

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

3 Ben J. G. Sutherland\*, Ciro Rico<sup>†‡</sup>, Céline Audet<sup>§</sup> and Louis Bernatchez\*

4 \* Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, QC, Canada G1V  
5 0A6

6 † School of Marine Studies, Molecular Diagnostics Laboratory, The University of the South Pacific,  
7 Laucala Campus, Suva, Fiji

8 ‡ Department of Wetland Ecology, Estación Biológica de Doñana (EBD-CSIC), c/Américo Vespucio s/n,  
9 41092 Sevilla, Spain

10 § Institut des Sciences de la Mer de Rimouski, Université du Québec à Rimouski, Rimouski, QC, Canada  
11 G5L 3A1

12

13

14 **Data Deposition:** Raw sequence data for this study is available on SRA under BioProject PRJNA308100  
15 and accession SRP068206.

16

17 **Running title:** Sex, heterochiasmy and QTL in Charr

18 **Keywords:** heterochiasmy; salmon; sex chromosomes; QTL; whole genome duplication

19

20

21

22 **Author for Correspondence:**

23 Ben Sutherland

24 Institut de Biologie Intégrative et des Systèmes (IBIS)

25 Université Laval, Québec, QC, Canada

26 G1V 0A6

27 Email: ben.sutherland.1@ulaval.ca (BJGS)

28

## ABSTRACT

29 Whole genome duplication can have large impacts on genome evolution. However, much remains  
30 unknown about these impacts, such as the mechanisms of coping with a duplicated sex determination  
31 system, which may result in increased sex determination mechanism diversity. Sexual conflict (i.e. alleles  
32 having different optimums in each sex) can result in sequestration of genes into non-recombining sex  
33 chromosomes. Development of sex chromosomes may involve heterochiasmy (i.e. sex-specific  
34 recombination rate), which is also poorly understood. Family Salmonidae is a model system for these  
35 phenomena, having undergone autotetraploidization and subsequent rediploidization in most of the  
36 genome at the base of the lineage. The salmonid master sex determining gene is known, and many species  
37 have non-homologous sex chromosomes, putatively due to transposition of this gene. In this study, we  
38 identify the sex chromosome of Brook Charr *Salvelinus fontinalis* and compare sex chromosome  
39 identities across the lineage (eight species, four genera). Although non-homology is frequent, homologous  
40 sex chromosomes and other consistencies are present in distantly related species, indicating probable  
41 convergence on specific sex and neo-sex chromosomes. We also characterize strong heterochiasmy with  
42 2.7-fold more crossovers in maternal than paternal haplotypes with paternal crossovers biased to  
43 chromosome ends. Y chromosome crossovers are restricted to a single end of the chromosome, and this  
44 chromosome contains a large interspecific inversion, although its status between males and females  
45 remains unknown. Finally, we identify QTL for 21 unique growth, reproductive and stress-related  
46 phenotypes to improve knowledge of the genetic architecture of these traits important to aquaculture and  
47 evolution.

48

## INTRODUCTION

49 Characterizing the genetic architecture of ecologically-relevant phenotypes is essential for organism and  
50 genome evolution research (Rogers and Bernatchez 2007; Gagnaire *et al.* 2013) and selective breeding  
51 (Yáñez *et al.* 2014). Regions of the genome associated with specific traits can be identified by  
52 quantitative trait loci (QTL) analysis (Mackay 2001) or genome-wide association studies (GWAS; Bush  
53 and Moore 2012). Mapped traits can include morphological, behavioral, physiological or molecular  
54 phenotypes, but must show sufficient heritable variation. Power to detect QTL is determined by the  
55 number of individuals in the study (Henning *et al.* 2014), the effect size of the QTL, allele frequencies  
56 (Mackay *et al.* 2009) and the degree of polygenic control of the trait (Rockman 2012; Ashton *et al.* 2016).  
57 QTL mapping precision depends on recombination frequency (Mackay *et al.* 2009) as well as map density,  
58 although QTL are often only in linkage with causative mutations, which are rarely identified (Slate 2005).  
59 Trait genetic architecture can differ between families or populations (e.g. Santure *et al.* 2015) but  
60 parallelism and shared QTL can be identified (e.g. Laporte *et al.* 2015; Larson *et al.* 2015). It is therefore  
61 valuable to analyze multiple crosses to understand the broader implications of a QTL (e.g. Hecht *et al.*  
62 2012; Palti *et al.* 2015; Lv *et al.* 2016), to more accurately determine the amount of variation explained by  
63 the QTL (Slate 2005), and to identify QTL that are not dependent on specific genetic backgrounds, which  
64 is particularly valuable for marker-assisted selection (Lv *et al.* 2016).

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

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

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

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

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

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

115 chromosomes and others have rediploidized (Allendorf and Thorgaard 1984; Allendorf *et al.* 2015; May  
116 and Delany 2015; Lien *et al.* 2016). Salmonid sex determination is genetically controlled (Davidson *et al.*  
117 2009) by a truncated gene from the *interferon-response factor* transcription factor family, *sdY* (sexually  
118 dimorphic on the Y-chromosome; Yano *et al.* 2012a). *sdY* may be a salmonid innovation as it has not yet  
119 been identified in the non-duplicated sister group for the salmonid WGD, Northern Pike *Esox lucius*  
120 (Yano *et al.* 2012b). Male genome-specific conservation of *sdY* occurs in more than ten salmonid species,  
121 but some exceptions exist, including the Lake Whitefish *Coregonus clupeaformis* and European  
122 Whitefish *C. lavaretus* (Yano *et al.* 2012b), and some Atlantic Salmon *Salmo salar* and Sockeye Salmon  
123 *Oncorhynchus nerka* individuals (Eisbrenner *et al.* 2013; Larson *et al.* 2016). Sex chromosomes are not  
124 homologous among many salmonid species, potentially due to transposition of *sdY* between chromosomes  
125 (Woram *et al.* 2003). Additional evidence for transposition includes repetitive flanking regions with  
126 putative transposable elements (Brunelli *et al.* 2008; Lubieniecki *et al.* 2015) and sequence conservation  
127 that abruptly stops outside of the sex determination cassette (Faber-Hammond *et al.* 2015). This  
128 transposition to different chromosomes may be delaying Y degeneration (Yano *et al.* 2012b; Lubieniecki  
129 *et al.* 2015). In general, the salmonids are at an early stage of sex chromosome evolution (Phillips and  
130 Ihssen 1985; Yano *et al.* 2012b) where sex chromosomes are homomorphic (Devlin *et al.* 1998; Phillips  
131 and Ráb 2001; Davidson *et al.* 2009). Male salmonids have low recombination rates relative to females  
132 with crossover events primarily occurring at telomeric regions, as observed in Rainbow Trout *O. mykiss*  
133 (Sakamoto *et al.* 2000) and Atlantic Salmon (Moen *et al.* 2004). Heterochiasmy is not viewed in the sister  
134 species of the salmonid WGD Northern Pike (Rondeau *et al.* 2014) and therefore salmonids are a valuable  
135 model to study the evolution and effects of heterochiasmy in relation to sex determination post-WGD.

136         The combination of characterizing heterochiasmy, sex chromosome identity and the genetic  
137 architecture for reproductive, growth and stress response traits provides much-needed information  
138 regarding the function of the Brook Charr *Salvelinus fontinalis* genome post-duplication. The goals of this  
139 study were to use a high-density genetic map for Brook Charr (Sutherland *et al.* 2016) to (a) identify the  
140 sex-linked chromosome; (b) quantify heterochiasmy in this mapping family while correcting for probable  
141 genotyping errors; and (c) search for growth, stress resistance and reproduction-related QTL. Furthermore,  
142 using the recent characterization of homology to ancestral chromosomes and homeolog identification  
143 among the salmonids (Sutherland *et al.* 2016), we subsequently compare identities of sex chromosomes  
144 and identified QTL across the salmonids to identify consistencies, and discuss the implications of  
145 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.

## 209 QTL analysis

210 The effect of sex on each phenotype was tested using linear models in R (R Development Core Team  
211 2017). If a marginal effect of sex was found ( $p \leq 0.20$ ), sex was included in the model as a covariate for  
212 the phenotype to reduce residual variation and improve power to identify the QTL (Broman and Sen  
213 2009). The R/qtl function *scanone* with permutation testing (10,000 permutations;  $p \leq 0.05$ ) was used to  
214 identify the presence of a single QTL within each linkage group (Broman *et al.* 2003). Chromosome-wide  
215 significance was tested in the same way but per chromosome (10,000 permutations;  $p \leq 0.01$ ). Confidence  
216 interval estimates (95%) for QTL positions were identified using *summary.scanone* calculating LOD  
217 support intervals with a 1.5 LOD drop. Sex-specific phenotypes (i.e. sperm diameter and concentration,  
218 egg diameter) were tested in only one sex, and therefore had smaller sample sizes. Percent variance  
219 explained by the identified QTL was performed using *makeqtl* and *fitqtl* within R/qtl, including all  
220 genome and chromosome-wide QTL per trait in the formula ( $\text{trait} \sim \text{QTL}_1 + \text{QTL}_2 + \text{QTL}_n$ ), as well as sex  
221 as a covariate when required. Phenotypic effects were estimated by calculating the differences between  
222 the mean phenotype values among the genotype groups for the marker closest to the identified QTL,  
223 including only individuals that were successfully genotyped. For markers that only segregate in one  
224 parent (i.e. *nn x np*) only two phenotype by genotype averages are given, one for the homozygote and one  
225 for the heterozygote offspring. Alternatively, for markers segregating in both parents (i.e. *hk x hk* or *ef x*  
226 *eg*), three phenotype averages are given, two for the alternate homozygotes and one for the heterozygote  
227 in *hk x hk* marker types and two for the alternate heterozygotes and one for the homozygote in *ef x eg*  
228 marker types. Sex-specific averages were calculated when the QTL required sex as a covariate in the  
229 model. RAD tags for all alleles and associated QTL results are available in File S2.

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

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



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

246

#### 247 **Data availability**

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

251 [https://github.com/bensutherland/sfon\\_pqtl/](https://github.com/bensutherland/sfon_pqtl/)

252

## 253 **RESULTS**

### 254 **Sex-linked chromosome in Brook Charr**

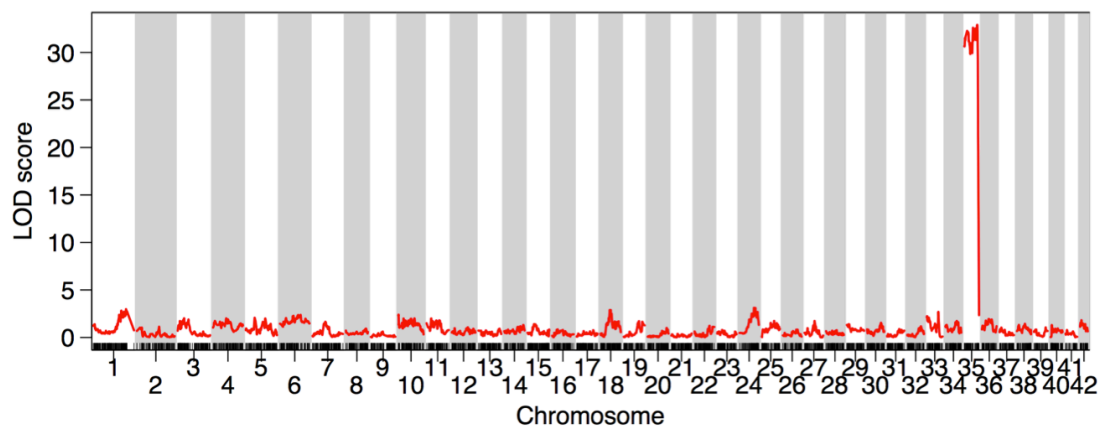
255 Sex was highly associated with the majority of Brook Charr (BC) linkage group (LG) BC35, indicating  
256 that this is the sex-linked chromosome in Brook Charr (Figure 1). Linkage across the entire LG until the  
257 LOD score decreases at the distal end can be explained by male salmonid-specific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

262 BC35 is an acrocentric chromosome homologous to the Northern Pike chromosome 15.1  
263 (homeolog naming from Sutherland *et al.* 2016). There are no other sex chromosomes in the other  
264 salmonid species with high-density genetic maps available with the chromosome arm containing the sex  
265 determining region homologous to BC35 (Table 1). Arctic Charr has a sex chromosome that is comprised  
266 of a triple fused chromosome (although this may vary across populations) that contains 15.1 in the fusion,  
267 but in Arctic Charr this is not the chromosome arm that holds the sex determining region, which is held  
268 within the arm on the other side of the chromosome AC04p (Nugent *et al.* 2016). Other salmonids have  
269 different sex chromosomes, as shown in Table 1, including Lake Whitefish (3.1; Gagnaire *et al.* 2013),  
270 Atlantic Salmon (9.1-20.2; Artieri *et al.* 2006; Lien *et al.* 2011), Rainbow Trout (14.2; Palti *et al.* 2015),  
271 Coho Salmon *O. kisutch* (3.1; Phillips *et al.* 2005; Kodama *et al.* 2014), Chinook Salmon *O. tshawytscha*  
272 (23.2; Phillips *et al.* 2005; Naish *et al.* 2013; Briec *et al.* 2014) or Sockeye Salmon (3.1-19.1; Larson *et al.*  
273 *et al.* 2016). This further refines previous observations of the general lack of homology in the sex  
274 chromosomes of the salmonids (Woram *et al.* 2003). Some information on sex chromosomes identities  
275 across *Salmo*, *Salvelinus* and *Oncorhynchus* have been previously reported (Phillips 2013) and most of

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

279 Considering the importance of inversions to sex chromosome formation through the reduction of  
280 recombination between X and Y (Lahn *et al.* 2001; van Doorn and Kirkpatrick 2007; Berset-Brandli *et al.*  
281 2008), it is interesting to note that Brook Charr has a species-specific inversion in BC35 in the female  
282 map (15.1; see Figure 5 in Sutherland *et al.* 2016). As is usual for salmonid linkage maps, the male-  
283 specific map was not produced as the low recombination frequency resulted in poorly placed male-  
284 specific markers (Sutherland *et al.* 2016), and so it is not possible to check whether this inversion is  
285 heterozygous within the species, but this will be valuable to investigate in future studies.

286



287

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

290

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

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

300

301

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	<i>Coregonus clupeaformis</i>	LW25	<u>3.1</u>	A	Linkage	(Gagnaire <i>et al.</i> 2013)
Atlantic Salmon	<i>Salmo salar</i>	Ssa02	<u>9.1-20.2</u>	F	FISH & Linkage	(Artieri <i>et al.</i> 2006; Phillips <i>et al.</i> 2009; Lien <i>et al.</i> 2011)
Arctic Charr	<i>Salvelinus alpinus</i>	AC04	<u>1.2-19.1-15.1</u>	F	Linkage	(Nugent <i>et al.</i> 2016)
Brook Charr	<i>Salvelinus fontinalis</i>	BC35	<u>15.1</u>	A	Linkage	(Sutherland <i>et al.</i> 2016) and <i>current paper</i>
Rainbow Trout	<i>Oncorhynchus mykiss</i>	OmySex (29)	<u>14.2</u>	A	FISH & Linkage	(Phillips <i>et al.</i> 2006; Rexroad <i>et al.</i> 2008; Palti <i>et al.</i> 2015)
Coho Salmon	<i>O. kisutch</i>	Co30	<u>3.1</u>	A	FISH & Linkage	(Phillips <i>et al.</i> 2005; Kodama <i>et al.</i> 2014)
Chinook Salmon	<i>O. tshawytscha</i>	Ots17	<u>23.2</u>	A	FISH & Linkage	(Phillips <i>et al.</i> 2005; Naish <i>et al.</i> 2013; Brieuc <i>et al.</i> 2014)
Sockeye Salmon	<i>O. nerka</i>	So09	<u>3.1-19.1*</u>	A	Linkage	(Larson <i>et al.</i> 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

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

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

335

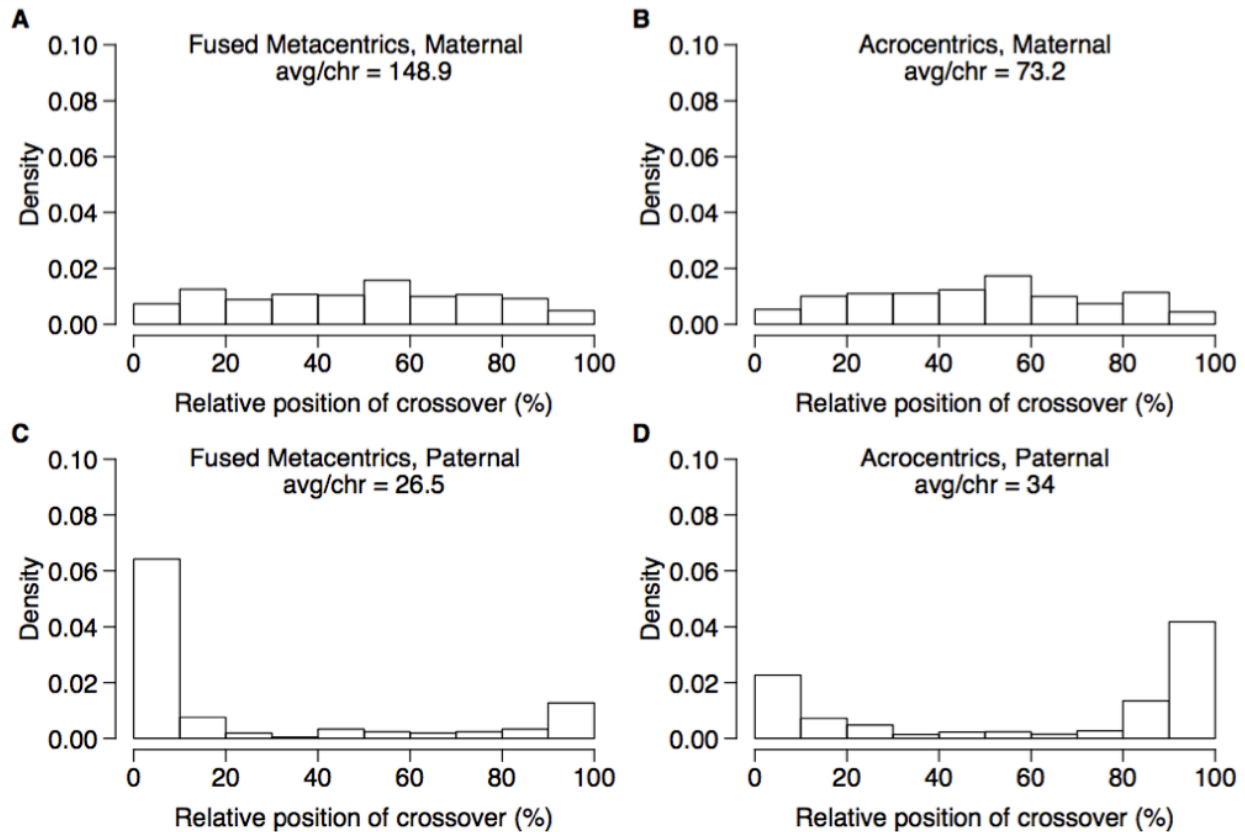
### 336 **QTL identification: growth, reproduction and stress response**

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

342 Several traits showed sexual dimorphism and therefore required sex as a model covariate. These  
343 included weight, length, liver weight, hepatosomatic index, hematocrit, change in osmolality and cortisol  
344 from stressor, resting plasma chloride, hepatic glycogen, *insulin-like growth factor 1* and *igf receptor 1*  
345 (Table S1). Specific growth rate, condition factor, change in chloride, resting plasma osmolality and  
346 glucose, and *growth hormone receptor* gene expression did not show sexual dimorphism. Traits with high  
347 phenotypic correlation included length and weight ( $r = 0.90$  at T1), and liver weight and hepatosomatic  
348 index ( $r = 0.85$ ; Figure S1). Specific growth rate T1-T2 was negatively correlated with weight at T1 ( $r = -$   
349  $0.64$ ), suggesting that larger individuals measured at T1 subsequently grew slower than smaller  
350 individuals. Other traits generally were not as highly correlated ( $r < 0.35$ ). Even though the phenotypes  
351 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.

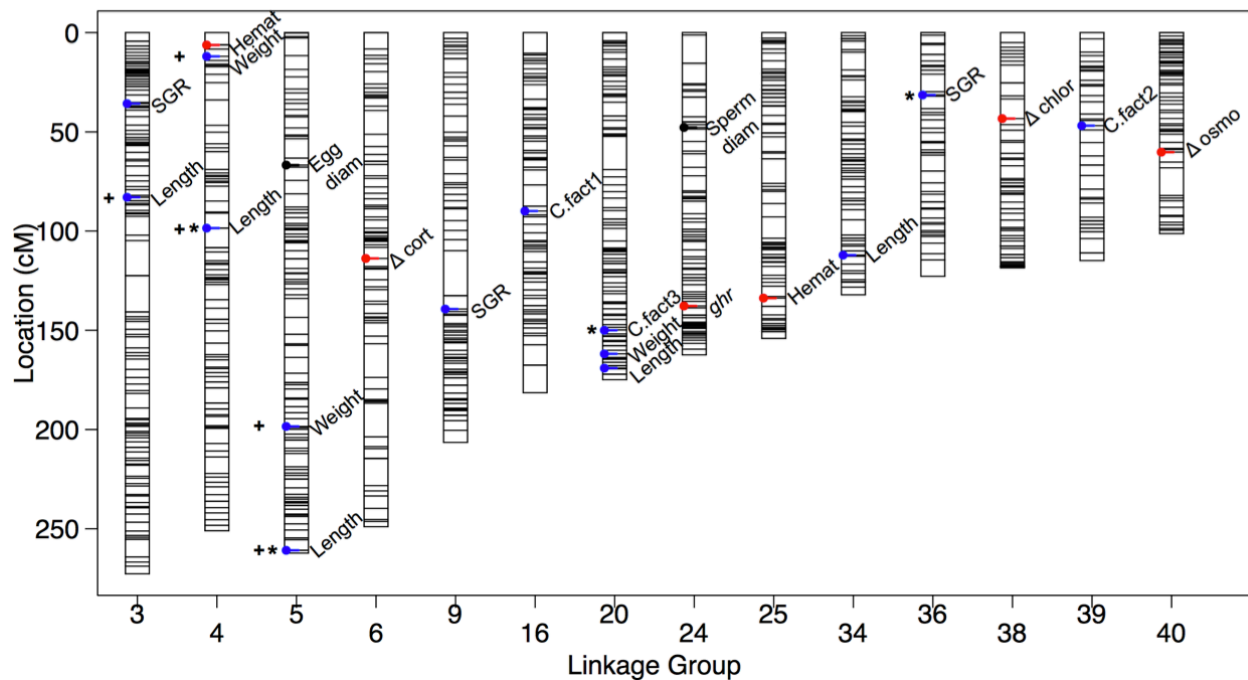
363

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  $nn \times np$ ; informative in both parents  $ef \times eg$ ; and semi-informative  $hk \times hk$ ). Interestingly, when including  
368 markers only polymorphic in the female (i.e.  $nn \times np$ ) 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

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

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

381



382

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

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

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

414 **Table 2.** Identified QTL in Brook Charr with positions, percent variance explained (PVE) and the effect  
 415 of the allelic state on the trait. Sex was included as a covariate when required, and in these cases the  
 416 allelic effect is given for both males and females, and the PVE from sex is also given. When sex was not  
 417 required as a covariate, the second averages are displayed as NA and the first averages represent both  
 418 sexes. The phenotype average for the homozygote common allele (aa avg) is shown for comparison to the  
 419 largest effect size (effect ♀ or ♂). LG = linkage group; Pos = cM position; CI = confidence interval; PVE  
 420 = percent variance explained; sex.cov = sex included as a covariate. QTL significance is displayed as  
 421 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	effect	aa	effect
								avg ♀	♀	avg ♂	♂
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				
<i>ghr</i>	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				



## DISCUSSION

422  
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 (Yañez *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 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



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

694

695

## ACKNOWLEDGEMENTS

696 This work was funded by a Fonds de Recherche du Québec Nature et Technologies (FRQNT) research  
697 grant awarded to Céline Audet, Louis Bernatchez and Nadia Aubin-Horth, a grant from the Société de  
698 Recherche et de Développement en Aquaculture Continentale (SORDAC) awarded to Louis Bernatchez  
699 and Céline Audet, and a grant from the Spanish Ministry of Education (Grant PR2010-0601) awarded to  
700 Ciro Rico. Thanks to G. Côté for laboratory assistance, M. Laporte for discussion on QTL and for  
701 comments on the manuscript, M. Lamothe and T. Gosselin for discussion on double recombinants and  
702 genotyping errors in RADseq data and to T. Gosselin for exporting the required files from STACKs for  
703 QTL analysis. During this work, BJGS was supported by an NSERC postdoctoral fellowship, and then an  
704 FRQS postdoctoral fellowship.

705

706

## REFERENCES

- 707 Allendorf F. W., Thorgaard G. H., 1984 Tetraploidy and the evolution of salmonid fishes. In: Turner BJ  
708 (Ed.), *Evolutionary genetics of fishes*, Plenum Publishing Corporation, New York, pp. 1–53.
- 709 Allendorf F. W., Bassham S., Cresko W. A., Limborg M. T., Seeb L. W., Seeb J. E., 2015 Effects of  
710 crossovers between homeologs on inheritance and population genomics in polyploid-derived  
711 salmonid fishes. *J. Hered.* **106**: 217–227.
- 712 Andrews K. R., Good J. M., Miller M. R., Luikart G., Hohenlohe P. A., 2016 Harnessing the power of  
713 RADseq for ecological and evolutionary genomics. *Nat Rev Genet* **17**: 81–92.
- 714 Artieri C. G., Mitchell L. A., Ng S. H. S., Parisotto S. E., Danzmann R. G., Hoyheim B., Phillips R. B.,  
715 Morasch M., Koop B. F., Davidson W. S., 2006 Identification of the sex-determining locus of  
716 Atlantic Salmon (*Salmo salar*) on chromosome 2. *Cytogenet Genome Res* **112**: 152–159.
- 717 Ashton D. T., Ritchie P. A., Wellenreuther M., 2016 15 years of QTL studies in fish: challenges and  
718 future directions. *Mol. Ecol.*
- 719 Bachtrog D., 2006 A dynamic view of sex chromosome evolution. *Current Opinion in Genetics &*  
720 *Development* **16**: 578–585.
- 721 Barson N. J., Aykanat T., Hindar K., Baranski M., 2015 Sex-dependent dominance at a single locus  
722 maintains variation in age at maturity in salmon. *Nature* **528**: 405–408.
- 723 Beavis W. D., 1997 QTL Analyses: Power, Precision, and Accuracy. In: Paterson AH (Ed.), *Molecular*  
724 *Dissection of Complex Traits*, CRC Press, Boca Raton, pp. 145–159.
- 725 Berset-Brandli L., Jaquier J., Broquet T., Ulrich Y., Perrin N., 2008 Extreme heterochiasmy and nascent  
726 sex chromosomes in European tree frogs. *Proceedings of the Royal Society B: Biological Sciences*  
727 **275**: 1577–1585.
- 728 Brandvain Y., Coop G., 2012 Scrambling eggs: Meiotic drive and the evolution of female recombination  
729 rates. *Genetics* **190**: 709–723.
- 730 Brelsford A., Stöck M., Betto-Colliard C., Dubey S., Dufresnes C., Jourdan-Pineau H., Rodrigues N.,

- 731 Savary R., Sermier R., Perrin N., 2013 Homologous sex chromosomes in three deeply divergent  
732 anuran species. *Evolution* **67**: 2434–2440.
- 733 Briec M. S. O., Briec M. S. O., Waters C. D., Waters C. D., Seeb J. E., Seeb J. E., Naish K. A., 2014 A  
734 dense linkage map for Chinook Salmon (*Oncorhynchus tshawytscha*) reveals variable chromosomal  
735 divergence after an ancestral whole genome duplication event. *G3 - Genes|Genomes|Genetics* **4**: 447–  
736 460.
- 737 Broman K. W., Sen S., 2009 *A Guide to QTL Mapping with R/qlt*. Springer, New York.
- 738 Broman K. W., Murray J. C., Sheffield V. C., White R. L., Weber J. L., 1998 Comprehensive human  
739 genetic maps: Individual and sex-specific variation in recombination. *The American Journal of*  
740 *Human Genetics* **63**: 861–869.
- 741 Broman K. W., Wu H., Sen S., Churchill G. A., 2003 R/qlt: QTL mapping in experimental crosses.  
742 *Bioinformatics* **19**: 889–890.
- 743 Brunelli J. P., Wertzler K. J., Sundin K., Thorgaard G. H., 2008 Y-specific sequences and polymorphisms  
744 in Rainbow Trout and Chinook Salmon. *Genome* **51**: 739–748.
- 745 Bush W. S., Moore J. H., 2012 Chapter 11: Genome-Wide Association Studies. *PLoS Comput Biol* **8**:  
746 e1002822.
- 747 Carvalho A., 2002 Origin and evolution of the *Drosophila* Y chromosome. *Current Opinion in Genetics &*  
748 *Development* **12**: 664–668.
- 749 Catchen J. M., Amores A., Hohenlohe P., Cresko W., Postlethwait J. H., 2011 Stacks: building and  
750 genotyping loci *de novo* from short-read sequences. *G3 - Genes|Genomes|Genetics* **1**: 171–182.
- 751 Charlesworth B., 1991 The evolution of sex chromosomes. *Science* **251**: 1030–1033.
- 752 Charlesworth D., Charlesworth B., 1980 Sex differences in fitness and selection for centric fusions  
753 between sex-chromosomes and autosomes. *Genet. Res.* **35**: 205–214.
- 754 Charlesworth D., Charlesworth B., 2005 Sex chromosomes: Evolution of the weird and wonderful.  
755 *Current Biology* **15**: R129–R131.
- 756 Charlesworth D., Charlesworth B., Marais G., 2005 Steps in the evolution of heteromorphic sex  
757 chromosomes. *Heredity* **95**: 118–128.
- 758 Chen S., Zhang G., Shao C., Huang Q., Liu G., Zhang P., Song W., An N., Chalopin D., Volf J.-N., Hong  
759 Y., Li Q., Sha Z., Zhou H., Xie M., Yu Q., Liu Y., Xiang H., Wang N., Wu K., Yang C., Zhou Q.,  
760 Liao X., Yang L., Hu Q., Zhang J., Meng L., Jin L., Tian Y., Lian J., Yang J., Miao G., Liu S., Liang  
761 Z., Yan F., Li Y., Sun B., Zhang H., Zhang J., Zhu Y., Du M., Zhao Y., Schartl M., Tang Q., Wang J.,  
762 2014 Whole-genome sequence of a flatfish provides insights into ZW sex chromosome evolution and  
763 adaptation to a benthic lifestyle. *Nat Genet* **46**: 253–260.
- 764 Clarke J. T., Lloyd G. T., Friedman M., 2016 Little evidence for enhanced phenotypic evolution in early  
765 teleosts relative to their living fossil sister group. *Proc. Natl. Acad. Sci. U.S.A.*
- 766 Crête-Lafrenière A., Weir L. K., Bernatchez L., 2012 Framing the Salmonidae Family phylogenetic  
767 portrait: a more complete picture from increased taxon sampling. *PLoS ONE* **7**: e46662.
- 768 Davidson W. S., Huang T.-K., Fujiki K., Schalburg von K. R., Koop B. F., 2009 The sex determining loci  
769 and sex chromosomes in the Family Salmonidae. *Sexual Development* **3**: 78–87.
- 770 Davidson W. S., Koop B. F., Jones S. J. M., Iturra P., Vidal R., Maass A., Jonassen I., Lien S., Omholt S.  
771 W., 2010 Sequencing the genome of the Atlantic Salmon (*Salmo salar*). *Genome Biol.* **11**: 403.
- 772 Devlin R. H., Stone G. W., Smailus D. E., 1998 Extensive direct-tandem organization of a long repeat  
773 DNA sequence on the Y chromosome of Chinook Salmon (*Oncorhynchus tshawytscha*). *J Mol Evol*  
774 **46**: 277–287.
- 775 Eisbrenner W. D., Botwright N., Cook M., Davidson E. A., Dominik S., Elliott N. G., Henshall J., Jones S.  
776 L., Kube P. D., Lubieniecki K. P., Peng S., Davidson W. S., 2013 Evidence for multiple sex-  
777 determining loci in Tasmanian Atlantic Salmon (*Salmo salar*). **113**: 86–92.
- 778 Faber-Hammond J. J., Phillips R. B., Brown K. H., 2015 Comparative analysis of the shared sex-

- 779 determination region (SDR) among salmonid fishes. *Genome Biology and Evolution* **7**: 1972–1987.
- 780 Faber-Hammond J., Phillips R. B., Park L. K., 2012 The Sockeye Salmon neo-Y chromosome is a fusion  
781 between linkage groups orthologous to the Coho Y chromosome and the long arm of Rainbow Trout  
782 chromosome 2. *Cytogenet Genome Res* **136**: 69–74.
- 783 Furman B., Evans B. J., 2016 Sequential turnovers of sex chromosomes in African clawed frogs  
784 (*Xenopus*) suggest some genomic regions are good at sex determination. *G3 -*  
785 *Genes|Genomes|Genetics*.
- 786 Gagnaire P.-A., Normandeau E., Pavey S. A., Bernatchez L., 2013 Mapping phenotypic, expression and  
787 transmission ratio distortion QTL using RAD markers in the Lake Whitefish (*Coregonus*  
788 *clupeaformis*). *Mol. Ecol.* **22**: 3036–3048.
- 789 Grützner F., Rens W., Tsend-Ayush E., El-Mogharbel N., O'Brien P. C. M., Jones R. C., Ferguson-Smith  
790 M. A., Marshall Graves J. A., 2004 In the platypus a meiotic chain of ten sex chromosomes shares  
791 genes with the bird Z and mammal X chromosomes. *Nature* **432**: 913–917.
- 792 Hackett C. A., Broadfoot L. B., 2003 Effects of genotyping errors, missing values and segregation  
793 distortion in molecular marker data on the construction of linkage maps. **90**: 33–38.
- 794 Haldane J., 1922 Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics* **12**: 101–109.
- 795 Hecht B. C., Thrower F. P., Hale M. C., Miller M. R., Nichols K. M., 2012 Genetic architecture of  
796 migration-related traits in rainbow and steelhead trout, *Oncorhynchus mykiss*. *G3 -*  
797 *Genes|Genomes|Genetics* **2**: 1113–1127.
- 798 Henning F., Lee H. J., Franchini P., Meyer A., 2014 Genetic mapping of horizontal stripes in Lake  
799 Victoria cichlid fishes: benefits and pitfalls of using RAD markers for dense linkage mapping. *Mol.*  
800 *Ecol.* **23**: 5224–5240.
- 801 Jiang C., Zeng Z. B., 1995 Multiple trait analysis of genetic mapping for quantitative trait loci. *Genetics*  
802 **140**: 1111–1127.
- 803 Johnston S. E., Berenos C., Slate J., Pemberton J. M., 2016 Conserved genetic architecture underlying  
804 individual recombination rate variation in a wild population of Soay sheep (*Ovis aries*). *Genetics* **203**:  
805 583–598.
- 806 Johnstone R., Simpson T. H., Youngson A. F., 1978 Sex reversal in salmonid culture. *Aquaculture* **13**:  
807 115–134.
- 808 Kirkpatrick M., 2016 The evolution of genome structure by natural and sexual selection. *J. Hered* **108**: 3–  
809 11.
- 810 Kitano J., Peichel C. L., 2011 Turnover of sex chromosomes and speciation in fishes. *Environ Biol Fish*  
811 **94**: 549–558.
- 812 Kitano J., Ross J. A., Mori S., Kume M., Jones F. C., Chan Y. F., Absher D. M., Grimwood J., Schmutz J.,  
813 Myers R. M., Kingsley D. M., Peichel C. L., 2009 A role for a neo-sex chromosome in stickleback  
814 speciation. *Nature* **461**: 1079–1083.
- 815 Kodama M., Briec M. S. O., Devlin R. H., Hard J. J., Naish K. A., 2014 Comparative mapping between  
816 Coho Salmon (*Oncorhynchus kisutch*) and three other salmonids suggests a role for chromosomal  
817 rearrangements in the retention of duplicated regions following a whole genome duplication event.  
818 *G3 - Genes|Genomes|Genetics* **4**: 1717–1730.
- 819 Kondo M., 2006 Genomic organization of the sex-determining and adjacent regions of the sex  
820 chromosomes of medaka. *Genome Research* **16**: 815–826.
- 821 Kondo M., Nagao E., Mitani H., Shima A., 2001 Differences in recombination frequencies during female  
822 and male meioses of the sex chromosomes of the medaka, *Oryzias latipes*. *Genet Res* **78**.
- 823 Küttner E., Nilsson J., Skúlason S., Gunnarsson S., Ferguson M. M., Danzmann R. G., Civetta A., 2011  
824 Sex chromosome polymorphisms in Arctic Charr and their evolutionary origins. *Genome* **54**: 852–  
825 861.
- 826 Lahn B. T., Pearson N. M., Jegalian K., 2001 The human Y chromosome, in the light of evolution. *Nat*

- 827 Rev Genet **2**: 207–216.
- 828 Laporte M., Rogers S. M., Dion-Côté A.-M., Normandeau E., Gagnaire P.-A., Dalziel A. C., Chebib J.,  
829 Bernatchez L., 2015 RAD-QTL mapping reveals both genome-level parallelism and different genetic  
830 architecture underlying the evolution of body shape in Lake Whitefish (*Coregonus clupeaformis*)  
831 species pairs. *G3 - Genes|Genomes|Genetics* **5**: 1481–1491.
- 832 Larson W. A., McKinney G. J., Limborg M. T., Everett M. V., Seeb L. W., Seeb J. E., 2015 Identification  
833 of multiple QTL hotspots in Sockeye Salmon (*Oncorhynchus nerka*) using Genotyping-by-  
834 Sequencing and a dense linkage map. *J. Hered.* **107**: 122-133.
- 835 Larson W. A., McKinney G. J., Seeb J. E., Seeb L. W., 2016 Identification and characterization of sex-  
836 associated loci in Sockeye Salmon using genotyping-by-sequencing and comparison with a sex-  
837 determining assay based on the *sdY* gene. *J. Hered.* **107**: 559-566.
- 838 Lenormand T., 2003 The evolution of sex dimorphism in recombination. *Genetics* **163**: 811–822.
- 839 Lenormand T., Dutheil J., 2005 Recombination difference between sexes: a role for haploid selection. *Plos*  
840 *Biol* **3**: e63.
- 841 Lenormand T., Engelstädter J., Johnston S. E., Wijnker E., Haag C. R., 2016 Evolutionary mysteries in  
842 meiosis. *Phil. Trans. R. Soc. B* **371**: 20160001
- 843 Lien S., Gidskehaug L., Moen T., Ben J Hayes, Berg P. R., Davidson W. S., Omholt S. W., Kent M. P.,  
844 2011 A dense SNP-based linkage map for Atlantic Salmon (*Salmo salar*) reveals extended  
845 chromosome homeologies and striking differences in sex-specific recombination patterns. *BMC*  
846 *Genomics* **12**: 615.
- 847 Lien S., Koop B. F., Sandve S. R., Miller J. R., Kent M. P., Nome T., Hvidsten T. R., Leong J. S.,  
848 Minkley D. R., Zimin A., Grammes F., Grove H., Gjuvsland A., Walenz B., Hermansen R. A.,  
849 Schalburg von K., Rondeau E. B., Di Genova A., Samy J. K. A., Olav Vik J., Vigeland M. D., Caler  
850 L., Grimholt U., Jentoft S., Våge D. I., de Jong P., Moen T., Baranski M., Palti Y., Smith D. R.,  
851 Yorke J. A., Nederbragt A. J., Tooming-Klunderud A., Jakobsen K. S., Jiang X., Fan D., Hu Y.,  
852 Liberles D. A., Vidal R., Iturra P., Jones S. J. M., Jonassen I., Maass A., Omholt S. W., Davidson W.  
853 S., 2016 The Atlantic Salmon genome provides insights into rediploidization. *Nature* **533**: 200–205.
- 854 Limborg M. T., Seeb L. W., Seeb J. E., 2016 Sorting duplicated loci disentangles complexities of  
855 polyploid genomes masked by genotyping by sequencing. *Mol. Ecol.* **25**: 2117–2129.
- 856 Lubieniecki K. P., Lin S., Cabana E. I., Li J., Lai Y. Y. Y., Davidson W. S., 2015 Genomic instability of  
857 the sex-determining locus in Atlantic Salmon (*Salmo salar*). *G3 - Genes|Genomes|Genetics* **5**: 2513–  
858 2522.
- 859 Lv W., Zheng X., Kuang Y., Cao D., Yan Y., Sun X., 2016 QTL variations for growth-related traits in  
860 eight distinct families of Common Carp (*Cyprinus carpio*). *BMC Genet* **17**: 65.
- 861 Mable B. K., 2004 'Why polyploidy is rarer in animals than in plants': myths and mechanisms. *Biological*  
862 *Journal of the Linnean Society* **82**: 453–466.
- 863 Mackay T. F., 2001 The genetic architecture of quantitative traits. *Annu. Rev. Genet.* **35**: 303–339.
- 864 Mackay T. F. C., Stone E. A., Ayroles J. F., 2009 The genetics of quantitative traits: challenges and  
865 prospects. *Nat Rev Genet* **10**: 565–577.
- 866 Mank J. E., Avise J. C., 2009 Evolutionary diversity and turn-over of sex determination in teleost fishes.  
867 *Sex Dev* **3**: 60–67.
- 868 Marshall Graves J. A., Peichel C. L., 2010 Are homologies in vertebrate sex determination due to shared  
869 ancestry or to limited options? *Genome Biol.* **11**: 205.
- 870 May B., Delany M. E., 2015 Meiotic models to explain classical linkage, pseudolinkage, and  
871 chromosomal pairing in tetraploid derivative salmonid genomes: II. Wright is still right. *J. Hered.*  
872 **106**: 762–766.
- 873 McKinney G. J., Seeb L. W., Larson W. A., Gomez Uchida D., Limborg M. T., Briec M. S. O., Everett  
874 M. V., Naish K. A., Waples R. K., Seeb J. E., 2016 An integrated linkage map reveals candidate

- 875 genes underlying adaptive variation in Chinook Salmon (*Oncorhynchus tshawytscha*). Mol. Ecol.  
876 Resour. **16**: 769–783.
- 877 Moen T., Hoyheim B., Munck H., Gomez-Raya L., 2004 A linkage map of Atlantic Salmon (*Salmo salar*)  
878 reveals an uncommonly large difference in recombination rate between the sexes. Anim. Genet. **35**:  
879 81–92.
- 880 Moghadam H. K., Ferguson M. M., Danzmann R. G., 2007 Linkage variation at the sex-determining locus  
881 within Fraser strain Arctic charr *Salvelinus alpinus*. Journal of Fish Biology **71**: 294–301.
- 882 Muller H. J., 1925 Why polyploidy is rarer in animals than in plants. The American Naturalist **59**: 346–  
883 353.
- 884 Myosho T., Takehana Y., Hamaguchi S., 2015 Turnover of sex chromosomes in Celebensis group  
885 Medaka fishes. G3 - Genes|Genomes|Genetics **5**: 2685–2691.
- 886 Nagler J. J., Bouma J., Thorgaard G. H., Dauble D. D., 2001 High incidence of a male-specific genetic  
887 marker in phenotypic female Chinook Salmon from the Columbia River. Environ. Health Perspect.  
888 **109**: 67–69.
- 889 Naish K. A., Phillips R. B., Briec M. S. O., Newton L. R., Elz A. E., Park L. K., 2013 Comparative  
890 genome mapping between Chinook Salmon (*Oncorhynchus tshawytscha*) and Rainbow Trout (*O.*  
891 *mykiss*) based on homologous microsatellite loci. G3 - Genes|Genomes|Genetics **3**: 2281–2288.
- 892 Nei M., 1969 Linkage modification and sex difference in recombination. Genetics **63**: 681–699.
- 893 Nugent C. M., Easton A. A., Norman J. D., Ferguson M. M., Danzmann R. G., 2016 A SNP based linkage  
894 map of the Arctic Charr (*Salvelinus alpinus*) genome provides insights into the diploidization process  
895 after whole genome duplication. G3 - Genes|Genomes|Genetics.
- 896 Ohno S., 1970 *Evolution by Gene Duplication*. Springer Berlin Heidelberg, Berlin, Heidelberg.
- 897 Orr H. A., 1990 “Why polyploidy is rarer in animals than in plants” revisited. The American Naturalist  
898 **136**: 759–770.
- 899 Otto S. P., 2007 The Evolutionary Consequences of Polyploidy. Cell **131**: 452–462.
- 900 Palti Y., Vallejo R. L., Gao G., Liu S., Hernandez A. G., Rexroad C. E. III, Wiens G. D., 2015 Detection  
901 and validation of QTL affecting bacterial cold water disease resistance in Rainbow Trout using  
902 restriction-site associated DNA sequencing. PLoS ONE **10**: e0138435.
- 903 Pennell M. W., Kirkpatrick M., Otto S. P., Vamosi J. C., Peichel C. L., Valenzuela N., Kitano J., 2015 Y  
904 fuse? Sex chromosome fusions in fishes and reptiles. PLoS Genet **11**: e1005237.
- 905 Perrin N., 2009 Sex reversal: a fountain of youth for sex chromosomes? Evolution **63**: 3043–3049.
- 906 Phillips R., Ráb P., 2001 Chromosome evolution in the Salmonidae (Pisces): an update. Biol Rev Camb  
907 Philos Soc **76**: 1–25.
- 908 Phillips R. B., 2013 Evolution of the sex chromosomes in salmonid fishes. Cytogenet Genome Res **141**:  
909 177–185.
- 910 Phillips R. B., Ihssen P. E., 1985 Identification of sex chromosomes in lake trout (*Salvelinus namaycush*).  
911 Cytogenet. Cell Genet. **39**: 14–18.
- 912 Phillips R. B., Keatley K. A., Morasch M. R., Ventura A. B., Lubieniecki K. P., Ben F Koop, Danzmann  
913 R. G., Davidson W. S., 2009 Assignment of Atlantic Salmon (*Salmo salar*) linkage groups to specific  
914 chromosomes: Conservation of large syntenic blocks corresponding to whole chromosome arms in  
915 Rainbow Trout (*Oncorhynchus mykiss*). BMC Genet **10**: 46.
- 916 Phillips R. B., Morasch M. R., Park L. K., Naish K. A., Devlin R. H., 2005 Identification of the sex  
917 chromosome pair in Coho Salmon (*Oncorhynchus kisutch*): lack of conservation of the sex linkage  
918 group with Chinook Salmon (*Oncorhynchus tshawytscha*). Cytogenet Genome Res **111**: 166–170.
- 919 Phillips R. B., Nichols K. M., DeKoning J. J., Morasch M. R., Keatley K. A., Rexroad C., Gahr S. A.,  
920 Danzmann R. G., Drew R. E., Thorgaard G. H., 2006 Assignment of Rainbow Trout linkage groups  
921 to specific chromosomes. Genetics **174**: 1661–1670.
- 922 R Development Core Team, 2017 R: A language and environment for statistical computing.

- 923 Rexroad C. E., Palti Y., Gahr S. A., Vallejo R. L., 2008 A second generation genetic map for Rainbow  
924 Trout (*Oncorhynchus mykiss*). BMC Genet **9**: 74.
- 925 Rexroad C. E., Vallejo R. L., Liu S., Palti Y., Weber G. M., 2012 QTL affecting stress response to  
926 crowding in a Rainbow Trout broodstock population. BMC Genet **13**: 97.
- 927 Rockman M. V., 2012 The QTN program and the alleles that matter for evolution: all that's gold does not  
928 glitter. Evolution **66**: 1–17.
- 929 Roco Á. S., Olmstead A. W., Degitz S. J., Amano T., Zimmerman L. B., Bullejos M., 2015 Coexistence of  
930 Y, W, and Z sex chromosomes in *Xenopus tropicalis*. Proceedings of the National Academy of  
931 Sciences **112**: E4752–E4761.
- 932 Rogers S. M., Bernatchez L., 2007 The genetic architecture of ecological speciation and the association  
933 with signatures of selection in natural Lake Whitefish (*Coregonus* sp. *Salmonidae*) species pairs.  
934 Molecular Biology and Evolution **24**: 1423–1438.
- 935 Rondeau E. B., Minkley D. R., Leong J. S., Messmer A. M., Jantzen J. R., Schalburg von K. R., Lemon C.,  
936 Bird N. H., Koop B. F., 2014 The genome and linkage map of the Northern Pike (*Esox lucius*):  
937 conserved synteny revealed between the salmonid sister group and the Neoteleostei. PLoS ONE **9**:  
938 e102089.
- 939 Ross J. A., Urton J. R., Boland J., Shapiro M. D., Peichel C. L., 2009 Turnover of sex chromosomes in the  
940 stickleback fishes (*Gasterosteidae*). PLoS Genet **5**: e1000391–12.
- 941 Sakamoto T., Danzmann R. G., Gharbi K., Howard P., Ozaki A., Khoo S. K., Woram R. A., Okamoto N.,  
942 Ferguson M. M., Holm L. E., Guyomard R., Hoyheim B., 2000 A microsatellite linkage map of  
943 Rainbow Trout (*Oncorhynchus mykiss*) characterized by large sex-specific differences in  
944 recombination rates. Genetics **155**: 1331–1345.
- 945 Santure A. W., Poissant J., De Cauwer I., Oers K., Robinson M. R., Quinn J. L., Groenen M. A. M.,  
946 Visser M. E., Sheldon B. C., Slate J., 2015 Replicated analysis of the genetic architecture of  
947 quantitative traits in two wild great tit populations. Mol. Ecol. **24**: 6148–6162.
- 948 Sauvage C., Vagner M., Derôme N., Audet C., Bernatchez L., 2012a Coding gene single nucleotide  
949 polymorphism mapping and quantitative trait loci detection for physiological reproductive traits in  
950 Brook Charr, *Salvelinus fontinalis*. G3 - Genes|Genomes|Genetics **2**: 379–392.
- 951 Sauvage C., Vagner M., Derôme N., Audet C., Bernatchez L., 2012b Coding gene SNP mapping reveals  
952 QTL linked to growth and stress response in Brook Charr (*Salvelinus fontinalis*). G3 -  
953 Genes|Genomes|Genetics **2**: 707–720.
- 954 Session A. M., Uno Y., Kwon T., Chapman J. A., Toyoda A., Takahashi S., Fukui A., Hikosaka A.,  
955 Suzuki A., Kondo M., van Heeringen S. J., Quigley I., Heinz S., Ogino H., Ochi H., Hellsten U.,  
956 Lyons J. B., Simakov O., Putnam N., Stites J., Kuroki Y., Tanaka T., Michiue T., Watanabe M.,  
957 Bogdanovic O., Lister R., Georgiou G., Paranjpe S. S., van Kruijsbergen I., Shu S., Carlson J.,  
958 Kinoshita T., Ohta Y., Mawaribuchi S., Jenkins J., Grimwood J., Schmutz J., Mitros T., Mozaffari S.  
959 V., Suzuki Y., Haramoto Y., Yamamoto T. S., Takagi C., Heald R., Miller K., Haudenschild C.,  
960 Kitzman J., Nakayama T., Izutsu Y., Robert J., Fortriede J., Burns K., Lotay V., Karimi K., Yasuoka  
961 Y., Dichmann D. S., Flajnik M. F., Houston D. W., Shendure J., DuPasquier L., Vize P. D., Zorn A.  
962 M., Ito M., Marcotte E. M., Wallingford J. B., Ito Y., Asashima M., Ueno N., Matsuda Y., Veenstra  
963 G. J. C., Fujiyama A., Harland R. M., Taira M., Rokhsar D. S., 2016 Genome evolution in the  
964 allotetraploid frog *Xenopus laevis*. Nature **538**: 336–343.
- 965 Singer A., Perlman H., Yan Y., Walker C., Corley-Smith G., Brandhorst B., Postlethwait J., 2002 Sex-  
966 specific recombination rates in zebrafish (*Danio rerio*). Genetics **160**: 649–657.
- 967 Slate J., 2005 Quantitative trait locus mapping in natural populations: progress, caveats and future  
968 directions. Mol. Ecol. **14**: 363–379.
- 969 Slate J., 2008 Robustness of linkage maps in natural populations: a simulation study. Proceedings of the  
970 Royal Society B: Biological Sciences **275**: 695–702.
- 971 Steinemann M., Steinemann S., 1998 Enigma of Y chromosome degeneration: neo-Y and neo-X

- 972 chromosomes of *Drosophila miranda* a model for sex chromosome evolution. *Genetica* **102-103**:  
973 409–420.
- 974 Stöck M., Horn A., Grossen C., Lindtke D., Sermier R., Betto-Colliard C., Dufresnes C., Bonjour E.,  
975 Dumas Z., Luquet E., Maddalena T., Sousa H. C., Martinez-Solano I., Perrin N., 2011 Ever-young  
976 sex chromosomes in European tree frogs. *Plos Biol* **9**: e1001062–9.
- 977 Stöck M., Savary R., Betto-Colliard C., Biollay S., Jourdan-Pineau H., Perrin N., 2013 Low rates of X-Y  
978 recombination, not turnovers, account for homomorphic sex chromosomes in several diploid species  
979 of Palearctic green toads (*Bufo viridis* subgroup). *Journal of Evolutionary Biology* **26**: 674–682.
- 980 Sutherland B. J. G., Gosselin T., Normandeau E., Lamothe M., Isabel N., Audet C., Bernatchez L., 2016  
981 Salmonid chromosome evolution as revealed by a novel method for comparing RADseq linkage maps.  
982 *Genome Biology and Evolution*: **8**: 3600-3617.
- 983 Taylor J. S., Braasch I., Frickey T., Meyer A., Van de Peer Y., 2003 Genome duplication, a trait shared by  
984 22,000 species of ray-finned fish. *Genome Research* **13**: 382–390.
- 985 Thorgaard G. H., 1977 Heteromorphic sex chromosomes in male Rainbow Trout. *Science* **196**: 900–902.
- 986 Trivers R., 1998 Sex differences in rates of recombination and sexual selection. In: Michod R, Levin D  
987 (Eds.), *The evolution of sex*, pp. 270–286.
- 988 Tsai H. Y., Hamilton A., Guy D. R., Tinch A. E., Bishop S. C., Houston R. D., 2015 The genetic  
989 architecture of growth and fillet traits in farmed Atlantic Salmon (*Salmo salar*). *BMC Genet* **16**: 117–  
990 11.
- 991 van Doorn G. S., Kirkpatrick M., 2007 Turnover of sex chromosomes induced by sexual conflict. **449**:  
992 909–912.
- 993 van Ooijen J. W., 2006 JoinMap4: Software for the calculation of genetic linkage maps in experimental  
994 populations.
- 995 Wei T., Simko V., 2017 corrplot: Visualization of a correlation matrix.
- 996 Woram R. A., Gharbi K., Sakamoto T., Høyheim B., Holm L.-E., Naish K., McGowan C., Ferguson M.  
997 M., Phillips R. B., Stein J., Guyomard R., Cairney M., Taggart J. B., Powell R., Davidson W.,  
998 Danzmann R. G., 2003 Comparative genome analysis of the primary sex-determining locus in  
999 salmonid fishes. *Genome Research* **13**: 272–280.
- 1000 Wu R., Ma C.-X., Painter I., Zeng Z.-B., 2002 Simultaneous maximum likelihood estimation of linkage  
1001 and linkage phases in outcrossing species. *Theor Popul Biol* **61**: 349–363.
- 1002 Xu S., 2003 Theoretical basis of the Beavis effect. *Genetics* **165**: 2259–2268.
- 1003 Yano A., Guyomard R., Nicol B., Jouanno E., Quillet E., Klopp C., Cabau C., Bouchez O., Fostier A.,  
1004 Guiguen Y., 2012a An immune-related gene evolved into the master sex-determining gene in  
1005 Rainbow Trout, *Oncorhynchus mykiss*. *Curr. Biol.* **22**: 1423–1428.
- 1006 Yano A., Nicol B., Jouanno E., Quillet E., Fostier A., Guyomard R., Guiguen Y., 2012b The sexually  
1007 dimorphic on the Y-chromosome gene (*sdY*) is a conserved male-specific Y-chromosome sequence in  
1008 many salmonids. *Evol Appl* **6**: 486–496.
- 1009 Yáñez J. M., Houston R. D., Newman S., 2014 Genetics and genomics of disease resistance in salmonid  
1010 species. *Front. Genet.* **5**: 415.
- 1011 Yoshida K., Makino T., Yamaguchi K., Shigenobu S., Hasebe M., Kawata M., Kume M., Mori S., Peichel  
1012 C. L., Toyoda A., Fujiyama A., Kitano J., 2014 Sex chromosome turnover contributes to genomic  
1013 divergence between incipient stickleback species. *PLoS Genet* **10**: e1004223–16.
- 1014

- 1015 **SUPPLEMENTAL MATERIAL**
- 1016 **Table S1.** Phenotype average, standard deviation and sample size in males and females. Phenotypes  
1017 showing any differences between males and females ( $p \leq 0.2$ ) included sex as a covariate in the model.  
1018 Sex-specific phenotypes were only tested within the one sex and therefore had smaller sample sizes.
- 1019 **Table S2.** Complete QTL table with all identified genome- and chromosome-wide QTLs and associated  
1020 values, including marker sequence and SNP, and effect size of different genotypes.
- 1021 **Figure S1.** Correlation plot of phenotypes used in QTL analysis. Phenotype pairs that do not share any  
1022 individuals for correlation are shown with ‘?’.
- 1023 **Figure S2.** Heterochiasmy plots for individual chromosomes in the maternal (a) and paternal (b)  
1024 haplotypes. Grey boxes above the plot indicate probable residually tetraploid chromosome arms, and  
1025 centromere positions transferred from Chinook Salmon are indicated by stars. When Chinook Salmon  
1026 chromosomes were not fusions but were metacentrics ( $n = 2$ ), this is denoted by a ‘?’ to denote the  
1027 uncertainty as to the centromere position in Brook Charr.
- 1028 **File S1.** Required files for running Rqtl analysis (phenotype (.qua), map (.map) and genotype (.loc)). The  
1029 map file corresponds to the female map from Sutherland *et al.* 2016. See the Data Availability for code for  
1030 performing complete analysis with these files.
- 1031 **File S2.** Fasta file with RAD-seq tags output using the STACKs population module for alleles from all  
1032 individual offspring and parents.