# Sex chromosome evolution, heterochiasmy and physiological QTL in the salmonid Brook Charr *Salvelinus fontinalis*

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## ABSTRACT

Whole genome duplication can have large impacts on genome evolution. However, much remains unknown about these impacts, such as the mechanisms of coping with a duplicated sex determination system, which may result in increased sex determination mechanism diversity. Sexual conflict (i.e. alleles having different optimums in each sex) can result in sequestration of genes into non-recombining sex chromosomes. Development of sex chromosomes may involve heterochiasmy (i.e. sex-specific recombination rate), which is also poorly understood. Family Salmonidae is a model system for these phenomena, having undergone autotetraploidization and subsequent rediploidization in most of the genome at the base of the lineage. The salmonid master sex determining gene is known, and many species have non-homologous sex chromosomes, putatively due to transposition of this gene. In this study, we identify the sex chromosome of Brook Charr *Salvelinus fontinalis* and compare sex chromosome identities across the lineage (eight species, four genera). Although non-homology is frequent, homologous sex chromosomes and other consistencies are present in distantly related species, indicating probable convergence on specific sex and neo-sex chromosomes. We also characterize strong heterochiasmy with 2.7-fold more crossovers in maternal than paternal haplotypes with paternal crossovers biased to chromosome ends. Y chromosome crossovers are restricted to a single end of the chromosome, and this chromosome contains a large interspecific inversion, although its status between males and females remains unknown. Finally, we identify QTL for 21 unique growth, reproductive and stress-related phenotypes to improve knowledge of the genetic architecture of these traits important to aquaculture and evolution.

## INTRODUCTION

Characterizing the genetic architecture of ecologically-relevant phenotypes is essential for organism and genome evolution research (Rogers and Bernatchez 2007; Gagnaire *et al.* 2013) and selective breeding (Yáñez *et al.* 2014). Regions of the genome associated with specific traits can be identified by quantitative trait loci (QTL) analysis (Mackay 2001) or genome-wide association studies (GWAS; Bush and Moore 2012). Mapped traits can include morphological, behavioral, physiological or molecular phenotypes, but must show sufficient heritable variation. Power to detect QTL is determined by the number of individuals in the study (Henning *et al.* 2014), the effect size of the QTL, allele frequencies (Mackay *et al.* 2009) and the degree of polygenic control of the trait (Rockman 2012; Ashton *et al.* 2016). QTL mapping precision depends on recombination frequency (Mackay *et al.* 2009) as well as map density, although QTL are often only in linkage with causative mutations, which are rarely identified (Slate 2005). Trait genetic architecture can differ between families or populations (e.g. Santure *et al.* 2015) but parallelism and shared QTL can be identified (e.g. Laporte *et al.* 2015; Larson *et al.* 2015). It is therefore valuable to analyze multiple crosses to understand the broader implications of a QTL (e.g. Hecht *et al.* 2012; Palti *et al.* 2015; Lv *et al.* 2016), to more accurately determine the amount of variation explained by the QTL (Slate 2005), and to identify QTL that are not dependent on specific genetic backgrounds, which is particularly valuable for marker-assisted selection (Lv *et al.* 2016).

Advances in massively parallel sequencing (MPS) technology and comparative genomics have benefited QTL and association studies in several ways. MPS greatly increases marker density (Catchen *et al.* 2011; Ashton *et al.* 2016), provides markers with flanking sequence that can be aligned against reference genomes for integrating across species (Sutherland *et al.* 2016) or for identifying genes near QTL to inform on potential drivers underlying a trait (e.g. McKinney *et al.* 2016; Johnston *et al.* 2016). When causative mutations are not known, detecting orthologous QTL in other species can provide further evidence for a region or gene being related to a trait (Mackay 2001; Larson *et al.* 2015). As an example, QTL for recombination rate in mammalian model and non-model systems occur near the same genes (Johnston *et al.* 2016). Comparative genomics clearly has an important role in identifying drivers of trait variation.

Genetic architecture can be strongly affected by sexually antagonistic selection and sex determination. Sexually antagonistic alleles (i.e. alleles that benefit sexes differently) produce genetic conflict (Mackay 2001; Charlesworth *et al.* 2005), which can be resolved by the sequestration of alleles in non-recombining sex chromosomes. As an example of the effect this can have on genome architecture, the *Drosophila* Y chromosome is made almost exclusively of genes that have migrated from other chromosomes, presumably due to their specific benefit to males (Carvalho 2002). An additional benefit

occurs by constant sex-specific selection occurring for alleles on the Y (or W) chromosome, as these are always only in the heterogametic sex (Lahn *et al.* 2001). However, the lack of recombination between the sex chromosomes can also result in Y degeneration due to accumulation of mutations that are not able to be purged through recombination with X (Charlesworth 1991). A different resolution to genetic conflict involves sex-dependent dominance, whereby allelic dominance depends on the sex of the individual, which need not be on the sex chromosome (Barson *et al.* 2015). Much remains to be understood about resolving these conflicts.

Genome evolution can also be affected by large mutational forces, such as polyploidization events including whole genome duplication (WGD) (Ohno 1970), which may disrupt sex determination systems (Davidson *et al.* 2009). Although details of this disruption remain generally unknown, some hypotheses have been proposed involving the independent segregation of duplicated sex determining chromosomes or imbalances in gene dosages when X inactivation occurs (Muller 1925; Orr 1990; Davidson *et al.* 2009). Highly diverse sex determination systems are observed in teleosts (Marshall Graves and Peichel 2010), which may have been influenced by the teleost-specific WGD due to a post-WGD adoption of numerous different sex determination mechanisms (Mank and Avise 2009). Evolution of sex determination post-WGD may occur through the mutational disruption of one duplicated portion of the existing system, or by the development of a new system (Davidson *et al.* 2009), which can thus result in the evolution of new sex chromosomes.

Sex chromosome evolution may be facilitated by differences in recombination rates between the sexes (i.e. heterochiasmy) (Charlesworth *et al.* 2005). The evolution of heterochiasmy remains under investigation, although several explanations have been proposed (Lenormand and Dutheil 2005; Brandvain and Coop 2012; Lenormand *et al.* 2016). First, sexes can experience different extents of selection at haploid stages (Lenormand 2003), and heterochiasmy permits retention of epistatically-interacting alleles within a haplotype specifically within the sex experiencing more haploid selection (Lenormand and Dutheil 2005). Second, physical meiotic differences may play a role; female meiosis occurs with a long delay, and chiasma (i.e. locations where crossovers occur) stabilize chromatids during this process (Lenormand 2003; Lenormand *et al.* 2016). Third, recombination protects from meiotic drive, to which the sexes have different susceptibilities (Brandvain and Coop 2012). Other hypotheses have also been proposed (Trivers 1998; Lenormand 2003). However, in general it is unclear which of the above explanations have the largest influence, and thus the relationships between WGD, heterochiasmy and sex chromosome evolution require further study.

Salmonids (Family Salmonidae) are an ideal system to study genetic architecture and sex determination post-WGD (Davidson *et al.* 2010). The salmonid genome remains in a residually tetraploid state, where some chromosomal telomeric regions continue recombining between homeologous

chromosomes and others have rediploidized (Allendorf and Thorgaard 1984; Allendorf *et al.* 2015; May and Delany 2015; Lien *et al.* 2016). Salmonid sex determination is genetically controlled (Davidson *et al.* 2009) by a truncated gene from the *interferon-response factor* transcription factor family, *sdY* (sexually dimorphic on the Y-chromosome; Yano *et al.* 2012a). *sdY* may be a salmonid innovation as it has not yet been identified in the non-duplicated sister group for the salmonid WGD, Northern Pike *Esox lucius* (Yano *et al.* 2012b). Male genome-specific conservation of *sdY* occurs in more than ten salmonid species, but some exceptions exist, including the Lake Whitefish *Coregonus clupeaformis* and European Whitefish *C. lavaretus* (Yano *et al.* 2012b), and some Atlantic Salmon *Salmo salar* and Sockeye Salmon *Oncorhynchus nerka* individuals (Eisbrenner *et al.* 2013; Larson *et al.* 2016). Sex chromosomes are not homologous among many salmonid species, potentially due to transposition of *sdY* between chromosomes (Woram *et al.* 2003). Additional evidence for transposition includes repetitive flanking regions with putative transposable elements (Brunelli *et al.* 2008; Lubieniecki *et al.* 2015) and sequence conservation that abruptly stops outside of the sex determination cassette (Faber-Hammond *et al.* 2015). This transposition to different chromosomes may be delaying Y degeneration (Yano *et al.* 2012b; Lubieniecki *et al.* 2015). In general, the salmonids are at an early stage of sex chromosome evolution (Phillips and Ihssen 1985; Yano *et al.* 2012b) where sex chromosomes are homomorphic (Devlin *et al.* 1998; Phillips and Ráb 2001; Davidson *et al.* 2009). Male salmonids have low recombination rates relative to females with crossover events primarily occurring at telomeric regions, as observed in Rainbow Trout *O. mykiss* (Sakamoto *et al.* 2000) and Atlantic Salmon (Moen *et al.* 2004). Heterochiasmy is not viewed in the sister species of the salmonid WGD Northern Pike (Rondeau *et al.* 2014) and therefore salmonids are a valuable model to study the evolution and effects of heterochiasmy in relation to sex determination post-WGD.

The combination of characterizing heterochiasmy, sex chromosome identity and the genetic architecture for reproductive, growth and stress response traits provides much-needed information regarding the function of the Brook Charr *Salvelinus fontinalis* genome post-duplication. The goals of this study were to use a high-density genetic map for Brook Charr (Sutherland *et al.* 2016) to (*a*) identify the sex-linked chromosome; (*b*) quantify heterochiasmy in this mapping family while correcting for probable genotyping errors; and (*c*) search for growth, stress resistance and reproduction-related QTL. Furthermore, using the recent characterization of homology to ancestral chromosomes and homeolog identification among the salmonids (Sutherland *et al.* 2016), we subsequently compare identities of sex chromosomes and identified QTL across the salmonids to identify consistencies, and discuss the implications of consistencies as well as the observed heterochiasmy regarding sex chromosome evolution.

146 **METHODS**

147 **Fish and phenotyping**

148 Juvenile Brook Charr used in this study were the same individuals used to construct a low-density genetic
149 map and perform QTL analysis for 21 phenotypes (29 including repeated measurements occurring at three
150 time points; Table S1) by Sauvage *et al.* for growth (2012a) and reproductive QTL (2012b). Fish were
151 raised in tanks as previously described until 65-80 g, at which point weight, length and condition factor
152 were measured. These phenotypes were measured on the same fish two and six months after the initial
153 measurements. Growth rate was calculated between the multiple sampling times. At the final sampling,
154 all phenotypes were collected. Stress response was also evaluated at this final sampling through an acute
155 handling stress by reducing water levels, capturing fish without chasing and holding out of water for one
156 minute in order to phenotype the stress response using blood parameters chloride, osmolality and cortisol
157 before and after the stress. After fish had re-acclimatized, they were anaesthetized and killed by
158 decapitation as per regulations of Canadian Council of Animal Protection recommendations and protocols
159 approved by the University Animal Care Committee, as previously reported (Sauvage *et al.* 2012a). The
160 sex of each individual was determined by visual inspection of the gonads as reported by Sauvage *et al.*
161 (2012b).

162

163 **Genetic map and quality control of markers and phenotypes**

164 A recently developed high-density genetic map with 3826 markers was used with genotypes for 192
165 offspring (Sutherland *et al.* 2016). In brief, genotype data was obtained using the population module of
166 STACKS v.1.32 (Catchen *et al.* 2011), phased in JoinMap v.4.1 (van Ooijen 2006), and imported into
167 R/qtl (Broman *et al.* 2003) using the *read.cross* function with data interpreted as a four-way cross type in
168 the *mapqtl* format (see File S1 for map, genotype and phenotype input files).

169 All 29 phenotypes (including eight measures at multiple time points) related to blood parameters,
170 growth, growth-related gene expression, reproduction and stress response were used to search for QTL
171 (Table S1). Correlation between phenotypes was evaluated using Pearson correlation in R (R
172 Development Core Team 2017) and a correlation plot was generated using the R package *corrplot*
173 (v.0.77; Wei and Simko 2017). Phenotypes were inspected for normal distribution, and when required,
174 log transformed (Broman and Sen 2009). Outlier phenotype values (>3 SD from the mean) were removed
175 to prevent spurious associations (Broman and Sen 2009), including two individuals each for T1-T2 and
176 T2-T3 growth rates, two individuals for length at T2, four individuals for condition factor at T2, one
177 individual for change in osmolality and one individual for sperm diameter.

178 Markers present in the map were tested for segregation distortion by chi-square tests for
179 Mendelian segregation in R/qtl and removed when $p \leq 0.01$ (Broman and Sen 2009). A total of 157
180 markers with significant segregation distortion were removed, leaving a remainder of 3669 markers.
181 Proportions of identical genotypes were tested in R/qtl to ensure that there were no mis-labeled samples.
182 Recombination fraction between marker pairs was estimated using Expectation Maximization algorithm
183 within *est.rf* in R/qtl. The minimum number of obligate crossover events was calculated per individual
184 using *count.XO* in R/qtl, and an outlier sample with 1093 crossovers was removed (other samples had
185 mean and median crossovers of 101 and 83, respectively, before correcting for unlikely double
186 crossovers).
187
188 **Recombination rate**
189 To characterize heterochiasmy in the mapping family parents, the *plotGenotypes* function of R/qtl was
190 used to identify positions of crossovers per parental chromosome (total = 84 chromosomes per individual
191 offspring) and modified to export these positions (see Data Availability section for all code used in the
192 analysis). Male-specific markers were not included in the original map due to low recombination rate and
193 poor positioning (Sutherland *et al.* 2016), and therefore to avoid bias of including female-specific but not
194 male-specific markers, crossovers were evaluated in a map with only markers informative in both sexes
195 (i.e. *ef* x *eg* and *hk* x *hk*). Furthermore, as recombination rates can be inflated by a genotyping error
196 appearing to be flanked by two false recombination events (Hackett and Broadfoot 2003; Slate 2008),
197 which can also occur in RAD-seq data (Andrews *et al.* 2016), an additional correction was made to more
198 accurately quantify heterochiasmy. Specifically, per individual and per phased haplotype within
199 individual the number of crossovers within 50 cM of each crossover were counted and crossovers were
200 only considered when the number of crossovers was odd, suggesting that a true phase change occurred. If
201 the number was even, these crossovers were not counted as they probably reflect a genotyping error since
202 crossover interference is expected within the salmonids and double crossovers therefore should not occur.
203 This is similar to the approach used by Johnston *et al.* (2016) to avoid false double crossovers by only
204 including crossovers that flank more than a single marker. Subsequently, the cumulative number of
205 crossovers for fused metacentric and acrocentric chromosome were calculated and cumulatively displayed
206 in positions as a percentage of the total chromosome length. The corrected crossover counts were used to
207 calculate the female:male recombination rates of the parents. This was also conducted without cumulating
208 and displayed on a per chromosome per haplotype basis.

**QTL analysis**

The effect of sex on each phenotype was tested using linear models in R (R Development Core Team 2017). If a marginal effect of sex was found ($p \leq 0.20$), sex was included in the model as a covariate for the phenotype to reduce residual variation and improve power to identify the QTL (Broman and Sen 2009). The R/qtl function *scanone* with permutation testing (10,000 permutations; $p \leq 0.05$) was used to identify the presence of a single QTL within each linkage group (Broman *et al.* 2003). Chromosome-wide significance was tested in the same way but per chromosome (10,000 permutations; $p \leq 0.01$). Confidence interval estimates (95%) for QTL positions were identified using *summary.scanone* calculating LOD support intervals with a 1.5 LOD drop. Sex-specific phenotypes (i.e. sperm diameter and concentration, egg diameter) were tested in only one sex, and therefore had smaller sample sizes. Percent variance explained by the identified QTL was performed using *makeqtl* and *fitqtl* within R/qtl, including all genome and chromosome-wide QTL per trait in the formula (trait ~ $QTL_1 + QTL_2 + QTL_n$), as well as sex as a covariate when required. Phenotypic effects were estimated by calculating the differences between the mean phenotype values among the genotype groups for the marker closest to the identified QTL, including only individuals that were successfully genotyped. For markers that only segregate in one parent (i.e. *nn* x *np*) only two phenotype by genotype averages are given, one for the homozygote and one for the heterozygote offspring. Alternatively, for markers segregating in both parents (i.e. *hk* x *hk* or *ef* x *eg*), three phenotype averages are given, two for the alternate homozygotes and one for the heterozygote in *hk* x *hk* marker types and two for the alternate heterozygotes and one for the homozygote in *ef* x *eg* marker types. Sex-specific averages were calculated when the QTL required sex as a covariate in the model. RAD tags for all alleles and associated QTL results are available in File S2.

To identify the sex chromosome, offspring sex was coded as a binary trait to identify linkage to any of the LG by QTL mapping as described above (Broman and Sen 2009). Furthermore, the effect of a QTL may vary depending on the sex of an individual in a non-additive manner (Broman and Sen 2009) due to genetic variation in sexual dimorphism for the trait (e.g. loci that have a different effect in males and females Mackay 2001). Therefore, QTL by sex interaction effects were inspected per trait by subtracting an additive model (*genotype* and *sex*) from a full model (*genotype*, *sex* and a *sex-by-genotype* interaction term) as described by Broman and Sen (2009) . If the additive model is largely driving the effect, the model with only the interaction effect will not be significant. Significant interaction effects were only considered when the full model was found to be significant (Broman and Sen 2009).

Identities of sex chromosomes of other species were obtained from references listed in Table 1. For Atlantic Salmon, Artieri *et al.* (2006) identify that the sex determining region is on the long (q) arm of chromosome Ssa02, and Lien *et al.* identify that Ssa02q is homeologous to Ssa12q, indicating that the chromosome arm holding the sex determining region corresponds to the ancestral chromosome 9.1

(Sutherland *et al.* 2016). Other species were directly obtained from references in Table 1. Correspondence between Arctic Charr *S. alpinus* and Brook Charr were identified indirectly through other species shared between Nugent *et al.* (2016) and Sutherland *et al.* (2016).

**Data availability**

The raw data for this study is available in the NCBI SRA in BioProject PRJNA308100 and accession SRP068206. All input files used for the analysis are in the supplementary files (File S1) and all code used to perform analyses is available on Github at the following link:

https://github.com/bensutherland/sfon_pqtl/

## RESULTS

**Sex-linked chromosome in Brook Charr**

Sex was highly associated with the majority of Brook Charr (BC) linkage group (LG) BC35, indicating that this is the sex-linked chromosome in Brook Charr (Figure 1). Linkage across the entire LG until the LOD score decreases at the distal end can be explained by male salmonid-specific low recombination rate and male bias of crossovers towards telomeric regions (Sakamoto *et al.* 2000). The drop in LOD suggests the far end of the chromosome is pseudoautosomal, which even occurs in the highly differentiated mammalian X/Y chromosomes in a recombinogenic distal region of the Y chromosome (Lahn *et al.* 2001). Many of the recombination events in BC35 were at a similar section of the LG (~90-110 cM; Figure S2B).

BC35 is an acrocentric chromosome homologous to the Northern Pike chromosome 15.1 (homeolog naming from Sutherland *et al.* 2016). There are no other sex chromosomes in the other salmonid species with high-density genetic maps available with the chromosome arm containing the sex determining region homologous to BC35 (Table 1). Arctic Charr has a sex chromosome that is comprised of a triple fused chromosome (although this may vary across populations) that contains 15.1 in the fusion, but in Arctic Charr this is not the chromosome arm that holds the sex determining region, which is held within the arm on the other side of the chromosome AC04p (Nugent *et al.* 2016). Other salmonids have different sex chromosomes, as shown in Table 1, including Lake Whitefish (3.1; Gagnaire *et al.* 2013), Atlantic Salmon (9.1-20.2; Artieri *et al.* 2006; Lien *et al.* 2011), Rainbow Trout (14.2; Palti *et al.* 2015), Coho Salmon *O. kisutch* (3.1; Phillips *et al.* 2005; Kodama *et al.* 2014), Chinook Salmon *O. tshawytscha* (23.2; Phillips *et al.* 2005; Naish *et al.* 2013; Brieuc *et al.* 2014) or Sockeye Salmon (3.1-19.1; Larson *et al.* 2016). This further refines previous observations of the general lack of homology in the sex chromosomes of the salmonids (Woram *et al.* 2003). Some information on sex chromosomes identities across *Salmo, Salvelinus* and *Oncorhynchus* have been previously reported (Phillips 2013) and most of

the results correspond with those here, with the exception of the Brook Charr sex chromosome, which the two studies identify as corresponding to opposite arms of the Arctic charr sex chromosome. This is possibly due to a population polymorphism, but more work would be needed to confirm this.

Considering the importance of inversions to sex chromosome formation through the reduction of recombination between X and Y (Lahn *et al.* 2001; van Doorn and Kirkpatrick 2007; Berset-Brandli *et al.* 2008), it is interesting to note that Brook Charr has a species-specific inversion in BC35 in the female map (15.1; see Figure 5 in Sutherland *et al.* 2016). As is usual for salmonid linkage maps, the male-specific map was not produced as the low recombination frequency resulted in poorly placed male-specific markers (Sutherland *et al.* 2016), and so it is not possible to check whether this inversion is heterozygous within the species, but this will be valuable to investigate in future studies.

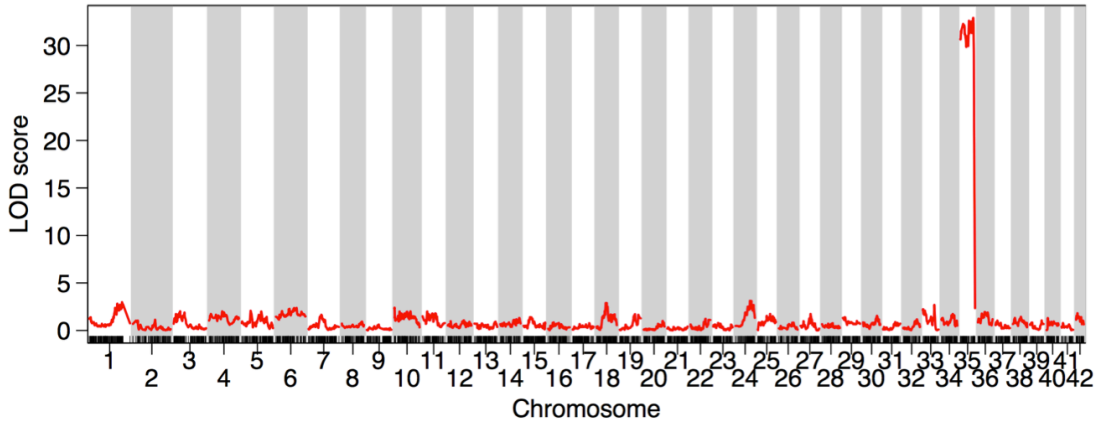



**Figure 1.** The acrocentric linkage group BC35 is highly associated with sex in Brook Charr. Due to low recombination in males, high linkage is viewed across the majority of the linkage group.

**Sex-specific recombination rate and positions of crossovers**

Crossovers occurred 2.7-fold more often in the maternal haplotypes (total = 3679) than in the paternal (total = 1368; Figure 2) based on the phased haplotypes of 169 individual offspring (Wu *et al.* 2002; Sutherland *et al.* 2016). The double recombinant correction (see Methods) in the autosomes removed 606 and 682 crossover events due to probable genotyping errors from the dam and sire, respectively, providing a more accurate estimation of the heterochiasmy ratio, although the trends regarding the crossover positions remained similar. Crossovers were biased towards the center of the linkage groups in the dam and towards the external 20% of the linkage groups in the sire (Figure 2). This bias is similar to that observed in Rainbow Trout (Sakamoto *et al.* 2000) although reasons for it remain unknown.

302 **Table 1.** Salmonid sex chromosomes from high-density genetic maps named with Northern Pike
303 designations (ancestral). The chromosome arm that contains the sex determining region is underlined, and
304 the fusion status of the chromosome and original reference are provided. Ancestral chromosomes are
305 defined by Sutherland *et al*. (2016) and are based on Northern Pike chromosomes from Rondeau *et al*.
306 (2014).
307

| Common name | Scientific name | Linkage group (sex) | Ancestral | Fused (F) or acrocentric (A) | Evidence type | Citations |
|---|---|---|---|---|---|---|
| Lake Whitefish | *Coregonus clupeaformis* | LW25 | 3.1 | A | Linkage | (Gagnaire *et al.* 2013) |
| Atlantic Salmon | *Salmo salar* | Ssa02 | 9.1-20.2 | F | FISH & Linkage | (Artieri *et al.* 2006; Phillips *et al.* 2009; Lien *et al.* 2011) |
| Arctic Charr | *Salvelinus alpinus* | AC04 | 1.2-19.1-15.1 | F | Linkage | (Nugent *et al.* 2016) |
| Brook Charr | *Salvelinus fontinalis* | BC35 | 15.1 | A | Linkage | (Sutherland *et al.* 2016) and *current paper* |
| Rainbow Trout | *Oncorhynchus mykiss* | OmySex (29) | 14.2 | A | FISH & Linkage | (Phillips *et al.* 2006; Rexroad *et al.* 2008; Palti *et al.* 2015) |
| Coho Salmon | *O. kisutch* | Co30 | 3.1 | A | FISH & Linkage | (Phillips *et al.* 2005; Kodama *et al.* 2014) |
| Chinook Salmon | *O. tshawytscha* | Ots17 | 23.2 | A | FISH & Linkage | (Phillips *et al.* 2005; Naish *et al.* 2013; Brieuc *et al.* 2014) |
| Sockeye Salmon | *O. nerka* | So09 | 3.1-19.1* | A | Linkage | (Larson *et al.* 2016) |

308

309 Separating chromosomes into fused metacentric (n = 8) and acrocentric chromosomes (n = 34)
310 indicated a higher heterochiasmy ratio in fused metacentric than acrocentric chromosomes (5.6-fold and
311 2.2-fold, respectively). The male had fewer crossovers per chromosome in the fused metacentrics (mean =
312 26.5) than acrocentrics (mean = 32.9), even though fused metacentrics are comprised of two acrocentric
313 chromosomes combined and thus are longer. In contrast, the female had approximately twice as many
314 crossovers per fused metacentric chromosome (mean = 148.9) than acrocentric (mean = 70.5). The lower
315 recombination in the paternal fused metacentrics than the paternal acrocentrics is probably due to missing
316 regions of the genetic map that are residually tetraploid that were removed during marker filtering due to
317 quality filtering, as the map was produced using a diploid cross (see Limborg *et al.* 2016). Inspection of

individual chromosomes indicates that the chromosomes expected to still exhibit residual tetraploidy (Sutherland *et al.* 2016) all show an absence of crossovers in the male relative to the chromosomes expected to have returned to a diploid state (Figure S2). The missing regions in the 16 (of 50) chromosome arms expected to be residually tetraploid will result in an inflation of the heterochiasmy ratio, as male crossovers will be specifically underestimated for these arms. Regardless, most chromosomes are not residually tetraploid and heterochiasmy can be viewed in these other chromosomes. In summary, the male has fewer recombination events than the female and the crossovers are biased to the distal portions of the chromosomes.

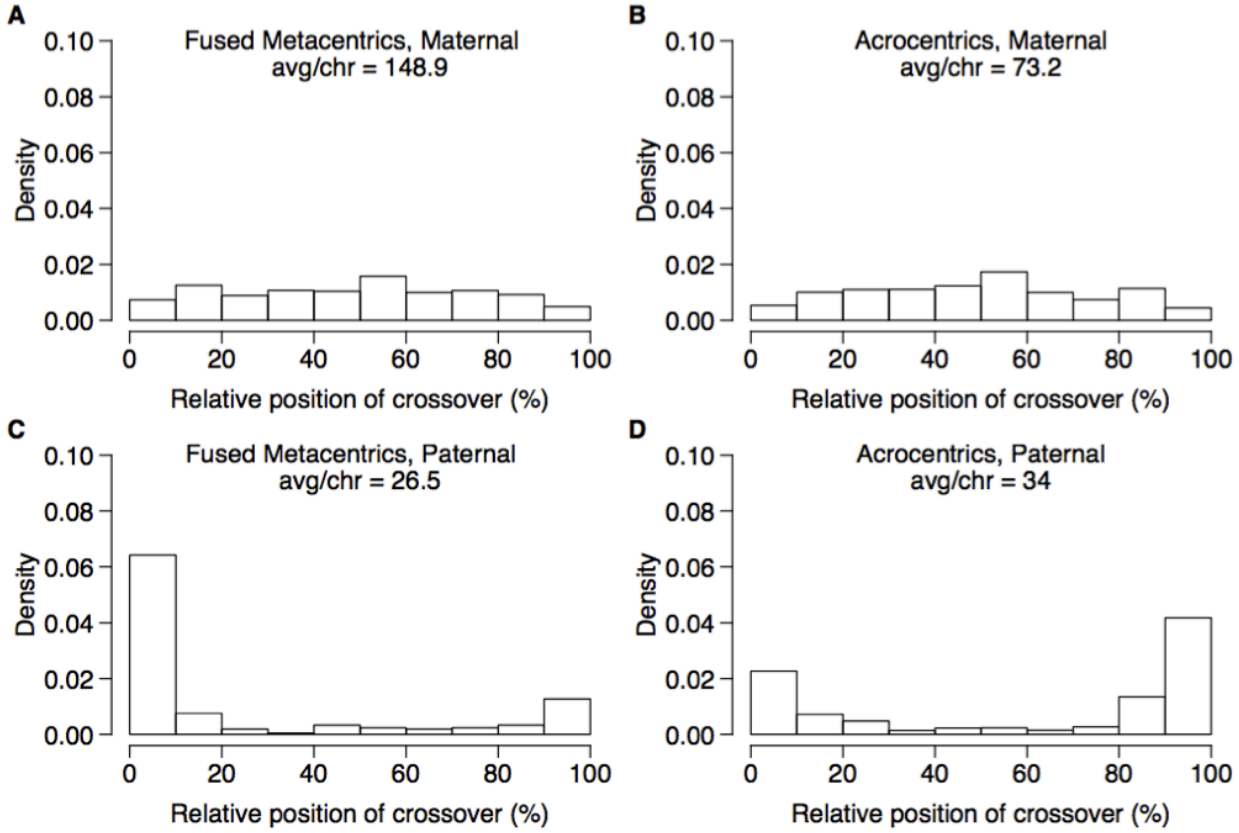
The identified sex chromosome had more crossovers than the average male acrocentric chromosomes (sex = 71, other acrocentrics average = 32.9), and all of the crossover events in the sex chromosome occurred at one end of the chromosome and almost no crossovers occurred in the rest of the chromosome (Figure S2). This bias to only a single end of each chromosome in the male map was consistent throughout all of the chromosomes with crossovers present. Using the positions of centromeres determined for Chinook Salmon (Brieuc *et al.* 2014), and placing them in the corresponding position on the Brook Charr map using map correspondence (Sutherland *et al.* 2016), indicates that the end of the acrocentric chromosomes where the crossovers occur is the opposite end to that containing the probable centromere (see Figure S2).

**QTL identification: growth, reproduction and stress response**

Genome-wide significant QTL were identified for weight, length, condition factor, specific growth rate, and liver weight (Table 2; Table S2). A total of 29 QTL were found to be significant at the chromosome-wide level ($p \leq 0.01$), and these included QTL for phenotypes egg and sperm diameter, change in cortisol, chloride and osmolality after an acute handling stress, *growth hormone receptor* gene expression and hematocrit (Table 2). In total, QTL were identified on 14 of the 42 Brook Charr linkage groups (Figure 3).

Several traits showed sexual dimorphism and therefore required sex as a model covariate. These included weight, length, liver weight, hepatosomatic index, hematocrit, change in osmolality and cortisol from stressor, resting plasma chloride, hepatic glycogen, *insulin-like growth factor 1* and *igf receptor 1* (Table S1). Specific growth rate, condition factor, change in chloride, resting plasma osmolality and glucose, and *growth hormone receptor* gene expression did not show sexual dimorphism. Traits with high phenotypic correlation included length and weight ($r = 0.90$ at T1), and liver weight and hepatosomatic index ($r = 0.85$; Figure S1). Specific growth rate T1-T2 was negatively correlated with weight at T1 ($r = -0.64$), suggesting that larger individuals measured at T1 subsequently grew slower than smaller individuals. Other traits generally were not as highly correlated ($r < 0.35$). Even though the phenotypes were not highly correlated, QTL were identified for condition factor and weight in the same region of

352 BC20, and QTL affecting hematocrit and weight (r = 0.24) were found in the same region on BC04
353 (Figure 3).
354




355

356 **Figure 2**. Maternal and paternal cumulative crossover positions across the chromosomes. The position of
357 each crossover is expressed as a percent of the total crossover length and cumulated for all crossovers
358 within each chromosome type, specifically fused metacentric chromosomes (A,C) and acrocentric
359 chromosomes (B,D) in the maternal and paternal haplotypes, respectively. Maternal haplotypes had 2.7-
360 fold more crossovers than paternal haplotypes, with the maternal crossovers occurring throughout the
361 chromosome and the paternal crossovers restricted mainly to the first and/or last 20% of the linkage
362 groups.
363

364 A few specific trait-linkage group combinations had elevated LOD across a large portion of the
365 LG. This was observed for length and weight on BC03, BC04 and BC05. To determine if this was due to
366 one specific marker type, the three marker types were each tested independently for QTL (female-specific
367 *nn* x *np*; informative in both parents *ef* x *eg*; and semi-informative *hk* x *hk*). Interestingly, when including
368 markers only polymorphic in the female (i.e. *nn* x *np*) these elevated LOD baselines were not observed
369 (*data not shown*). It is possible that this may be therefore due to an effect originating from paternal alleles,
370 which have strong linkage across the entire LG. As these QTL explained a substantial amount of variance

for these three traits, the full analysis includes these markers, but the exact locations within the LG of these QTL cannot be determined without additional families or crosses (markers noted in Figure 3).

A substantial amount of trait variance for length at T2 within this mapping family was explained by five QTL. Together with the additive sex covariate, this collectively explained 54.5% of the trait variation. The QTL with broad elevated LOD on BC04 and BC05 (see above) individually explained 5.5 and 8.2% of the variation, respectively. QTL for condition factor varied depending on the sampling time (T1-T3), where each time point had a QTL at a different LG that explained over 10% of the trait variation within the time point. However, trait variation was small for this trait and therefore effect sizes of the QTL were also small (Table 2). QTL for specific growth rate (SGR) were identified on BC03, BC09 and BC36, with three QTL explaining 26% of the SGR (T2-T3) trait variation (Table 2).

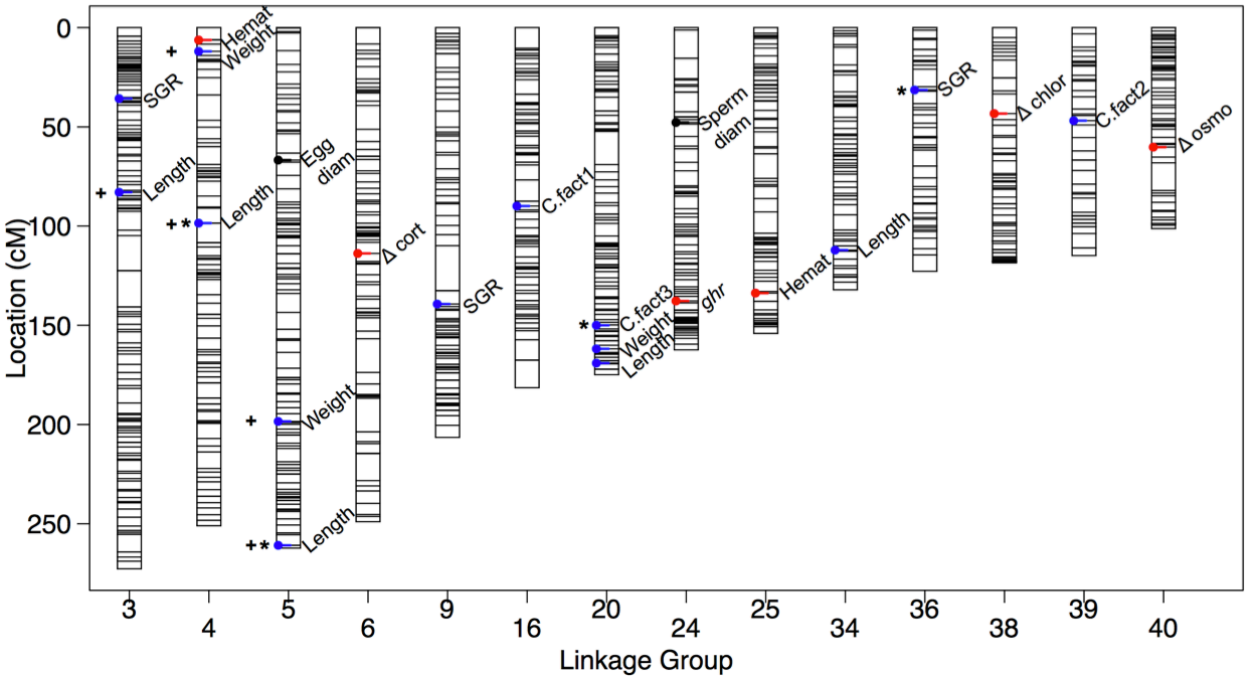



**Figure 3.** All identified QTL plotted on the Brook Charr genetic map. QTL for growth related traits are shown in blue, reproductive in black, and blood or stress-related in red. QTL with asterisks are at the genome-wide significance level, and the rest are chromosome-wide. QTL with broad confidence intervals discussed in the Results are denoted with a positive symbol (+). More details on phenotypes can be found in Table S1 and on QTL can be found in Table 2 and Table S2.

Reproductive traits were sex-specific and therefore had approximately half of the individuals as the traits with values in both sexes, and thus had less statistical power, reducing the ability to detect QTL. Nonetheless, enough power was present in the data to detect chromosome-wide significant QTL for egg and sperm diameter (Table 2; Figure 3). A QTL for egg diameter was identified at 67 cM of BC05, explaining 39% of the trait variation and a QTL for sperm diameter was identified at 48 cM of BC24, explaining 33% of the trait variation. Neither of these reproductive-related traits mapped to the sex-linked chromosome (BC35). A QTL for *growth hormone receptor* (*ghr*) gene expression was identified at 138 cM of BC24 explaining 24% of the trait variation.

Stress response QTL were identified only at the chromosome-wide significance level. This included responses of cortisol (114 cM of BC06), chloride (43 cM of BC38), and osmolality (60 cM on BC40; Table 2; Figure 3). Change in cortisol from acute handling stress was highly dependent on sex; the identified QTL explained 9% of the trait variation whereas sex explained 43% (total PVE = 58%; Table 2). Females heterozygous at the marker closest to the QTL increased cortisol by 2.3 μg/dL plasma more than the homozygote, and heterozygous males had 0.65 μg/dL lower than the homozygote. To further demonstrate the large sex effect, averaging the two genotypes shows that females increased blood cortisol by 8.6 μg/dL whereas males only increase by 0.43 μg/dL (i.e. 20-fold higher cortisol response in females). Osmolality change (mmol/kg) was also affected by sex but to a lesser extent than was cortisol. More specifically, the identified QTL explained 14% of the trait variance and sex explained 14.6%. For this QTL, both sexes showed suggestive additive effects with the heterozygote having a value in between the two homozygotes (Table S2). Chloride change (mmol/L) was not sex-dependent, and the identified QTL on BC38 explained 18% of the trait variation. Chloride reduced in the heterozygote individuals by 2.58 mmol/L whereas it stayed approximately the same in the homozygote (0.3 mmol/L; Table 2). Resting blood hematocrit was affected by sex, and two QTL were identified at the chromosome-wide level (23 cM on BC04 and 14 cM on BC25). Together with the sex covariate, these explained 43% of the trait variation, with each QTL explaining approximately 12%. Together, these results indicate the importance of including sex as covariate in these models. These markers will provide targets for selective breeding.

**Table 2.** Identified QTL in Brook Charr with positions, percent variance explained (PVE) and the effect of the allelic state on the trait. Sex was included as a covariate when required, and in these cases the allelic effect is given for both males and females, and the PVE from sex is also given. When sex was not required as a covariate, the second averages are displayed as NA and the first averages represent both sexes. The phenotype average for the homozygote common allele (aa avg) is shown for comparison to the largest effect size (effect ♀ or ♂). LG = linkage group; Pos = cM position; CI = confidence interval; PVE = percent variance explained; sex.cov = sex included as a covariate. QTL significance is displayed as genome wide $p \leq 0.01$ ***; $p \leq 0.05$ **; or chromosome-wide $p \leq 0.01$ *.

| Phenotype | LG | Pos | 95% CI | Marker | QTL pval | Tot. PVE | Ind. PVE | aa avg ♀ | effect ♀ | aa avg ♂ | effect ♂ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Weight (g) T2 | 4 | 28.3 | 16-214 | 7187 | * | 42.8 | 9.5 | 126 | +22.1 | 129.5 | +52.1 |
| | 5 | 198 | 39-262 | 125487 | * | | 8.1 | 121.4 | +27.9 | 145.3 | +30.6 |
| | 20 | 162 | 105-175 | 6352 | * | | 6.2 | 133.9 | -2.2 | 171.2 | -22.8 |
| | | | | sex.cov | | | 9.5 | | | | |
| Length (cm) T2 | 3 | 83 | 19-267 | 90770 | * | 54.5 | 2.0 | 22.4 | -0.3 | 23.6 | +0.4 |
| | 4 | 115 | 16-215 | 66075 | ** | | 5.5 | 21.8 | +0.9 | 23.6 | +0.2 |
| | 5 | 261 | 185-262 | 85980 | *** | | 8.2 | 21.7 | +1.1 | 23.6 | +0.3 |
| | 20 | 169 | 99-175 | 60142 | * | | 4.1 | 21.7 | +0.8 | 22.9 | +1.3 |
| | 34 | 112 | 85-132 | 120757 | * | | 3.1 | 22.5 | -1.1 | 24.0 | +0.7 |
| | | | | sex.cov | | | 13.5 | | | | |
| Cond. Fact. T1 | 16 | 89.9 | 48-105 | 118085 | * | 10.0 | 10.0 | 1.0 | -0.02 | NA | NA |
| Cond. Fact. T2 | 39 | 46.9 | 35-83 | 39977 | * | 10.3 | 10.3 | 1.2 | -0.03 | NA | NA |
| Cond. Fact. T3 | 20 | 150 | 116-175 | 55565 | ** | 12.2 | 12.2 | 1.1 | +0.04 | NA | NA |
| SGR T2-T3 | 3 | 35.7 | 18-85 | 115199 | * | 26.0 | 6.8 | 0.6 | +0.10 | NA | NA |
| | 9 | 139 | 89-189 | 128240 | * | | 5.3 | 0.6 | +0.04 | NA | NA |
| | 36 | 31.4 | 1-80 | 30493 | *** | | 5.3 | 0.6 | -0.09 | NA | NA |
| Egg diameter | 5 | 66.8 | 41.7-185 | 37572 | * | 39.0 | 38.95 | 4.0 | -0.046 | NA | NA |
| Sperm diameter | 24 | 47.8 | 0-59.8 | 202134 | * | 32.6 | 32.59 | NA | NA | 2.9 | -0.001 |
| Δ cortisol | 6 | 114 | 108-135 | 113752 | * | 57.6 | 9.0 | 7.4 | +2.3 | 0.8 | -0.6 |
| | | | | sex.cov | | | 43.0 | | | | |
| Δ chloride | 38 | 43.3 | 25.3-60.9 | 116693 | * | 18.5 | 18.5 | 0.3 | -2.9 | NA | NA |
| Δ osmolality | 40 | 60.3 | 26.3-82.1 | 52306 | * | 31.1 | 14.0 | 15.2 | -3.4 | -5.4 | +4.6 |
| | | | | sex.cov | | | 14.6 | | | | |
| *ghr* | 24 | 138 | 47.8-153 | 141355 | * | 23.8 | 23.8 | -3.9 | +0.2 | NA | NA |
| Hematocrit | 4 | 22.6 | 16.3-161 | 105237 | * | 42.8 | 12.0 | 35.3 | +0.9 | 39.6 | -1.7 |
| | 25 | 139 | 113-159 | 1153 | * | | 12.4 | 35.1 | +0.5 | 38.3 | -0.2 |
| | | | | sex.cov | | | 13.9 | | | | |

422 **DISCUSSION**

423 Salmonids are a model system for studying the effects of whole genome duplication (WGD) on genome
424 evolution, sex determination and speciation. Specifically, the evolution of sex determination after WGD
425 and its interaction with heterochiasmy remain active areas of research. In this study we identify the sex-
426 linked chromosome and strong heterochiasmy using the high-density genetic map of Brook Charr. Female
427 recombination rates were 2.7-fold higher than those in the male, and male recombination was highly
428 biased to chromosome ends. Using recently established correspondence among salmonid chromosomes,
429 we show that the Brook Charr sex chromosome is not the same chromosome arm linked to sex in any
430 other species characterized with high-density genetic maps. However, this chromosome arm (ancestral
431 15.1) is contained in a fusion within a triple chromosome fused sex chromosome of the congener Arctic
432 Charr. In other salmonid species, some consistencies in sex chromosomes can be viewed, even as distant
433 as Lake Whitefish and members of the genus *Oncorhynchus* (discussed below). We additionally evaluate
434 linkage to 29 reproductive, stress and growth phenotypes (21 not including phenotypes measured at
435 multiple time points) and identify 29 genome- or chromosome-wide QTL on 14 of 42 linkage groups and
436 compare these to known QTL in other salmonids. This work provides markers for selective breeding of
437 Brook Charr as well as insight into the role of heterochiasmy in sex determination and genome evolution
438 in a post-WGD salmonid genome.

439 **Sex determination post whole genome duplication**

440 In species with genetic sex determination, WGD generates multiple copies of sex chromosomes and this
441 may present challenges to the new lineage (Davidson *et al.* 2009) such as unbalanced gametes and
442 independent segregation of sex chromosomes (Muller 1925) or disruptions in dosage balance in species
443 with heteromorphic sex chromosomes (Orr 1990). However, polyploidization also poses other challenges
444 independent to the duplicated sex chromosome system (Mable 2004; Otto 2007). Nonetheless, ancestral
445 polyploidization resulting in paleopolyploid lineages has occurred throughout plant and animal evolution,
446 with some notable examples in vertebrates including the teleost-specific duplication (3R; Taylor *et al.*
447 2003), the salmonid-specific duplication (4R; Allendorf and Thorgaard 1984), and an allopolyploidization
448 within the *Xenopus* genus (Session *et al.* 2016). Debate still exists on the effect of polyploidization on
449 diversification (e.g. Clarke *et al.* 2016). Diversification may involve sex determination systems, as
450 paleopolyploids may develop a wide variety of sex determination systems, for example in the teleosts
451 (Mank and Avise 2009). A wide variety of sex chromosomes are used in different teleost groups including
452 stickleback species (Ross *et al.* 2009; Kirkpatrick 2016) and Medaka, in which *Oryzias latipes* sex
453 chromosome is LG1 with a probable translocation of the sex gene to a neo-sex chromosome (Kondo 2006)
454 whereas other members such as those of the Medaka *celebensis* group initially used LG24 as the sex

455 chromosome and then transitioned to LG10 in several other species (Myosho *et al.* 2015). Ancestral
456 allotetraploids can also develop new sex determination systems. For example, the African clawed frog
457 *Xenopus laevis* (ZZ/ZW), has a probable translocated W-specific region that is out of synteny with the
458 sister group to the polyploidization and with the homeolog of the sex chromosome (Session *et al.* 2016).
459 However, variable sex determination systems are not unique to paleopolyploid lineages. For example, *X.*
460 *tropicalis* did not undergo the allotetraploid event but has a unique sex determination system that involves
461 W, Z, and Y chromosomes, where the presence of a single Y can override the feminizing presence of two
462 W chromosomes in triploids (Roco *et al.* 2015). The extent of the involvement of polyploidization on the
463 lability of sex determination systems remains to be determined.

464 Taxa with high rates of turnover in sex chromosomes have indicated that some chromosomes are
465 more likely to become sex chromosomes. This is possibly due to favourable gene content, for example
466 when an autosome contains sexually antagonistic genes it can be repeatedly selected to become a sex
467 chromosome (Marshall Graves and Peichel 2010). A comparative analysis of teleosts indicates repeated
468 independent evolution of the same chromosomes as sex chromosomes throughout evolutionary history
469 (see Table 2 in Marshall Graves and Peichel 2010). Therefore it is not only the master sex determining
470 gene that can be repeatedly utilized by evolution, but also certain chromosomes due to the gene content,
471 which can occur over large evolutionary distance. For example, the teleost tongue sole *Cynoglossus*
472 *semilaevis* and the chicken *Gallus gallus* (both ZZ/ZW) independently evolved sex chromosomes in
473 homologous chromosomes (Chen *et al.* 2014). Similarly, species from three anuran genera that have
474 diverged for over 210 million years (*Bufo*, *Hyla* and *Rana* spp.) all have sex-linked markers that map to
475 the same *X. tropicalis* chromosome containing *doublesex and mab-3 related transcription factor 1*
476 (DMRT1) and a large region homologous to the avian sex chromosome (Brelsford *et al.* 2013), which the
477 authors suggest is due to independent evolution to the same sex chromosome across the different genera.
478 The platypus *Ornithorhynchus anatinus* has five Y and five X chromosomes, all of which are independent
479 but form a chain at meiosis to co-segregate all together into sperm; this system connects the two sex
480 determination types, with the most degenerate sex chromosome as homologous to the Z chromosome of
481 birds and the least degenerate as that homologous to the X chromosome of mammals (Grützner *et al.*
482 2004; Charlesworth and Charlesworth 2005). Finally, although at least three non-homologous sex
483 chromosomes exist within *Xenopus*, the sex determining region of *X. borealis* shares orthologous genes to
484 mammals including humans (Furman and Evans 2016). In summary, repeated, independent evolution of
485 the same sex chromosome or use of the same set of specific genes for sex determination therefore has
486 been documented across a variety of animal taxa.

487 Salmonids have genetically controlled sex determination with XX/XY systems (Thorgaard 1977;
488 Davidson *et al.* 2009), but putative translocation of the sex determining gene to different autosomes has

489 resulted in many different sex chromosomes in the different lineages (Woram *et al.* 2003) and even within
490 the same species (Küttner *et al.* 2011; Eisbrenner *et al.* 2013). However, comparing across the phylogeny
491 indicates some noteworthy consistencies. First, several species use 3.1 as the sex chromosome, or have
492 this chromosome fused with the sex chromosome, including the neo-Y of Sockeye Salmon and the sex
493 chromosome of Coho Salmon (Faber-Hammond *et al.* 2012), as well as the sex-linked LG in Lake
494 Whitefish (Gagnaire *et al.* 2013), identified as 3.1 during map comparisons (Sutherland *et al.* 2016).
495 Relative to the variability seen in sex chromosomes in the salmonids, this is a striking consistency
496 considering that these species have diverged for approximately 50 million years (Crête-Lafrenière *et al.*
497 2012). This consistency may indicate that either a) 3.1 is an ancestral sex chromosome in the salmonids;
498 or b) the different species converged on this chromosome independently as it contains a gene complement
499 that is highly beneficial to be present as a sex chromosome (Marshall Graves and Peichel 2010; Chen *et al.*
500 2014; Furman and Evans 2016). Second, the Brook Charr sex chromosome (15.1) is fused within the sex
501 chromosome of Arctic Charr, but is not the same arm as that containing the sex marker in Arctic Charr
502 (Nugent *et al.* 2016), indicating one of these is a neo-sex chromosome. Furthermore, the middle
503 chromosome arm fused in the Arctic Charr triple chromosome fusion is 19.1, which is the neo-Y of
504 Sockeye Salmon (Table 1). These observations provide further evidence for the fusion of specific
505 chromosomes together that are beneficial for maintaining within the sex chromosome environment.
506 Finally, intraspecific polymorphism in sex chromosomes occurs in Arctic Charr (Moghadam *et al.* 2007;
507 Küttner *et al.* 2011), and Icelandic Arctic Charr were identified as having a sex chromosome as one of the
508 two homeologs AC01 or AC21 instead of AC04, and state that this is homologous to the sex chromosome
509 of Atlantic Salmon Ssa02 (Küttner *et al.* 2011), which is 9.1 (Sutherland et al 2016), again indicating the
510 potential re-use of the same chromosome as the sex chromosome.

511 The presence of both the Y chromosome of Brook Charr and the neo-Y of Sockeye Salmon as
512 putative neo-Y chromosomes of Arctic Charr is worth further investigation because neo-Y chromosomes
513 can influence phenotypic divergence and reproductive isolation, as observed in sympatric Threespine
514 Stickleback *Gasterosteus aculeatus* populations for male courtship displays and hybrid instability,
515 respectively (Kitano *et al.* 2009; Kitano and Peichel 2011). These consistencies across a phylogeny can
516 provide insight into speciation. For example, the Threespine Stickleback and Ninespine Stickleback
517 *Pungitius pungitius* have two different sex chromosomes (LG19 and LG12, respectively), and the
518 Blackspotted Stickleback *G. wheatlandi* has a fused Y-chromosome made up of these two linkage groups
519 (Ross *et al.* 2009), to which the authors suggest multiple independent recruitment of LG12 as the sex or
520 neo-Y chromosome. Other sticklebacks have different sex chromosomes, such as the Brook Stickleback
521 *Culaea inconstans*, and the Fourspine Stickleback *Apeltes quadracus*, which has a ZW sex determination
522 system (Ross *et al.* 2009). The salmonid variety and consistencies identified here provide another group

523 for analyzing sex chromosome differences in relation to gene content and speciation, and in salmonids
524 also occurs with the salmonid-specific WGD. As more salmonid genomes are characterized, it will
525 become clearer whether certain sex chromosomes are ancestral or have independently evolved, and
526 whether there is a favourable gene content within often-viewed sex chromosomes.

527 In the context of sex chromosome fusions and residual tetraploidy, several additional observations
528 on the nature of salmonid sex chromosomes can be made from the present analysis (four genera; eight
529 species; Table 1). First, sex chromosomes with fusions only occur in species specific fusions in the data
530 here; the three fused sex chromosomes (in Atlantic Salmon, Arctic Charr, and Sockeye Salmon) are
531 products of species-specific fusions and not in conserved fusions (Sutherland *et al.* 2016). Arctic Charr *S.*
532 *alpinus* has a sex chromosome that in some individuals involved three fused chromosomes (Nugent *et al.*
533 2016), and all available evidence suggests this is a species-specific fusion given that these fusions are not
534 present in the more basally diverging Atlantic Salmon nor the congener Brook Charr (Sutherland *et al.*
535 2016). Y fusions are the most common of sex chromosome fusions (Pennell *et al.* 2015) and can permit
536 other sexually antagonistic genes to be linked to the non-recombining regions (Charlesworth and
537 Charlesworth 1980; Charlesworth *et al.* 2005). Y fusions may also occur due to drift with only slightly
538 deleterious effects (Kirkpatrick 2016), as males have increased fusion prevalence in general and increased
539 repeat content (and thus fusion potential) in degenerating Y (Pennell *et al.* 2015). However, since the same
540 chromosomes that are involved in Y fusions in some species are the sex chromosomes in others (e.g. 15.1
541 or 19.1, discussed above), it suggests that these fusions could have an adaptive advantage, such as the
542 movement of an autosome with alleles under sexually antagonistic selection into the Y chromosome
543 environment, as discussed by Charlesworth and Charlesworth (1980) and Kirkpatrick (2016). When
544 recombination is low in males (i.e. heterochiasmy), this Y fusion holds an additional chromosome in a
545 constantly lower recombination environment as it will always be present in males. The use of the same
546 chromosomes as sex chromosomes and as fusion partners within the salmonids merits further study.
547 Secondly, chromosomes with regions of residual tetraploidy can become sex chromosomes; two of the
548 seven identified sex chromosomes (Chinook Ots17 (23.2) and Atlantic Salmon Ssa02q (9.1)) are
549 chromosomes known to exhibit residual tetraploidy (Brieuc *et al.* 2014; Allendorf *et al.* 2015; Sutherland
550 *et al.* 2016; Lien *et al.* 2016), therefore suggesting that exhibiting residual tetraploidy does not prevent a
551 chromosome from becoming a sex chromosome.

552 Translocation of a sex determining gene to an autosome and the adoption of the autosome as a
553 new sex chromosome may be possible if the gene moves into linkage with a locus that is under sexually
554 antagonistic selection (van Doorn and Kirkpatrick 2007). The probability of this adoption is increased
555 with the occurrence of an inversion in the region which will increase linkage by reducing recombination
556 (van Doorn and Kirkpatrick 2007), but it requires that the benefit of the new chromosome is greater than

557 that existing on the original sex chromosome. Interestingly, in the unique sex chromosome of Brook Charr
558 (15.1), there is a large inversion in relation to the other salmonids (Sutherland *et al.* 2016). However, this
559 is an interspecific inversion and has not yet been determined whether it is also heterozygous within the
560 species due to low recombination and resultant challenges of generating male maps. To further
561 characterize this, genome sequence for both the X and Y chromosomes of Brook Charr will be valuable.

562 The salmonids, being at an early stage of sex chromosome evolution (Phillips and Ihssen 1985)
563 provide a good system to study sex chromosome evolution (van Doorn and Kirkpatrick 2007). As we
564 observed here, reduced recombination occurs consistently in male salmonids, being restricted to the
565 telomeric region opposite the centromere, resulting in a lack of recombination between X and Y in the
566 middle of the chromosome. This may facilitate sex chromosome formation, with tight linkage developing
567 across the entire Y chromosome (Haldane 1922; Nei 1969; Lenormand 2003). Heterochiasmy is not only
568 restricted to the sex chromosome, but rather occurs throughout the genome, as has been viewed in several
569 systems with developing sex chromosomes, such as the European tree frog *Hyla arborea* (Berset-Brandli
570 *et al.* 2008), Medaka (see Kondo *et al.* 2001; Kondo 2006), zebrafish *Danio rerio* (Singer *et al.* 2002), and
571 the salmonids of genera *Oncorhynchus* (Sakamoto *et al.* 2000), *Salmo* (Moen *et al.* 2004) and *Salvelinus*
572 (present study). However, heterochiasmy also occurs in systems with fully developed sex chromosomes,
573 such as humans, where females have ~1.6-fold higher rates than males, which recombine predominantly at
574 telomeric regions (Broman *et al.* 1998).

575 Y degeneration can occur from a lack of recombination in sex chromosomes (Charlesworth 1991;
576 Charlesworth *et al.* 2005), and this can also result in degeneration of fused neo-Y, when present. Neo-Y
577 degeneration has occurred rapidly in achiasmate male species such as *Drosophila miranda*, having
578 degenerated after only 1-2 My in the non-recombining state (Steinemann and Steinemann 1998;
579 Charlesworth and Charlesworth 2005). In species with heterochiasmy, even before large degeneration,
580 accumulated substitutions can occur throughout a neo-Y and increased sex-biased gene expression occurs
581 for genes within the neo-Y than the other autosomes, as observed in stickleback (Yoshida *et al.* 2014).
582 These changes are not only degenerative, migration to the Y, and preservation of male-beneficial genes on
583 the Y also occurs, as well as dosage compensation and migration of female-beneficial genes to the X
584 (Bachtrog 2006). Many changes can occur between X and Y when crossovers do not occur throughout the
585 chromosomes.

586 In the salmonids, sex chromosome turnover by *sdY* translocation may restart the process of Y
587 degeneration (Yano *et al.* 2012b). In species with heterochiasmy rather than achiasmy, occasional
588 crossover between X and Y would also reduce sex chromosome heteromorphism and Y degeneration. This
589 may be the reason for sex chromosomes remaining homomorphic in green toad species (*Bufo viridis*) all
590 which have the same sex chromosomes (Stöck *et al.* 2013), and in several members of the *Hyla* genus of

591 European tree frogs, which also all share the same sex chromosomes (Stöck *et al.* 2011). Regeneration of
592 Y chromosomes by occasional crossover is termed the 'fountain-of-youth' hypothesis, and is particularly
593 likely for species with the possibility of sex reversal, as recombination rate is based on phenotypic sex
594 rather than genetic sex (discussed in Perrin 2009). Some salmonid sex chromosomes are heteromorphic
595 (Davidson *et al.* 2009) and accumulate repeats (Devlin *et al.* 1998), this may suggest in some species this
596 regeneration is not occurring. Lack of recombination will be accentuated by inversion accumulation and
597 other differentiation between sex chromosomes reducing meiotic pairing and crossovers. Sex reversal is
598 possible in salmonids (Johnstone *et al.* 1978) and has been observed in the wild, for example in Chinook
599 Salmon (Nagler *et al.* 2001), but the greater extent of this occurring in nature in other salmonids is yet to
600 be determined. Relative effects of sex chromosome turnovers, occasional X/Y crossovers, and large sex
601 chromosomal polymorphisms merits further investigation for which the salmonids are a good model
602 system. The extent of Y or neo-Y degeneration, gene migration, or other aspects of sex chromosome
603 evolution have not yet been explored comparatively in the salmonids. As these aspects may differ among
604 species depending on the length of time the chromosome has been used as the Y chromosome, further
605 investigation into interspecific differences (e.g. 3.1 sex chromosome in both Lake Whitefish and members
606 of *Oncorhynchus*), or intraspecific differences between populations having different sex chromosomes
607 (Eisbrenner *et al.* 2013), will be valuable to determine the history of the sex chromosome evolution in the
608 salmonids.

609 **QTL mapping, hotspots and consistencies with other species**

610 Knowledge on the genetic architecture of important traits in the salmonids is improving, for example for
611 aquaculture-related traits such as disease resistance (Yáñez *et al.* 2014) and stress tolerance (Rexroad *et al.*
612 2012), and ecologically-relevant traits such as age-at-sea (Barson *et al.* 2015) and body shape evolution
613 (Laporte *et al.* 2015). In the present study we improve the understanding of genetic architecture of growth,
614 reproductive and stress-response traits by identifying QTL on 14 of the 42 LGs in the Brook Charr linkage
615 map (four fused metacentric and 10 acrocentric chromosomes). This improves the previous analysis of
616 these traits on a low-density map (Sauvage *et al*. 2012a, 2012b) and brings the QTL for these phenotypes
617 into the context of the more characterized high-density map generated here (e.g. with information on
618 correspondence of arms with other salmonids, probable residual tetraploidy and centromere positions,
619 ancestral chromosomes, and identified sex chromosome).

620 Although correlated phenotypes clustered on the map as expected (e.g. length, weight), no
621 clustering was observed for blood and stress-related parameters (i.e. hematocrit, change in cortisol,
622 chloride and osmolality), with each trait having a QTL on a different chromosome. As pleiotropy can
623 occur with both positive and negative genetic correlations between traits with common underlying biology
624 (Mackay *et al.* 2009). This is important to consider in marker-assisted selection, to identify QTL useful for

625 simultaneous selective breeding of multiple traits and to avoid negative correlations between desirable
626 traits (Lv *et al.* 2016). Mapping multiple correlated traits simultaneously can help define regions (Jiang
627 and Zeng 1995). However, it can be difficult to determine whether two traits are truly pleiotropic or
628 whether causal variants for each trait are in in tight linkage, especially when a QTL region is wide
629 (Mackay *et al.* 2009).

630 Consistencies in QTL across multiple species can be useful for identifying regions of the genome
631 with highly conserved roles. Several QTL hotspots have been identified within *Oncorhynchus*, specifically
632 for thermotolerance, length and weight on So6b (Hecht *et al.* 2012), So7a (except weight; also viewed in
633 Rainbow Trout and Chinook Salmon), and So11b (see Larson *et al.* 2015). The corresponding Brook
634 Charr LGs to So6 and So11b (Sutherland *et al.* 2016) did not contain any QTL in the present study, but
635 the corresponding LG to So7a (BC34) contains a length QTL (Table 2). This further implicates this
636 chromosome (ancestral 10.2) as having an evolutionary conserved influence on salmonid growth.

637 Weight and growth are expected to be highly polygenic traits, therefore requiring many
638 individuals to have sufficient power to identify loci of minor effect (Rockman 2012; Ashton *et al.* 2016).
639 For example, sample sizes of at least 500 individuals may be required to identify QTL accounting for less
640 than 5% of the total phenotypic variance (Mackay 2001). This means that often only large effect QTL are
641 identified, leading to the misconception that these are the norm and to an inflation of the actual percent
642 variance explained by the QTL (Beavis 1997; Xu 2003). High powered studies can identify more QTL,
643 such as a recent study in Atlantic Salmon with 1695 offspring and 20 sires, which identified four
644 chromosomes harboring major effect growth QTL (Tsai *et al.* 2015). Similarly, a study in Common Carp
645 *Cyprinus carpio* with 522 offspring and eight families identified 10 genome-wide and 28 chromosome-
646 wide significant QTL for three growth traits, with 30/50 chromosomes containing suggestive QTL (Lv *et*
647 *al.* 2016). Nonetheless, QTL can be detected with fewer individuals. For example, QTL for polygenic
648 traits growth rate, behavior and morphology were identified in Lake Whitefish with 102 individuals in the
649 mapping family (Gagnaire *et al.* 2013; Laporte *et al.* 2015). Furthermore in the present study we identified
650 QTL for many of the traits with 169 or fewer individuals. Since the effect of a QTL can differ in different
651 genetic backgrounds due to epistasis (Mackay 2001), it is therefore important to evaluate the effect of
652 markers in different crosses with different genetic backgrounds to better understand the broader use of the
653 marker (Lv *et al.* 2016).

654 The precision of mapping QTL within a family depends on recombination rate (Mackay 2001;
655 Mackay *et al.* 2009). Therefore the low number of crossovers in male salmonids will reduce the overall
656 precision of trait mapping. This effect of heterochiasmy has been used by recent salmonid studies to use a
657 two-stage approach by initially using a sire-based analysis with few markers per chromosome to identify
658 chromosomes of interest followed by a dam-based analysis to more finely resolve the QTL positions (Tsai

659 *et al.* 2015). Heterochiasmy is therefore important to consider when designing QTL experiments for
660 species exhibiting this trait. In the present study, several QTL with very broad regions of elevated LOD
661 were identified (e.g. for length on BC03, BC04, and BC05), which may be due to low recombination and
662 paternally associated haplotypes (see Results). In contrast, many of the other identified QTL in this study
663 have small confidence intervals and high percent variance explained, and therefore will be useful for
664 selective breeding (Table 2; Figure 3).

665 Although QTL mapping connects nucleotide sequence with trait variation, it generally ignores
666 intermediate phenotypes that can be very useful in determining underlying drivers of traits, and the use of
667 the expression levels of gene transcripts as traits to identify eQTL can provide information on the
668 intermediate steps to generate a phenotype (Mackay *et al.* 2009). Traits queried in eQTL experiments have
669 the additional information on gene location in the genome, providing information on cis or trans-eQTL
670 (Mackay *et al.* 2009). This will be an important next step in determining the underlying causes of the
671 genotype-phenotype interaction in Brook Charr.
672

673 **CONCLUSIONS**

674 The relationships between sex chromosomes, heterochiasmy and polyploidization have important
675 influences on genome architecture for key biological traits, but much remains unknown about these
676 interactions. Here we identify the sex-linked chromosome in Brook Charr and compare sex chromosome
677 identities across the salmonids to investigate consistencies. Although many different chromosomes are
678 used as sex chromosomes in salmonids, some consistencies can be identified, even in lineages that have
679 diverged for ~50 million years, *Coregonus* and *Oncorhynchus*. Sex chromosomes that are contained
680 within fused chromosomes thus far are only observed in species-specific fusions and not in conserved
681 fusions. Heterochiasmy, or differences in recombination rate between sexes, may play an important role in
682 the evolution of sex chromosomes. Heterochiasmy is viewed here in the *Salvelinus* genus, and in other
683 salmonid genera *Oncorhynchus* and *Salmo*, where male recombination is much lower than female, and
684 crossovers are restricted to telomeric regions. Inversions are also important for sex chromosome evolution,
685 and the Brook Charr sex chromosome from the female map exhibits a large interspecific inversion,
686 although the intraspecific polymorphism of this inversion has not yet been determined. Additional analysis
687 of salmonid genomes is needed to understand the effect of the mobile sex determining gene on phenomena
688 such as Y degeneration. To improve the characterization of important traits and potential for selective
689 breeding, we additionally identify 29 QTL across the genome for growth, reproduction, and stress-
690 response traits, several of which having high PVE and well-refined intervals. Hotspots for multiple traits
691 were not common, but we identify that an earlier identified hotspot in *Oncorhynchus* also contains a

length QTL in Brook Charr, further indicating the importance of this chromosome region and the value of identifying orthologous QTL with comparative genomics.

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## SUPPLEMENTAL MATERIAL

**Table S1.** Phenotype average, standard deviation and sample size in males and females. Phenotypes showing any differences between males and females ($p \leq 0.2$) included sex as a covariate in the model. Sex-specific phenotypes were only tested within the one sex and therefore had smaller sample sizes.

**Table S2.** Complete QTL table with all identified genome- and chromosome-wide QTLs and associated values, including marker sequence and SNP, and effect size of different genotypes.

**Figure S1.** Correlation plot of phenotypes used in QTL analysis. Phenotype pairs that do not share any individuals for correlation are shown with '?'.

**Figure S2.** Heterochiasmy plots for individual chromosomes in the maternal (a) and paternal (b) haplotypes. Grey boxes above the plot indicate probable residually tetraploid chromosome arms, and centromere positions transferred from Chinook Salmon are indicated by stars. When Chinook Salmon chromosomes were not fusions but were metacentrics (n = 2), this is denoted by a '?' to denote the uncertainty as to the centromere position in Brook Charr.

**File S1.** Required files for running Rqtl analysis (phenotype (.qua), map (.map) and genotype (.loc)). The map file corresponds to the female map from Sutherland *et al.* 2016. See the Data Availability for code for performing complete analysis with these files.

**File S2.** Fasta file with RAD-seq tags output using the STACKs population module for alleles from all individual offspring and parents.