that are each on different chromosomes. Goodness of fit of F2 progeny haplotype frequencies across the hexavalent were determined by a χ^2 test with 10,000 permutations.

Results

Segregation of duplicated locus pairs across linkage maps

A total of 319 duplicated locus pairs were tested for non-random segregation (Table 4.4). The number of duplicated loci tested and the number of homeologous chromosome arm pairs represented by these loci varied among linkage maps. Rainbow-cutthroat F1 hybrid male parents had the greatest number of non-randomly segregating duplicated loci and one F1 hybrid female parent also exhibited non-random segregation (Table 4.4, Table 4.5). Alleles at these duplicated locus pairs segregated into a significant excess of non-parental haplotypes in rainbow-cutthroat F1 parental gametes, indicating pseudolinkage (Table 4.4, Table 4.5). Male gametes, but not female gametes, from intraspecific F1hybrid parents showed non-random segregation. Parents that were not F1 hybrids segregated all duplicated locus pairs randomly into gametes, with the exception of one brook charr male and one Atlantic salmon female (Table 4.4, Table 4.5).

Segregation across the eight Oncorhynchus homeologous chromosome pairs

We found substantial pseudolinkage across all eight *Oncorhynchus* homeologous chromosome pairs in both rainbow-cutthroat F1 hybrid male parents (Supplementary material S4.2), with the exception of RYHyb07-RYHyb18 in the Family 2 male parent. All loci mapped to RHYyb18 exhibited segregation distortion in this male parent; therefore, tests were not performed across RYHyb07-RYHyb18. Approximately 82% of all locus comparisons across the eight *Oncorhynchus* chromosome pairs showed pseudolinkage in the rainbow-cutthroat hybrid males. Rainbow and Yellowstone cutthroat trout alleles were highly correlated between loci within homeologous chromosomes pairs.

All intraspecific F1 hybrid male parents produced gametes that showed non-random segregation of alleles across some, but not all, of the eight *Oncorhynchus* homeologous chromosome pairs (Supplementary material S4.2). Alleles segregated non-randomly within three chromosome pairs in the Lot25 rainbow trout male parent (Omy07-Omy18, Omy12-Omy13, and Omy13-Omy17), within two chromosome pairs in the Lot44 rainbow trout male parent (Omy01-Omy23 and Omy02-Omy03), and within three chromosome pairs in the Arctic charr family 3 male parent (AC1-AC21, AC3-AC24, and AC13-AC34).

Three of the six male parents that were not F1 hybrids showed non-random segregation within some of the homeologous chromosome pairs (Supplementary material S4.2). In the brook charr, alleles segregated non-randomly within the same homeologous pair (BC1-BC21) in both male parents, and within two homeologous pairs (BC8-BC21 and BC18-BC25) in the male parent of Family HL3. One Atlantic salmon male (Family Br6) segregated alleles non-randomly into gametes at one homeologous pairing (SSa05-SSa02).

Extent of non-random segregation across Oncorhynchus homeologous chromosome pairs in males relative to females

Pseudolinkage in rainbow-cutthroat F1 hybrid males, relative to the hybrid female parent linkage maps, extended across all loci within the *Oncorhynchus* homeologous chromosome arm pair and across almost all loci in the adjacent arm in metacentric chromosomes (the arms not involved in pairings) (Supplementary material S4.3). Figure 4.2 shows the extent of pseudolinkage across a homeologous chromosome pair (RYHyb12-RYHyb13). Correlation coefficients in the rainbow-cutthroat male parents tended to be highest between loci mapping within each homeologous chromosome arm pair. In the rainbow-cutthroat Family 1 female parent, pseudolinkage of RYHyb15 and RYHyb21 occurred between loci mapping to the q arm of RYHyb15, the p arm of RYHyb21, and centromeres (Supplementary material S4.3). This result was compatible with the *Oncorhynchus* homeologous chromosome arms involved in pairings in these two metacentric chromosomes (Phillips *et al.* 2006).

The extent of non-random segregation across *Oncorhynchus* homeologous chromosomes in the rainbow trout intraspecific F1 hybrid male parents, relative to the female parent linkage maps, was more variable than the rainbow-cutthroat interspecific F1 hybrids (Supplementary material S4.3). Non-random segregation in the Lot25 male parent was inconsistent with the homeologous chromosome arms within the Omy07-Omy18 pair; involved only a single locus mapping to the q arm of Omy13 in the Omy12-Omy13 pair; and involved several loci within the homeologous chromosome arm pair Omy13-Omy17 (the p arm of Omy13 and q arm of Omy17), but also extended into the adjacent metacentric chromosome arms not involved in pairing. In addition, the Lot25 female parent showed non-random segregation at two locus comparisons in the homeologous pair Omy13-Omy17, although this parent was not an F1 intraspecific hybrid. However, the map position for these loci was inconsistent with the homeologous chromosome arms. Both of the parents for rainbow trout Lot44 showed non-random segregation at Omy01-Omy23 and Omy02-Omy03, although the female parent was not an F1 intraspecific hybrid. The map position of loci that segregated non-randomly in the female parent was more consistent with the homeologous chromosome arms, compared to the male parent. Comparisons were not performed in Arctic charr Family 3 because two or fewer markers were shared between the male and female parent linkage maps at each of the *Oncorhynchus* homeologous chromosomes pairs that showed evidence of non-random segregation in the male parent.

In the male parents that were not F1 hybrids, testing was limited to one homeologous pair (Ssa02-Ssa05) in Atlantic salmon Family Br6 (Supplementary material S4.3). One locus comparison segregated non-randomly between homeologs in the male parent and the map positions for these loci was not consistent with the homeologous chromosome arms. Testing of non-random segregation in brook charr male parents, relative to the female parent linkage maps, was not performed because parents shared two or fewer markers within each of the *Oncorhynchus* homeologous chromosomes pairs that showed evidence of non-random segregation in the male parent.

Extent of tetrasomic inheritance across single chromosome arms

Four duplicated loci (OMM1200, OMM1218, OMM1258, and OMM1274) in the homeologous chromosome arm pair RYHyb12q-RYHyb13q segregated tetrasomically into roughly 20% of the rainbow-cutthroat F1 hybrid female gametes (Table 4.6). Interestingly, OMM1200 segregated disomically into gametes in the male parent of Family 2. OMM1218 and OMM1258 segregated tetrasomically into hybrid male gametes, but alleles could not be assigned to the most likely locus, because male and female parents shared alleles, a parent had more than one allele copy, or there was no recombination between homologous chromosome arms in male parents. The four tetrasomically inherited duplicated loci mapped distally in RYHyb12q and RYHyb13q in the female F1 hybrids, indicating that recombination between homeologs occurred in the distal region of these chromosome arms (Figure 4.3). All loci mapping proximal to these duplicated loci segregated disomically into gametes.

Double-reduction

We found double-reduction in rainbow-cutthroat F1 hybrid gametes from both female parents, but not male parents, at duplicated loci that segregated tetrasomically in the homeologous chromosome arm pair RYHyb12q-RYHyb13q (Table 4.6). The female parent of Family 1 produced three and the female parent of Family 2 produced seven double-reduction gametes in this homeologous pairing. These double-reductions were not exclusive to species phase (rainbow or cutthroat) or to a homeologous chromosome arm (RYHyb12q or RYHyb13q). Double-reduction was also observed in a gamete from the Family 2 female parent at another duplicated locus (OMM1361) that segregated tetrasomically in the male and female parent (data not shown). OMM1361 is presumed to be located in the homeologous chromosome arm pair RYHyb13p-RYHyb17q (Guyomard *et al.* 2006). Interestingly, the F2 progeny that had the double-reduction at OMM1361 also had double-reduction at duplicated loci in RYHyb12q.

Incidence of alternate and adjacent disjunction

We established the putative centromere pairs between the homeologs RYHyb12 and RYHyb13 for 79 rainbow-cutthroat F1 hybrid female gametes between families. In the bivalent pairings, parental and non-parental centromere pairs segregated in equal frequency into female gametes (25 with parental and 26 with non-parental centromere pairs between homeologs). In the tetravalent pairings, five gametes had parental centromere pairs (indicating adjacent disjunction in anaphase I) and 23 gametes had non-parental centromere pairs (indicating alternate disjunction in anaphase I). Non-parental centromere pairs segregated at a significantly higher frequency than parental centromere pairs into female gametes in tetravalents ($P < 0.001$, Fisher's test). Across the 28 total tetravalents, 19 female gametes showed tetrasomic inheritance, all had non-parental centromere pairs between homeologs, and nine had double-reduction; five with parental and four with non-parental centromere pairs between homeologs.

Frequency of multiple crossover events within a homeologous arm pair

Eight of the 79 rainbow-cutthroat F1 hybrid female gametes with the putative centromere pair established between RYHyb12 and RYHyb13 had two crossovers on a homeologous arm (either in RYHyb12q or in RYHyb13q). Of these eight gametes, two showed disomic inheritance across all loci in the chromosome arm pairing, four showed tetrasomic inheritance at distal duplicated loci, and two showed double-reduction at distal duplicated loci. The double crossover in the gametes with tetrasomic inheritance and double-reduction involved one recombination event between homologous arms and one recombination event between homeologous arms. In addition, the double-reduction gametes that had two crossover events on a homeologous arm had non-parental centromere pairs between homeologs.

Segregation of alleles within a hexavalent

We found a deficit of non-parental haplotypes between loci across an extensive portion of the non-homeologs RYHyb12 and RYHyb17 in rainbow-cutthroat F1 hybrid male gametes, but not female gametes (Supplementary material S4.4). Parental alleles were highly correlated between loci in male gametes and the lowest observed f_{NP} across the homeologs was 0.30 and 0.25 in the male parent of Family 1 and the male parent of Family 2, respectively. Haplotype frequencies across RYHyb12, RYHyb13, and RYHyb17 deviated significantly from expected in hybrid male gametes (male Family 1, $P < 0.001$; male Family 2, $P < 0.001$; χ^2 tests), but not female gametes (Figure 4.4). Only non-parental haplotypes were observed in male gametes and over 70% of male gametes had R-Y-R and Y-R-Y haplotypes at the three loci tested across the RYHyb12-RYHyb13-RYHyb17 tetravalent, respectively.

Discussion

Here, we used the parental gametic phase from existing genetic linkage maps to identify the homeologs that recombine, to characterize this recombination, and to verify meiotic models of residual tetrasomic inheritance in autotetraploids. We found that the frequency of non-random segregation of alleles between homeologs was linked to genome divergence within parents. We also found that recombination between homeologs was limited to the eight homeologous chromosome arm pairs conserved in *Oncorhynchus* (Kodama *et al.* 2014), always involved a metacentric chromosome, was restricted to distal regions of chromosome arm pairs, and occurred in males and females. Pseudolinkage in male rainbow-cutthroat F1 hybrids was extensive across the eight homeologous chromosome pairings, extending across entire chromosomes, and pseudolinkage also occurred in an F1 hybrid female. Homeologous recombination caused double-reduction in the distal regions of chromosome arms in F1 hybrid female gametes, although double-reduction was not observed in male gametes. Double crossovers occurred within homeologous chromosome arm parings in several hybrid female gametes. In addition, homeologous chromosome arm pairing caused the formation of a hexavalent in F1 hybrid males. Taken together, these findings suggest that metacentric chromosomes have played an important role in maintaining homeologous pairings, allowing undifferentiated duplicated loci to be retained in distal chromosome regions through homeologous recombination.

Homeologous recombination increases with parental divergence

The frequency of non-random pairing between homeologs in meiosis I was linked to genetic divergence between parental sources. This result suggests that in genomes from diverse parental sources, homologous alleles in homeologous chromosome arm pairings may be more divergent than homeologous alleles, and this divergence could lead to an increased likelihood of non-random pairing and recombination between homeologs (Figure 4.5, panel 2b). Such genetic divergence may be expected to accrue between populations that do not interbreed. In populations descended from a sole source, the occasional pairing and recombination of homeologs in parental genomes may prevent the divergence of homologous alleles in homeologous chromosome arm pairings (Meirmans and Van Tienderen 2013). Consequently, random pairing between maternal and paternal homeologs may be expected in populations that are descended from a sole source when homeologous pairing does occur (Figure 4.5, panels 2a and 2b). Indeed, we found that parents that were not inter- or intraspecific F1 hybrids typically showed random segregation across all locus pairs, implying that recombination between homeologs was infrequent and/or occurred randomly between maternal and paternal homeologs, with the exception of Atlantic salmon and brook charr. It is intriguing that brook charr male parents showed non-random segregation even though this longstanding hatchery strain is several generations removed from a cross with a wild strain. Backcrossing has been found to erode non-random pairing of parental homeologs (Wright *et al.* 1983, Johnson *et al.* 1987); however, the polymorphisms causing non-random pairing may persist at low frequencies within populations for several generations (Davisson *et al.* 1973, Wright *et al.* 1983).

Eight homeologous chromosome arm pairs recombine

Our results indicate that homeologous recombination is limited to the same eight *Oncorhynchus* homeologous chromosome arms pairs, verifying the hypothesis Kodama *et al*. (2014). However, it is possible that other homeologs may recombine too because all chromosomes arms contain duplicated loci (Brieuc *et al.* 2014). Our analysis was limited to the linkage groups with duplicated markers in each parent, in addition to the eight homeologs. However, recombination between other homeologs may be unlikely, at least in *Oncorhynchus*, because such events would be expected to cause pseudolinkage in the rainbow-cutthroat hybrids. Only the eight homeologs identified herein as recombining were previously identified as forming pseudolinkage groups in the male hybrids (Ostberg *et al.* 2013). Although we found an Atlantic salmon female parent with non-random segregation at a duplicated locus pair on Ssa13 and Ssa15, possibly suggesting that these homeologs recombined, this homeology in Atlantic salmon is not well supported (Lien *et al.* 2011) and is not conserved in *Oncorhynchus*.

Metacentric chromosomes were involved in each of the homeologous pairings that recombined, strongly supporting the view that metacentric chromosomes have played a key role in maintaining homeologous pairings (Phillips *et al.* 2009). Metacentric chromosomes may provide the stability required for the formation of multivalents in prophase I and, thereby, retard diploidization by allowing homeologies to be preferentially retained (Brieuc *et al.* 2014). These multivalent formations are likely prevented following extensive rearrangement of metacentric chromosome arms through Robertsonian fusions and pericentric inversions. Such chromosome rearrangements are thought to have been widespread following the divergence of *Oncorhynchus* and Atlantic salmon from the last common ancestor (Phillips and Rab 2001). Rearrangement of metacentric chromosomes in Atlantic salmon could explain why some homeologous chromosome arm pairs conserved in *Oncorhynchus* are not supported in Atlantic salmon, while others are highly supported (Danzmann *et al.* 2008, Phillips *et al.* 2013, Brieuc *et al.* 2014, Kodama *et al.* 2014).

Recombination between homeologs occurs in distal chromosome arms regions

Our results in the rainbow-cutthroat F1 hybrids verify the meiotic model for residual tetrasomic inheritance in salmonids (Wright *et al.* 1983); recombination between homeologous chromosome arms in prophase I occurs in distal regions (Figure 4.3, Figure 4.5). While we confirmed that homeologous recombination was limited to the duplicated loci mapping distally in one chromosome arm pairing (RYHyb12q-RYHyb13q), our data suggest that recombination between homeologs was restricted to distal regions in each chromosome arm pairing that was pseudolinked. In these pairings, all loci mapped in each male parent segregated disomically into male gametes, indicating that recombination between homeologous chromosome arms occurred distally to these loci. Altogether, our findings suggest that homeologs have become sufficiently differentiated in proximal and medial chromosome arm regions, allowing disomic inheritance to be restored in these regions, but remain undifferentiated in distal regions as a consequence of recombination between homeologs, allowing tetrasomy to persist. Our findings support the mechanism for differentiation of homeologous chromosome arms predicted in the meiotic model for residual tetrasomic inheritance (Allendorf and Thorgaard 1984, Allendorf and Danzmann 1997). In this model, differentiation of homeologous arms depends on the recombination distance between homeologous loci and the centromere. Recombination between proximal loci and the centromere occurs less frequently, compared to distal regions, allowing differentiation of homeologous chromosome arms to be initiated in proximal regions. Differentiation between homeologs is expected to extend distally, but high frequency of homeologous recombination in distal arm regions retards differentiation.

Recombination between homeologs in the F1 hybrids occurred in male and female meiosis, suggesting that female gametogenesis has contributed to the retention of undifferentiated distal regions in homeologous chromosome arm pairings. To the best of our knowledge, homeologous recombination has not been demonstrated in females, although it has been suspected to occur (Danzmann *et al.* 2005). The chromosome arm region involved in homeologous recombination differed between sexes in one hybrid family. The female parent had an extended region of homeologous recombination compared to the male parent. Difference in the region of homeologous recombination between parents could be a consequence of the sex-specific difference in recombination rate in salmonids; male recombination rate in telomeric regions is higher than females, but lower elsewhere (Rexroad *et al.* 2008, Lien *et al.* 2011, Kodama *et al.* 2014). Alternatively, the difference in the region of homeologous recombination between parents could be a result of individual variation in recombination sites.

Pseudolinkage in rainbow-cutthroat hybrids

Pseudolinkage affected significant portions of chromosomes in rainbow-cutthroat hybrids, including the chromosome arm that was not involved in the homeologous pairing. This effect was prevalent in hybrid males, and is a consequence of their characteristically low recombination rate between homologs in proximal and medial chromosome arm regions, compared to females (Figure 4.2). The low frequency of recombination between homologs allows an extended chromosomal region adjacent to the area of recombination between homeologs to segregate intact into male gametes. As a result, a high proportion of male gametes was comprised of non-parental haplotypes across considerable portions of homeologous chromosome pairs, after alternate disjunction in anaphase I (Figure 4.5, panels 4c.1 – 4c.4). That is, in the hybrid males, the rainbow or cutthroat homeologs rarely co-segregated into the same gamete. The higher frequency of recombination in proximal and medial regions in females, relative to males, reduced the extent of the chromosomal region that segregated intact into female gametes. However, this intact region consisted of a substantial portion of the homeologous chromosome arm pair in the female that showed pseudolinkage between RYHyb15 and RYHyb21. These data taken together with the involvement of over half of the F1 hybrid linkage groups in homeologous pairings (15 linkage groups in total), indicate that non-parental haplotypes may be over-represented across extensive portions of the haploid genome in hybrid gametes.

Pseudolinkage depends on the frequency of recombination between the centromere and the locus under consideration, as well as the mode of disjunction in anaphase I. Loci near the centromere experience fewer crossover events than loci further from the centromere, and, therefore, proximal loci are more likely to segregate in the same parental phase as the centromere in anaphase I, compared to distal loci. Thus, pseudolinkage may be expected to have a greater affect in proximal regions of homeologous chromosome arms. However, distal pseudolinkage can occur if the locus is inherited disomically and if recombination between homologs is suppressed, such as in the rainbow-cutthroat hybrid males. For example, in the RYHyb12-RYHyb13 pairing, the distal duplicated locus OMM1200 segregated disomically into male gametes and was pseudolinked, but segregated tetrasomically into female gametes (Figure 4.3). This result indicates that pseudolinkage can have a distal effect, which may extend into the region of homeologous recombination.

Our results indicate that non-random pairing of homeologs and alternate disjunction in anaphase I do not necessarily lead to pseudolinkage. We found that all female gametes that segregated from tetravalents in the RYHyb12-RYHyb13 pairing had non-parental centromere pairs between the homeologs, indicating that alternate disjunction in anaphase I occurred after the homeologs recombined. However, the frequency of homeologous recombination in each female parent was not high enough to generate an excess of non-parental haplotypes between homeologs. When the frequency of homeologous recombination is increased, such as in the hybrid males at this pairing, then non-parental haplotypes between homeologs may segregate in excess and pseudolinkage may be observed.

Incidence of alternate and adjacent disjunction

Our result indicate that alternate disjunction in anaphase I occurs more frequently than adjacent disjunction, supporting the findings by Wright *et al*. (1983). In bivalents pairings, homeologs orient independently in the equatorial plane in metaphase I, and as a result parental and non-parental centromere pairs between homeologs are expected to segregate randomly into gametes with adjacent and/or alternate disjunction in anaphase I. However, the formation of tetravalents likely constrains the orientation of homeologs in the equatorial plane in metaphase I, resulting in parental and non-parental centromere pairs between homeologs to migrate to the same dividing cell following adjacent and alternate disjunction, respectively, in anaphase I (Figure 4.5, panel 3). While the frequency has not been reported, Wright *et al*. (1983) indicated that alternate disjunction was rare in salmonids. If the frequency of disjunction from tetravalent formation in anaphase I is characteristic of meiosis, in general, then our findings may suggest that adjacent disjunction in salmonids occurs more frequently than previously suspected.

Double-reduction

Double-reduction resulted in the inheritance of the distal portion of sister chromatids into the same gamete. This phenomenon is specific to autopolyploids and may occur only when multivalents form, homeologs recombine in prophase I, and sister chromatids of such homeologous recombination events migrate to the same pole in anaphase I (Mather 1936, Bever and Felber 1991) (Figure 4.5). Recombination between homeologs uncouples the distal portion of sister chromatids (i.e. region of homeologous recombination), allowing them to migrate to the same pole in anaphase I and assort together, but on different chromosomes, into the same gamete following anaphase II. Our results suggest that sister chromatids may be inherited through alternate and adjacent disjunction in anaphase I, depending on the number of crossovers that occurred in prophase I between the centromere and the locus under study. We found several double-reduction gametes with one crossover within a homeologous arm pair and parental centromere pairs between homeologs. Such gametes can form only through adjacent disjunction in anaphase I (Figure 4.5, panels 4d.2, and 4d.3). We also found several double-reduction gametes with two crossovers within a homeologous arm pair and non-parental centromere pairs between homeologs. Meiotic modelling suggested these double-reduction gametes likely occurred through alternate disjunction in anaphase I (Supplemental material S4.4a, panel 4a.3, and S4.4b, panel 4a.4).

Our results suggest that double-reduction may be common in genomes derived from two parental species. To the best of our knowledge, the frequency has not been reported for interspecific hybrids in salmonids. Studies using intraspecific crosses have found that double-reduction is rare (Wright *et al.* 1983, Allendorf and Danzmann 1997), although Johnson *et al*. (1987) found double-reduction in 4% of gametes from a male rainbow trout. Differences in the incidence of double-reduction among studies may be a consequence of recombination frequency between the centromere and the locus under consideration, as well as divergence between parental genomes. Indeed, we found higher frequencies of double-reduction as the recombination distance between the centromere increased. Our estimates of double-reduction is likely conservative because it was inferred only when the four alleles in an F2 offspring could be clearly assigned to each parent. While it was not observed in male gametes, the frequency of double-reduction is expected to be higher in males, compared to females, as a result of the higher frequency of homeologous recombination in males.

Multiple crossovers in homeologous pairings

Several gametes had multiple recombination events across chromosome arms involved in homeologous pairing in female rainbow-cutthroat hybrids, but not in males (Supplemental material S4.4). The female gametes with recombined homeologs had one crossover that occurred between homologs and a second crossover between homeologs that was distal to the first crossover (in the region of homeologous recombination). This finding supports two sequential stages of chromosome pairing with recombination proposed in the meiotic model for residual tetrasomic inheritance of Allendorf and Danzmann (1997). Sequential pairing between homologous arms, followed by secondary pairing between homeologous arms allows for the multiple recombination events observed in females. The absence of such events in males may be a consequence of their suppressed recombination in proximal regions of homologous arms. We acknowledge that the frequency of multiple crossovers in the chromosome arms involved in homeologous pairing (9%) was higher than that which is typically reported between homologous arms (Thorgaard 1983, Guyomard *et al.* 2006, Brieuc *et al.* 2014). However, the frequency of double crossovers between homologous arms in hybrid female gametes with disomic inheritance was typical (2.5%).

The lower proximal recombination frequency in males compared to females is often explained by multivalent formation. This view presumes that structural constraints suppress crossing over within multivalents (Sakamoto *et al.* 2000). However, the data presented here do not strongly support this view. Recombination occurred between homologs within multivalent formations, indicating that crossing over was not suppressed, at least in the gametes with multiple recombination events within the homeologous chromosome arm pair. Recombination suppression by multivalent formation is specific to homeologous pairings and does not account for suppressed recombination in males in proximal regions of chromosomes that do not form multivalents (Rexroad *et al.* 2008, Lien *et al.* 2011, Kodama *et al.* 2014).

Further, sex-specific differences in recombination rate is common across a wide range of taxa (Lenormand and Dutheil 2005). Differences in the distribution of recombination sites between sexes may be a more plausible mechanism for the lower rate of recombination in proximal regions in males (Lawrie *et al.* 1995, Kochakpour and Moens 2008). A largely telomeric distribution of recombination sites could allow homeologous pairings to form more readily in males, resulting in an overall impression of recombination suppression by multivalent formation.

Hexavalent formation

We have extended the meiotic model of tetrasomic inheritance to include a hexavalent formation. In the rainbow-cutthroat F1 hybrids, each arm on RYHyb13 forms a homeologous pairing with RYHyb12 and RYHyb17, which are non-homeologs. Our results showed that homeologous chromosome arm pairings biased the distribution of haplotypes in male gametes across these three chromosomes. Non-parental haplotypes segregated in excess into gametes in each homeologous arm pairing; however, parental haplotypes segregated in excess of between RYHyb12 and RYHyb17. These findings can be explained by hexavalent formation, followed by alternate disjunction in anaphase I. Hexavalent formation likely restricted the orientation of the homeologs in the equatorial plane in metaphase I. Following alternate disjunction from the hexavalent in anaphase I, Y-R-Y and R-Y-R haplotypes for RYHyb12-RYHyb13-RYHyb17, respectively, segregated in excess into male gametes. Consequently, parental haplotypes between RYHyb12 and RYHyb17 were constrained across large chromosomal portions as a result of the tetravalent formation, and this constraint caused an excess of parental haplotypes between RYHyb12 and RYHyb17 to co-segregate into gametes. Although non-parental haplotypes between RYHyb12 and RYHyb17 were deficient, these chromosomes were pseudolinked via a mutual association with RYHyb13. Pseudolinkage between non-homeologs is intriguing and may occur in other inter-specific hybrids, in particular, as well as other species.

Conclusions

Here, we have advanced the understanding of diploidization in the ancestrally autotetraploid salmonid genome and provided novel insight into the effects of incomplete re-diploidization on transmission genetics. Involvement of metacentric chromosomes in all the homeologous pairings that recombined could suggest that metacentric chromosomes have obstructed re-diploidization by supporting the formation of multivalents, allowing tetrasomy to persist in distal regions of these chromosome arms. We found that homeologous pairing and recombination can have far reaching effects across chromosomes and that the magnitude of these effects are contingent on the divergence between parental genomes and sex. These data suggest that recombination of homeologs could influence genome evolution in admixed populations. For example, if locally adapted genes interact between homeologs, then an excess of non-parental haplotypes in parental gametes could affect the fitness of progeny by interfering with epistatic interactions between homeologs. In addition, double-reduction increases homozygosity of duplicated locus pairs, and this can lead to an inbreeding effect by reducing the effective population size and promoting differentiation among populations (Bever and Felber 1991, Ronfort *et al.* 1998, Dufresne *et al.* 2014). We extended meiotic models of tetrasomic inheritance in salmonids by including double-reduction, multiple recombination events within a homeologous arm pair, and hexavalent formation. The meiotic models for tetrasomic inheritance developed herein have general application to inheritance studies in autotetraploids.

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Tables

Table 4.1. Glossary

Adjacent disjunction – Centromere of parental homeologs migrate to the same pole in the dividing cell during anaphase I.

Alternate disjunction – Centromere of parental homeologs migrate to opposite poles in the dividing cell during anaphase I.

Crossover interference – Formation of one chiasmata junction interferes with the formation of other junctions on the same chromosome arm.

Double-reduction - Two copies of the same allele, initially carried on sister chromatids prior to meiosis, segregate into the same gamete as a result of homeologous recombination.

Non-random pairing of homeologs – Preferential pairing of homeologs derived from the same parent, rather than between homeologs from opposite parents. Caused by greater sequence similarity, often encountered during hybrid crosses. Presumed to occur more frequently in males.

Non-parental haplotype – Haplotypes between chromosomes that differ from the original maternal and paternal haploid chromosomes.

Parental haplotype - Haplotypes between chromosomes that are the same as the original maternal and paternal haploid chromosomes.

Pseudolinkage – Excess of non-parental haplotypes resulting in statistical linkage between two differentiated loci that are not physically linked.

Random pairing of homeologs – Random pairing between any of the homeologous chromosomes. This pairing is more likely to occur between genomes derived from closely related individuals.

Residual tetrasomic inheritance – Tetrasomic inheritance for some loci in an individual where a component of the genome is diploid. Occurs when centromeres segregate disomically and undifferentiated distal duplicated loci segregate tetrasomically (compared to primary tetrasomy which involves tetrasomic segregation across entire chromosomes) (Allendorf and Thorgaard 1984). Residual tetrasomic segregation ratios may be observed when undifferentiated distal duplicated loci exhibit a mixture of disomic and tetrasomic inheritance (Allendorf and Thorgaard 1984).

Table 4.2. Eight major ancestral homeologous chromosome arm pairs comprising a significant proportion of duplicated markers that are conserved across Pacific salmonids, and their homologs in Atlantic salmon and charr. Arms of ancestral metacentric chromosomes involved in each pair in Pacific salmon are identified in bold.

Rainbow trout (Phillips *et al.* 2006)	Rainbow-cutthroat F1 hybrids (Ostberg *et al.* 2013)	Chinook salmon (Brieuc *et al.* 2014)	Coho salmon (Kodama *et al.* 2014)	Atlantic Salmon (Lien *et al.* 2011)	Arctic charr[1] (Timusk *et al.* 2011)	Brook charr[1] (Timusk *et al.* 2011)
Omy01q-Omy23a	**RYHyb01q**-RYHyb23a	**Ots06q**-Ots01q	**Co04b**-Co11a	Ssa18qa-Ssa01qa[2]	AC18-AC25	BC18-BC25
Omy02p-**Omy03p**	RYHyb02p-**RYHyb03p**	Ots23a-**Ots03p**	Co13b-**Co02a**	Ssa05q-Ssa02p	AC23-AC35; AC6-AC23	BC23-BC35; BC6-BC23
Omy06q-Omy26a	**RYHyb06q**-RYHyb26a	**Ots04q**-Ots12q	**Co03b**-Co08b	Ssa26a-Ssa11qb[2]	AC10-AC15	BC10-BC15
Omy07p-Omy18p	**RYHyb07p**-RYHyb18p	**Ots07p**-Ots14p	**Co05a**-Co16b	Ssa17qb-Ssa16qb[2]	AC12-AC27	BC12-BC27
Omy10q-**Omy19p**	RYHyb10q-**RYHyb19p**	Ots34a-**Ots11p**	Co12b-**Co07a**	Ssa08q-Ssa04p	AC13-AC34	BC13-BC34
Omy12q-Omy13q	**RYHyb12q**-RYHyb13q	**Ots09q**-Ots27a	**Co06b**-Co10b	Ssa03q-Ssa06p	AC20a-AC20b[3]	BC20a-BC20b[3]
Omy13p-**Omy17q**	RYHyb13p-**RYHyb17q**	Ots32a-**Ots02q**	Co20b-**Co01b**	Ssa12qa-Ssa02q[2]	AC1-AC21; AC8-AC21	BC1-BC21; BC8-BC21
Omy15q-**Omy21p**	RYHyb15q-**RYHyb21p**	Ots17a-**Ots15p**	Co19a-**Co09a**	Ssa17qb-Ssa07q	AC8-AC24; AC3-AC24	BC8-BC24; BC3-BC24

Metacentric chromosome arms in rainbow trout, F1 hybrids, Chinook salmon, and Atlantic salmon are identified as p and q, acrocentric chromosomes are identified as a, and the two sections of large acrocentric chromosomes in Atlantic salmon are identified as qa and qb. Metacentric chromosome arms in Coho salmon are identified as a and b.

[1] The affinities of Arctic and brook charr linkage groups to the ancestral homeologous arms are incompletely resolved. Linkage groups in Arctic and brook charr have yet to be assigned to specific chromosomes.

[2] Homeologous pairings not supported in Lien *et al*. (2011).

[3] Linkage group AC20 in Arctic charr is provisionally defined as a metacentric chromosome and the two arms are apparently homeologs. Linkage groups BC20a and BC20b are each provisionally defined as acrocentric chromosomes (Timusk *et al.* 2011).

Table 4.3. Summary of genetic linkage maps used to assess homeologous recombination.

Linkage maps[1]	Linkage map ID	No. of progeny	No. loci mapped[2]	No. linkage groups in map	Level of genome divergence in the F1 parent being mapped[3]	Parental phase[4]
Rainbow-cutthroat F1 hybrids	Family 1	54	383	28/29[5]	M & F: interspecific F1 hybrid	Known
(Ostberg *et al*. 2013)	Family 2	53			M & F: interspecific F1 hybrid	Known
Rainbow trout	Lot25	48	1127	29	M: intraspecific F1 hybrid; F: not F1 hybrid	Unknown
(Danzmann *et al*. 2006, 2008)	Lot44	86			M: intraspecific F1 hybrid; F: not F1 hybrid	Unknown
Chinook salmon	Family 1	48	335	34	M & F: not F1 hybrid	Known/
(Naish *et al*. 2013)						Unknown[6]
Atlantic Salmon	Family Br5	46	1396	29	M & F: not F1 hybrid	Unknown
(Danzmann *et al*. 2006, 2008)	Family Br6	46			M & F: not F1 hybrid	Unknown
Arctic charr (Woram *et al*. 2004,	Family 2	48	490	37	M: not F1 hybrid; F: intraspecific F1 hybrid	Unknown
Timusk *et al*. 2011)	Family 3	48			M: intraspecific F1 hybrid; F: not F1 hybrid	Unknown
Brook charr	Family HL3	114	133	35	M & F: not F1 hybrid	Unknown
(Timusk *et al*. 2011)	Family HL7	116			M & F: not F1 hybrid	Unknown

[1] The rainbow trout-Yellowstone cutthroat trout F1 hybrid maps were created using F0 male rainbow trout, Kamloops strain (Hayspur Hatchery, Idaho), and female Yellowstone cutthroat trout from Henry's Lake, Idaho. Rainbow trout maps were created using F1 hybrid males derived from two different strains backcrossed to pure strain females. The Arctic charr maps were developed using strains belonging to different phylogeographic groupings (Fraser and Nauyuk strains). The Arctic charr map for family 2 was created using an F1 Fraser-Nauyuk hybrid female backcrossed to a Fraser male and the map for family 3 was created using an F1 Fraser-Nauyuk hybrid male backcrossed to a Fraser female. The brook charr map was created using individuals from a longstanding hatchery strain that had been crossed with a wild strain on at least one occasion in the 1960s. The Chinook and Atlantic salmon maps were created from individuals derived from the same population, with no known hybrid history.

[2] Microsatellite, single nucleotide polymorphism, and allozyme loci.

[3] Male parent = M; female parent = F.

[4] The linkage maps with known parental phase were the only informative maps for confirming pseudolinkage.

[5] Variation in linkage group number is likely due to a Robertsonian polymorphism in the rainbow trout source stock (Ostberg *et al*. 2013).

[6] The parental phase in Chinook salmon was known across four chromosomes in the F1 female parent and across 15 chromosomes in the F1 male parent. The parental phase across the remaining Chinook salmon chromosomes was unknown.

Table 4.4. The total number of duplicated locus pairs exhibiting random (no significant deviations from the expected ratio of 1:1:1:1) and non-random (significant deviations from the expected ratio) segregation within sex and the number of unique homeologs represented.

		Random segregation		Non-random segregation[1]	
Linkage map	Sex	Duplicated loci	Unique homeolog pairs	Duplicated loci	Unique homeolog pairs
Rainbow-cutthroat	M	6	3	13[2]	5
F1 hybrids	F	21	6	1[2]	1
Rainbow trout	M	62	20	5	2
	F	60	21	0	0
Chinook salmon	M	12	6	0	0
	F	9	6	0	0
Atlantic salmon	M	31	14	0	0
	F	33	14	1	1
Arctic charr	M	15	10	4	2
	F	16	10	0	0
Brook charr	M	9	5	2	1
	F	20	10	0	0

[1] Significant after adjusting the FDR for 319 tests (α = 0.0079).

[2] Non-random segregation in rainbow-cutthroat F1 hybrids indicated pseudolinkage.

Table 4.5. Summary statistics (proportion of non-parental haplotypes, f_{NP}, and correlation coefficient, R) for duplicated locus pairs that segregated alleles non-randomly (deviated from the expected ratio of 1:1:1:1) after adjusting the FDR for 319 simultaneous tests (α = 0.0079).

Linkage map	Homeologous pair	Rainbow trout homeologs	Duplicated locus	Gamete counts				f_{NP}	R[1]
Rainbow-cutthroat F1 hybrid (phase known)				RR	RY	YR	YY		
Male1	RYHyb06q-RYHyb26a	Omy06q-Omy26a	OMM1512	1	21	15	1	0.947	-0.892
Male1	RYHyb10q-RYHyb19p	Omy10q-Omy19p	OMM1134	0	26	26	2	0.963	-0.929
Male1	RYHyb10q-RYHyb19p	Omy10q-Omy19p	OMM1528	0	22	22	1	0.978	-0.957
Male1	RYHyb12q-RYHyb13q	Omy12q-Omy13q	BHMS184	4	21	17	3	0.844	-0.687
Male2	RYHyb12q-RYHyb13q	Omy12q-Omy13q	OMM1200	0	20	23	2	0.956	-0.915
Male1	RYHyb13p-RYHyb17q	Omy13p-Omy17q	OMM5051	5	17	24	0	0.891	-0.800
Male1	RYHyb13p-RYHyb17q	Omy13p-Omy17q	OMM1035	0	22	16	5	0.884	-0.788
Male2	RYHyb13p-RYHyb17q	Omy13p-Omy17q	BX087664	5	21	15	5	0.783	-0.558
Male2	RYHyb13p-RYHyb17q	Omy13p-Omy17q	OMM5165	5	21	15	5	0.783	-0.558
Male1	RYHyb15q-RYHyb21p	Omy15q-Omy21p	OMM1077	3	23	25	1	0.923	-0.849
Male2	RYHyb15q-RYHyb21p	Omy15q-Omy21p	OMM1077	7	20	22	1	0.840	-0.704
Male1	RYHyb15q-RYHyb21q	Omy15q-Omy21p	OMM1764	3	14	16	1	0.882	-0.770
Male2	RYHyb15q-RYHyb21p	Omy15q-Omy21p	OMM1764	4	13	15	0	0.875	-0.777
Female1	RYHyb15q-RYHyb21p	Omy15q-Omy21p	OMM1077	10	16	21	5	0.712	-0.431
Rainbow trout (phase unknown)[2]				AA	AB	BA	BB		
Lot25 Male	Omy12q-Omy13q	Omy12q-Omy13q	OMM1274	5	23	16	4	0.813	0.618
Lot25 Male	Omy13p-Omy17q	Omy13p-Omy17q	OMM1064	4	16	18	8	0.739	0.489
Lot25 Male	Omy13p-Omy17q	Omy13p-Omy17q	SmaBFRO1	5	19	15	8	0.723	0.449
Lot25 Male	Omy13p-Omy17q	Omy13p-Omy17q	CR367013	5	15	17	7	0.727	0.427
Lot25 Male	Omy13p-Omy17q	Omy13p-Omy17q	CA358173	5	17	15	8	0.711	0.456
Atlantic salmon (phase unknown)[2]				AA	AB	BA	BB		
Br6 Female	Ssa13qa-Ssa15qb	Omy09q-Omy16q	StrA8	2	17	17	10	0.739	0.524

Arctic charr (phase unknown)[2]				AA	AB	BA	BB		
Male3	AC13-AC34	Omy10q-Omy19p	CL47450	7	20	15	6	0.729	0.453
Male3	AC13-AC34	Omy10q-Omy19p	OMM1412	7	19	15	6	0.723	0.443
Male3	AC13-AC34	Omy10q-Omy19p	OMM5017	5	18	14	7	0.727	0.453
Male3	AC1-AC21	Omy13p-Omy17q	OMM1330	2	18	12	8	0.750	0.524
Brook charr (phase unknown)[2]				AA	AB	BA	BB		
HL3 Male	BC1-BC21	Omy13p-Omy17q	BX311884	22	35	37	18	0.643	0.287
HL3 Male	BC1-BC21	Omy13p-Omy17q	Omy21INRA	22	36	37	20	0.635	0.270

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[1] *R* was significant for all tests.

[2] The two allele combinations occurring at the highest frequency (AA and BB or AB and BA) are assumed to represent recombinant gamete types when the parent phase is unknown.

Table 4.6. The frequency of disomy, tetrasomy, and double-reduction observed in female rainbow-cutthroat F1 hybrid gametes at four duplicated locus pairs where one locus maps distally to RYHyb12q and the other locus maps distally to RYHyb13q (see Figure 4.3).

		Genotype[1]		Frequency of inheritance in female gametes		
Family	Locus	Female parent	Male parent	Disomy	Tetrasomy	Double-reduction
Family 1	OMM1218	24/36	1357	0.72	0.21	0.07
Family 2	OMM1200	35/34	26/17	0.83	0.15	0.02
	OMM1218	46/37	1235	0.65	0.22	0.13
	OMM1258	23/15	3344	0.63	0.22	0.15
	OMM1274	34/16	2355	0.63	0.22	0.15

[1] The alleles at each duplicated locus are represented by a number within family. The two alleles preceding the backslash (/) represent the locus i and the two alleles following the backslash represent the locus ii in Figure 4.3. Alleles could not be assigned to the most likely locus for the male parent genotypes that do not have a backslash.

Figures

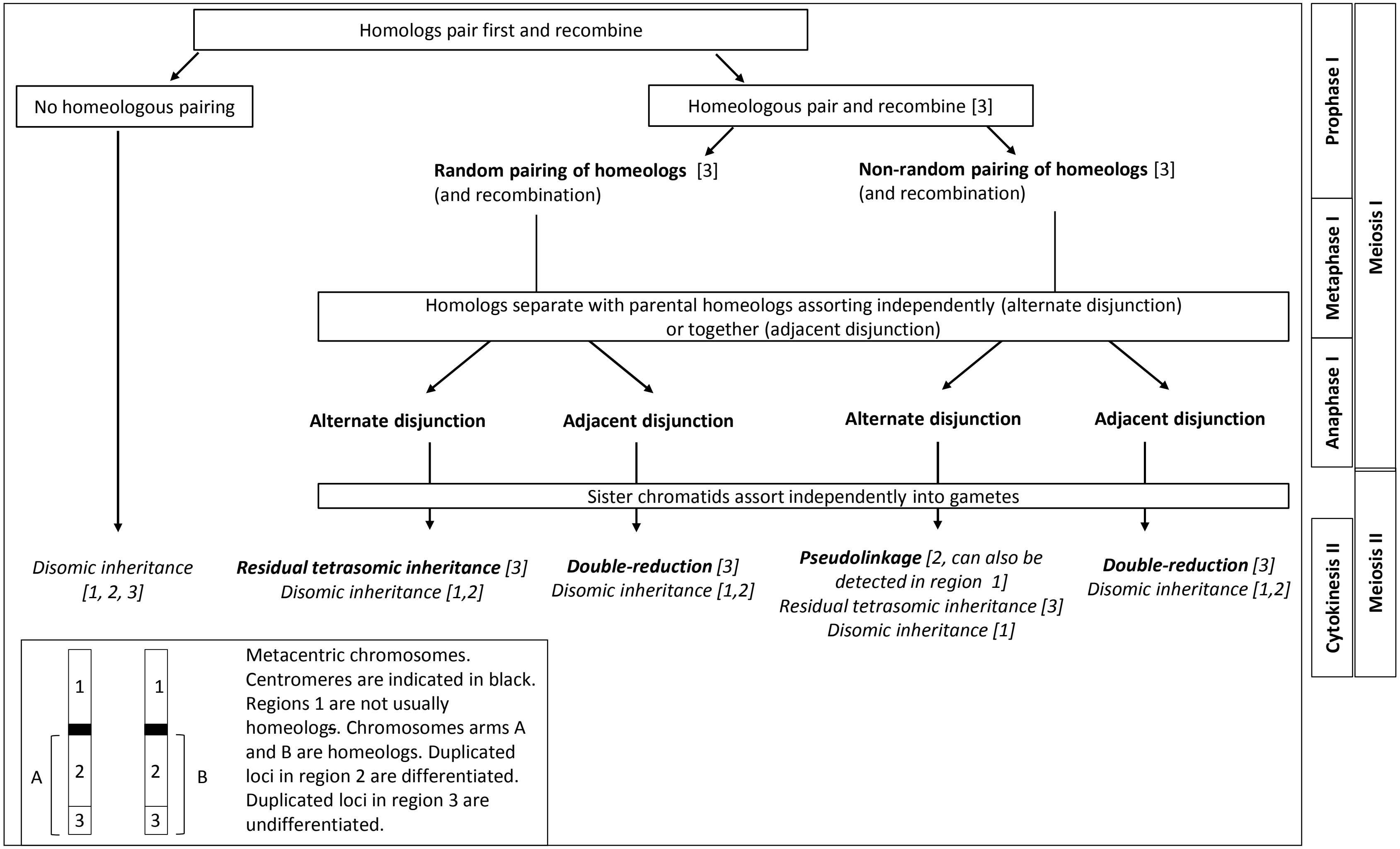


Figure 4.1. Flow diagram of meiosis identifying the events that lead to residual tetrasomic inheritance, pseudolinkage, and double-reduction. The inset represents two metacentric chromosomes having a homeologous arm pairing. These chromosomes are divided into three regions (identified

in the inset) and the specific events associated with each chromosome region are identified by numbers in brackets in the flow diagram. The pathway to double-reduction identified here is specific to one recombination events within a homeologous arm pair (double-reduction may occur through alternate disjunction when multiple recombination events have occurred within a homeologous arm pair).

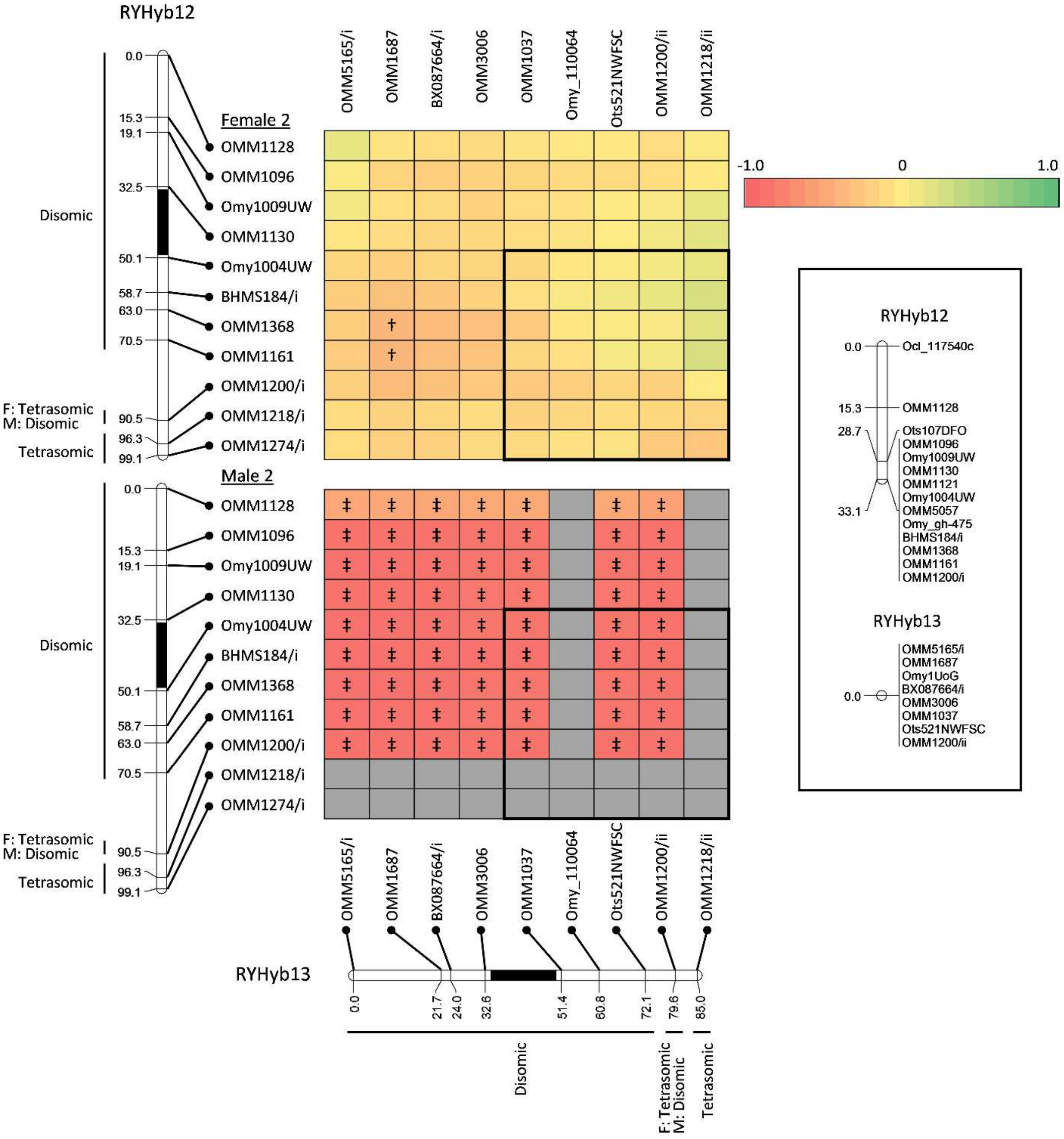


Figure 4.2. The extent of pseudolinkage and correlation coefficient of alleles between loci across the homeologous pairing RYHyb12q-RYHyb13q in rainbow-cutthroat F1 hybrid family 5. The female parent linkage maps are shown next to the male parent matrix and each locus is connected to the female map position. The male parent linkage maps are inset. † indicate significant correlations only and ‡ indicate significant correlations plus pseudolinkage between loci following the FDR adjustment. The heatmap calibration bar represents the range of possible correlation coefficients. Linkage maps are oriented p to q arm, putative centromere locations in female maps are shaded in black, and map distances are in centiMorgans. Pairwise comparisons between the homeologous chromosome arms are enclosed by a box in each matrix. Type of inheritance for each locus is indicated.

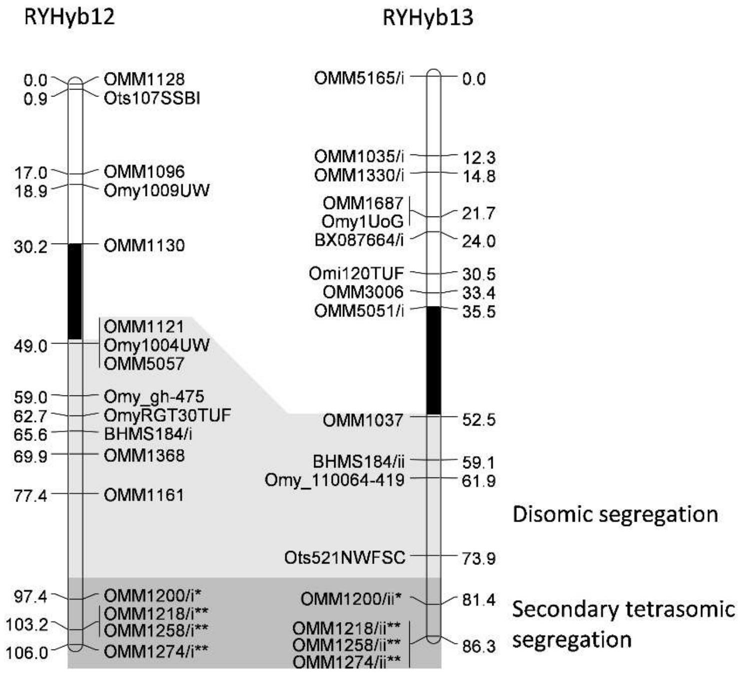


Figure 4.3. Distribution of loci at the RYHyb12q-RYHyb13q homeologous pairing that showed disomic (light grey) and tetrasomic (dark grey) segregation in rainbow-cutthroat F1 hybrids. Female-merged linkage maps are shown. Homologous chromosomes recombined within the light grey region. Recombination occurred between homologous and between homeologous chromosomes within the dark grey region; therefore these loci exhibited residual secondary tetrasomic inheritance. *Duplicated locus that segregated tetrasomically in female and disomically in males; ** duplicated locus that segregated tetrasomically in female and male. Linkage maps are oriented p to q arm, putative centromere locations are shaded in black, and map distances are in centiMorgans.

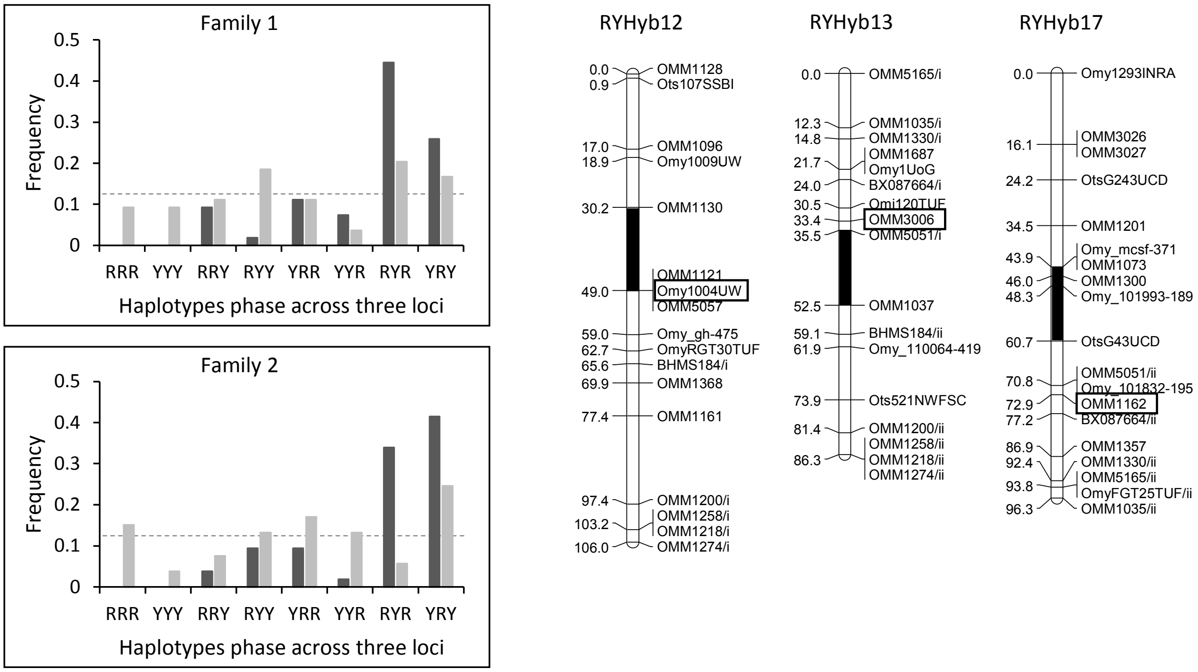
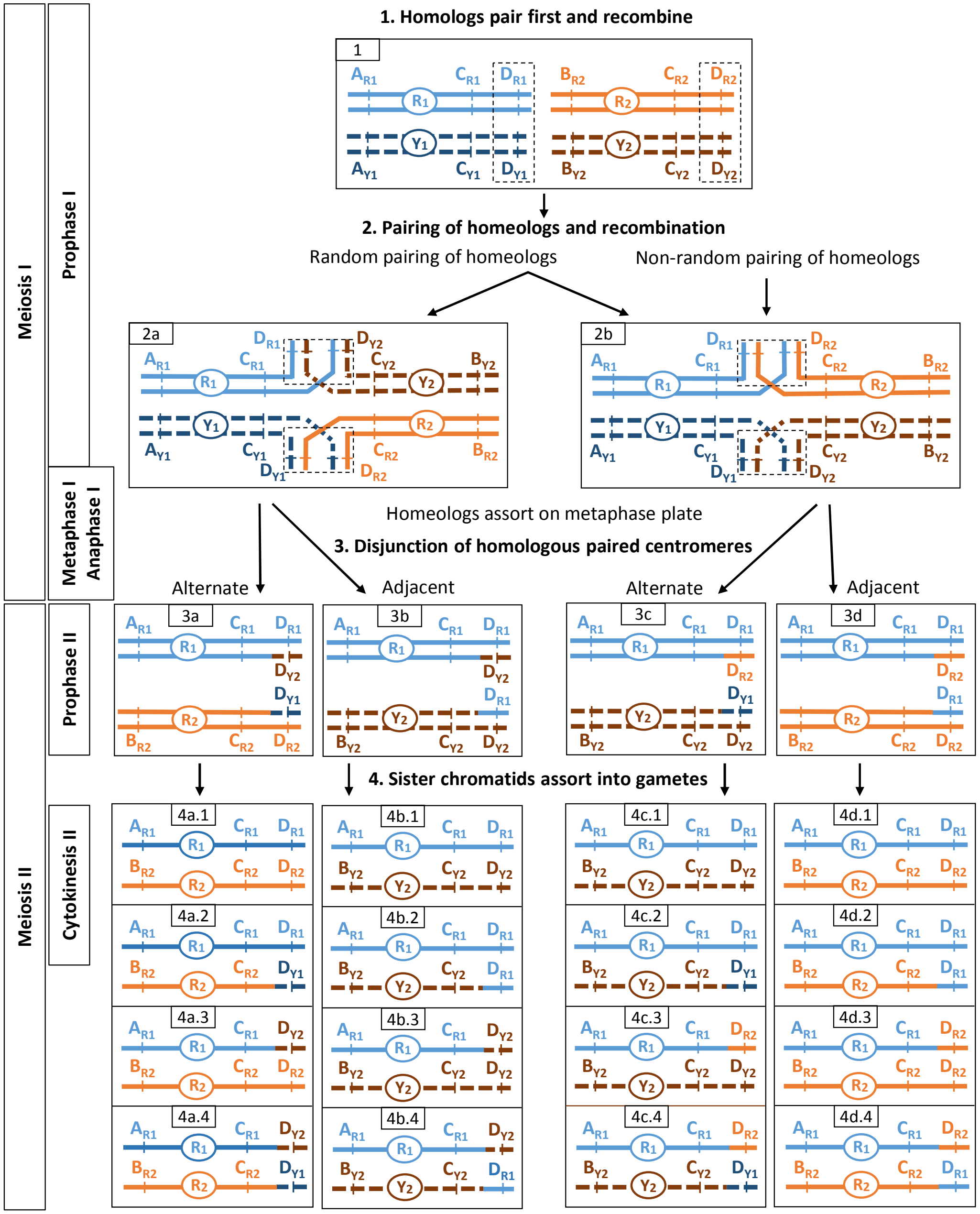


Figure 4.4. Frequency of parental and non-parental haplotypes at a hexavalent formation in rainbow-cutthroat F1 hybrid male (dark grey) and female (light grey) gametes across three disomically inherited loci mapping to RYHyb12, RYHyb13, and RYHyb17. Haplotypes are identified by parental gametic phase (R = rainbow trout, Y = Yellowstone cutthroat trout) and haplotypes across the three loci are presented in the order RYHyb12, RYHyb13, and RYHyb17. Eight haplotypes are expected; two parental (RRR and YYY) and six non-parental. The dashed line indicates the expected frequency with random segregation. Female-merged linkage maps are shown and the loci used in the analysis are boxed. Linkage maps are oriented p to q arm, putative centromere locations are shaded in black, and map distances are in centiMorgans.



1. Two rainbow trout (R_1 and R_2) and two cutthroat trout (Y_1 and Y_2) chromosomes are shown. Chromosome arms containing locus C and D are homeologs; the region inside dashed boxes are undifferentiated (locus D is duplicated) and the region between the centromere and dashed box are differentiated (locus C is duplicated). Locus A and B are non-duplicated and reside on chromosome arms that are not usually homeologs. Homologs (R_1/Y_1 and R_2/Y_2) pair first and may recombine anywhere. For simplicity, homologs are shown as non-recombining.

2. Homeologs pair and recombine. Random pairing of homeologs involves panels 2a and 2b. Non-random pairing of homeologs involves panel 2b only. Both homeologs are shown as recombining, although only one recombination event is necessary. Our empirical data indicate that recombination between homeologs is restricted to distal chromosome arm regions (i.e. duplicated locus D).

3. One of two meiosis I daughter cells from 2a and 2b each is shown following alternate and adjacent disjunction.

Randomly paired homeologs: Alternate disjunction results in daughter cells 3a and 3c. Adjacent disjunction results in daughter cells 3b and 3d.

Non-randomly paired homeologs: Alternate disjunction results in daughter cell 3c only. Adjacent disjunction results in daughter cell 3d only.

4. Four possible gamete types for each panel 3 daughter cell.

Randomly paired homeologs: Alternate disjunction results in residual tetrasomic segregation, panels 4a and 4c. Locus D segregates tetrasomically in gametes 4a.2, 4a.3, 4c.2, and 4c.3, and segregates disomically in gametes 4a.1 and 4c.1. Each chromosome in gametes 4a.4 and 4c.4 has a homeologous crossover at locus D. All other loci exhibit disomic inheritance. Adjacent disjunction results in double-reduction, panels 4b and 4d. Locus D exhibits double-reduction in gametes 4b.2, 4b.3, 4d.2, and 4d.3.

Non-random pairing of homeologs: Alternate disjunction results in pseudolinkage, panel 4c. The pool of gametes in 4c exhibit an excess of non-parental haplotypes at differentiated locus pairs between chromosomes results. Undifferentiated duplicated loci (for example, locus D) segregate tetrasomically. Alternate disjunction results in double-reduction, panel 4d. Locus D exhibits double-reduction in gametes 4d.2 and 4d.3.

Figure 4.5. (On previous page). Meiotic model demonstrating how recombination of randomly and non-randomly paired homeologs can lead to secondary tetrasomic inheritance, pseudolinkage, and double-reduction. This model follows the flow diagram in Figure 4.1. A homeologous chromosome pair (chromosome 1 in light and dark blue shades and chromosome 2 in light and dark orange shades) derived from a rainbow trout (solid line and lighter color shades) and cutthroat trout (dashed line and darker color shades) are shown in an individual. Centromeres are indicated by an ellipse (R = rainbow trout, Y = cutthroat trout). Locus subscripts indicate chromosome origin and species phase. The model incorporates empirical data from F1 hybrids

Supplementary Material

S4.1: Updated rainbow trout-Yellowstone cutthroat trout F1 hybrid linkage maps for the linkage groups RYHyb12, RYHyb13, and RYHyb17 in female and male parents of Families 1 and 2. Excel file with multiple worksheets.

S4.2: Non-random segregation and correlation coefficients between loci across Oncorhynchus homeologous chromosome pairs in gametes from male parents. Excel file with multiple worksheets.

S4.3: Extent of non-random segregation and correlation coefficients between loci across Oncorhynchus homeologous chromosome pairs in the male parent, relative to the female parent linkage map distances. Excel file with multiple worksheets.

S4.4: Pseudolinkage and correlation coefficients between loci across two non-homeologs (RYHyb12 and RYHyb17) within a tetravalent formation in rainbow-cutthroat F1 hybrid male and female parents. Excel file with multiple worksheets.

S4.5: Three meiotic models explaining how multiple cross-over events within a homeologous chromosome arms may have led to secondary tetrasomic segregation and double-reduction observed in female rainbow-cutthroat F1 hybrid gametes. PDF file with multiple pages.

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