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

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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

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28 **ABSTRACT**

29 Whole genome duplication can have large impacts on genome evolution, and much remains unknown
30 about these impacts. This includes the mechanisms of coping with a duplicated sex determination system
31 and whether this has an impact on increasing the diversity of sex determination mechanisms. Other
32 impacts include sexual conflict, where alleles having different optimums in each sex can result in
33 sequestration of genes into non-recombining sex chromosomes. Sex chromosome development itself may
34 involve sex-specific recombination rate (i.e. heterochiasmy), which is also poorly understood. Family
35 Salmonidae is a model system for these phenomena, having undergone autotetraploidization and
36 subsequent rediploidization in most of the genome at the base of the lineage. The salmonid master sex
37 determining gene is known, and many species have non-homologous sex chromosomes, putatively due to
38 transposition of this gene. In this study, we identify the sex chromosome of Brook Charr *Salvelinus*
39 *fontinalis* and compare sex chromosome identities across the lineage (eight species, four genera).
40 Although non-homology is frequent, homologous sex chromosomes and other consistencies are present in
41 distantly related species, indicating probable convergence on specific sex and neo-sex chromosomes. We
42 also characterize strong heterochiasmy with 2.7-fold more crossovers in maternal than paternal
43 haplotypes with paternal crossovers biased to chromosome ends. When considering only rediploidized
44 chromosomes, the overall heterochiasmy trend remains, although with only 1.9-fold more recombination
45 in the female than the male. Y chromosome crossovers are restricted to a single end of the chromosome,
46 and this chromosome contains a large interspecific inversion, although its status between males and
47 females remains unknown. Finally, we identify QTL for 21 unique growth, reproductive and stress-related
48 phenotypes to improve knowledge of the genetic architecture of these traits important to aquaculture and
49 evolution.

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50 **INTRODUCTION**

51 Characterizing the genetic architecture of ecologically-relevant phenotypes is essential for organism and
52 genome evolution research (Rogers and Bernatchez 2007; Gagnaire *et al.* 2013) and selective breeding
53 (Yáñez *et al.* 2014). Regions of the genome associated with specific traits can be identified by
54 quantitative trait loci (QTL) analysis (Mackay 2001) or genome-wide association studies (GWAS; Bush
55 and Moore 2012). Mapped traits can include morphological, behavioral, physiological or molecular
56 phenotypes, but must show sufficient heritable variation. Power to detect QTL is determined by the
57 number of individuals in the study (Slate 2013; Henning *et al.* 2014), the effect size of the QTL, allele
58 frequencies (Mackay *et al.* 2009), the degree of polygenic control of the trait (Rockman 2012; Ashton *et*
59 *al.* 2016), and the type of cross as well as the extent of the heritability of the trait (Mackay 2001). QTL
60 mapping precision depends on recombination frequency (Mackay *et al.* 2009) as well as map density,
61 although QTL are often only in linkage with causative mutations, which are rarely identified (Slate 2005).
62 Trait genetic architecture can differ between families or populations (e.g. Santure *et al.* 2015) but
63 parallelism and shared QTL can be identified (e.g. Laporte *et al.* 2015; Larson *et al.* 2015). It is therefore
64 valuable to analyze multiple crosses to understand the broader implications of a QTL (e.g. Hecht *et al.*
65 2012; Palti *et al.* 2015; Lv *et al.* 2016). This can reduce false positives that may occur from small sample
66 sizes (Slate 2013) by repeatedly identifying the same region associated to a trait, can more accurately
67 determine the amount of variation explained by the QTL (Slate 2005), and can identify QTL that are not
68 dependent on specific genetic backgrounds, which is particularly valuable for marker-assisted selection
69 (Lv *et al.* 2016).

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

80 Genetic architecture can be strongly affected by sexually antagonistic selection and sex
81 determination. Sexually antagonistic alleles (i.e. alleles that benefit sexes differently) produce genetic
82 conflict (Mackay 2001; Charlesworth *et al.* 2005), which can be resolved by the sequestration of alleles in

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83 non-recombining sex chromosomes. As an example of the effect this can have on genome architecture,
84 the *Drosophila* Y chromosome is made almost exclusively of genes that have migrated from other
85 chromosomes, presumably due to their specific benefit to males (Carvalho 2002). An additional benefit
86 occurs by constant sex-specific selection occurring for alleles on the Y (or W) chromosome, as these are
87 always only in the heterogametic sex (Lahn *et al.* 2001). However, the lack of recombination between the
88 sex chromosomes can also result in Y degeneration due to accumulation of mutations that are not able to
89 be purged through recombination with X (Charlesworth 1991). A different resolution to genetic conflict
90 involves sex-dependent dominance, whereby allelic dominance depends on the sex of the individual,
91 which need not be on the sex chromosome (Barson *et al.* 2015). Much remains to be understood about
92 resolving these conflicts.

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

104 Sex chromosome evolution may be facilitated by differences in recombination rates between the
105 sexes (i.e. heterochiasmy) (Charlesworth *et al.* 2005), where tight linkage forms along the sex
106 chromosome in the heterogametic sex (Haldane 1922). The evolution of heterochiasmy remains under
107 investigation, although several explanations have been proposed (Lenormand and Dutheil 2005;
108 Brandvain and Coop 2012; Lenormand *et al.* 2016). First, sexes can experience different extents of
109 selection at haploid stages (Lenormand 2003), and heterochiasmy permits retention of epistatically-
110 interacting alleles within a haplotype specifically within the sex experiencing more haploid selection
111 (Lenormand and Dutheil 2005). Second, physical meiotic differences may play a role; female meiosis
112 occurs with a long delay, and chiasma (i.e. locations where crossovers occur) stabilize chromatids during
113 this process (Lenormand 2003; Lenormand *et al.* 2016). Third, recombination protects from meiotic drive,
114 to which the sexes have different susceptibilities (Brandvain and Coop 2012; Johnston *et al.* 2017). Other
115 hypotheses have also been proposed (Trivers 1998; Lenormand 2003). However, in general it is unclear

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116 which of the above explanations have the largest influence, and thus the relationships between WGD,
117 heterochiasmy and sex chromosome evolution require further study.

118 Salmonids (Family Salmonidae) are an ideal system to study genetic architecture and sex
119 determination post-WGD (Davidson *et al.* 2010). The salmonid genome remains in a residually tetraploid
120 state, where some chromosomal telomeric regions continue recombining between homeologous
121 chromosomes and others have rediploidized (Allendorf and Thorgaard 1984; Allendorf *et al.* 2015; May
122 and Delany 2015; Lien *et al.* 2016). Salmonid sex determination is genetically controlled (Davidson *et al.*
123 2009) by a truncated gene from the *interferon-response factor* transcription factor family, *sdY* (sexually
124 dimorphic on the Y-chromosome; Yano *et al.* 2012a). *sdY* may be a salmonid innovation as it has not yet
125 been identified in the non-duplicated sister group for the salmonid WGD, Northern Pike *Esox lucius*
126 (Yano *et al.* 2012b). Male genome-specific conservation of *sdY* occurs in more than ten salmonid species,
127 but some exceptions exist, including the Lake Whitefish *Coregonus clupeaformis* and European
128 Whitefish *C. lavaretus* (Yano *et al.* 2012b), and some Atlantic Salmon *Salmo salar* and Sockeye Salmon
129 *Oncorhynchus nerka* individuals (Eisbrenner *et al.* 2013; Larson *et al.* 2016). Sex chromosomes are not
130 homologous among many salmonid species, potentially due to transposition of *sdY* between chromosomes
131 (Woram *et al.* 2003). Additional evidence for transposition includes repetitive flanking regions with
132 putative transposable elements (Brunelli *et al.* 2008) (Lubieniecki *et al.* 2015) and sequence conservation
133 that abruptly stops outside of the sex determination cassette (Faber-Hammond *et al.* 2015). This
134 transposition to different chromosomes may be delaying Y degeneration (Yano *et al.* 2012b; Lubieniecki
135 *et al.* 2015). In general, the salmonids are at an early stage of sex chromosome evolution (Phillips and
136 Ihssen 1985; Yano *et al.* 2012b) where sex chromosomes are homomorphic (Devlin *et al.* 1998; Phillips
137 and Ráb 2001; Davidson *et al.* 2009). Male salmonids have low recombination rates relative to females
138 with crossover events primarily occurring at telomeric regions, as observed in Rainbow Trout *O. mykiss*
139 (Sakamoto *et al.* 2000) and Atlantic Salmon (Moen *et al.* 2004). Heterochiasmy was principally observed
140 in one linkage group in Northern Pike (i.e. the sister species of the salmonid WGD), but in general was
141 much more equal between the sexes than previously observed in salmonids (Rondeau *et al.* 2014).
142 Salmonids are therefore a valuable model to study the evolution and effects of heterochiasmy in relation
143 to sex determination post-WGD.

144 The combination of characterizing heterochiasmy, sex chromosome identity and the genetic
145 architecture for reproductive, growth and stress response traits provides much-needed information
146 regarding the function of the Brook Charr *Salvelinus fontinalis* genome post-duplication. The goals of this
147 study are to use a high-density genetic map for Brook Charr (Sutherland *et al.* 2016) to (*a*) identify the
148 sex-linked chromosome; (*b*) quantify heterochiasmy in this mapping family while correcting for probable
149 genotyping errors; and (*c*) search for growth, stress resistance and reproduction-related QTL. Furthermore,

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150 using the recent characterization of homology to ancestral chromosomes and homeolog identification
151 among the salmonids (Sutherland *et al.* 2016), we subsequently compare identities of sex chromosomes
152 and identified QTL across the salmonids to identify consistencies. We then discuss the implications of sex
153 chromosome consistencies and heterochiasmy in relation to sex chromosome evolution in salmonids.
154

155 # METHODS

156 ## Fish and phenotyping

157 Juvenile Brook Charr used in this study were the same individuals used to construct a low-density genetic
158 map and perform QTL analysis for 21 phenotypes (29 including repeated measurements occurring at three
159 time points; Table S1) by Sauvage *et al.* for growth (2012a) and reproductive QTL (2012b), as well as to
160 produce a high-density genetic map (Sutherland *et al.* 2016). In brief, F_0 individuals were from a wild
161 anadromous population and a domestic population, and two of the F_1 individuals were crossed to produce
162 192 F_2 offspring. After filters for missing data per individual, 22 offspring were excluded, and one was
163 excluded due to abnormally high numbers of crossovers, leaving 169 individuals (Sutherland *et al.* 2016).
164 Fish were raised in tanks as previously described until 65-80 g, at which point weight, length and
165 condition factor were measured. These phenotypes were measured on the same fish two and six months
166 after the initial measurements. Growth rate was calculated between the multiple sampling times. At the
167 final sampling, all phenotypes were collected. Stress response was also evaluated at this final sampling
168 through an acute handling stress by reducing water levels, capturing fish without chasing and holding out
169 of water for one minute in order to phenotype the stress response using blood parameters chloride,
170 osmolality and cortisol before and after the stress. After fish had re-acclimatized, they were anaesthetized
171 and killed by decapitation as per regulations of Canadian Council of Animal Protection recommendations
172 and protocols approved by the University Animal Care Committee, as previously reported (Sauvage *et al.*
173 2012a). The sex of each individual was determined by visual inspection of the gonads as reported by
174 Sauvage *et al.* (2012b).
175

176 ## Genetic map and quality control of markers and phenotypes

177 A recently developed high-density genetic map with 3826 markers was used with genotypes for 192
178 offspring (Sutherland *et al.* 2016). Parents were diploid and therefore the map is probably missing
179 residually tetraploid regions because these would be removed due to too many alleles during genotyping
180 (see Sutherland *et al.* 2016 for more information). In brief, genotype data was obtained using the
181 population module of STACKS v.1.32 (Catchen *et al.* 2011), phased in JoinMap v.4.1 (van Ooijen 2006),

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182 and imported into R/qtl (Broman *et al.* 2003) using the *read.cross* function with data interpreted as a four-
183 way cross type in the *mapqtl* format (see File S1 for map, genotype and phenotype input files).

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

193 Markers present in the map were tested for segregation distortion by chi-square tests for
194 Mendelian segregation in R/qtl and removed when $p \leq 0.01$ (Broman and Sen 2009). A total of 157
195 markers with significant segregation distortion were removed, leaving a remainder of 3669 markers.
196 Proportions of identical genotypes were tested in R/qtl to ensure that there were no mis-labeled samples.
197 Recombination fraction between marker pairs was estimated using Expectation Maximization algorithm
198 within *est.rf* in R/qtl. The minimum number of obligate crossover events was calculated per individual
199 using *count.XO* in R/qtl, and an outlier sample with 1093 crossovers was removed (other samples had
200 mean and median crossovers of 101 and 83, respectively, before correcting for unlikely double
201 crossovers).

202

203 **Recombination rate**

204 To characterize heterochiasmy in the mapping family parents, the *plotGenotypes* function of R/qtl was
205 used to identify positions of crossovers per parental chromosome (total = 84 chromosomes per individual
206 offspring) and modified to export these positions (see Data Availability section for all code used in the
207 analysis). Male-specific markers were not included in the original map due to low recombination rate and
208 poor positioning (Sutherland *et al.* 2016), and therefore to avoid bias of including female-specific but not
209 male-specific markers, crossovers were evaluated in a map with only markers informative in both sexes
210 (i.e. *ef* x *eg* and *hk* x *hk*; see van Ooijen 2006 and Wu *et al.* 2002 for full explanation of marker types for a
211 cp cross type). Furthermore, as recombination rates can be inflated by a genotyping error appearing to be
212 flanked by two false recombination events (Hackett and Broadfoot 2003; Slate 2008), which can also
213 occur in RAD-seq data (Andrews *et al.* 2016), an additional correction was made to more accurately
214 quantify heterochiasmy. Specifically, per individual and per phased haplotype within individual,

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215 whenever a crossover is identified, a search within 50 cM of the crossover is conducted to identify if a
216 second crossover is near (or an even total of crossovers, resulting in no true phase change). If this number
217 is even, it suggests that the putative crossovers may have been due to a genotyping error, as double
218 crossovers are not expected due to crossover interference. As such, these crossovers would not be counted
219 in the total sum. This is similar to the approach used by Johnston *et al.* (2016) to avoid false double
220 crossovers by only including crossovers that flank more than a single marker. Subsequently, the
221 cumulative number of crossovers for fused metacentric and acrocentric chromosome were calculated and
222 cumulatively displayed in positions as a percentage of the total chromosome length. The corrected
223 crossover counts were used to calculate the female:male recombination rates of the parents. This was also
224 conducted without cumulating and displayed on a per chromosome per haplotype basis.
225
226 **QTL analysis**
227 The effect of sex on each phenotype was tested using linear models in R (R Development Core Team
228 2017). If a marginal effect of sex was found ($p \leq 0.20$), sex was included in the model as a covariate for
229 the phenotype to reduce residual variation and improve power to identify the QTL (Broman and Sen
230 2009). The R/qtl function *scanone* with permutation testing (10,000 permutations; $p \leq 0.05$) was used to
231 identify the presence of a single QTL within each linkage group (Broman *et al.* 2003). Chromosome-wide
232 significance was tested in the same way but per chromosome (10,000 permutations; $p \leq 0.01$). Confidence
233 interval estimates (95%) for QTL positions were identified using *summary.scanone* calculating LOD
234 support intervals with a 1.5 LOD drop. Sex-specific phenotypes (i.e. sperm diameter and concentration,
235 egg diameter) were tested in only one sex, and therefore had smaller sample sizes. Percent variance
236 explained by the identified QTL was performed using *makeqtl* and *fitqtl* within R/qtl, including all
237 genome and chromosome-wide QTL per trait in the formula (trait ~ $QTL_1 + QTL_2 + QTL_n$), as well as sex
238 as a covariate when required. Phenotypic effects were estimated by calculating the differences between
239 the mean phenotype values among the genotype groups for the marker closest to the identified QTL,
240 including only individuals that were successfully genotyped. For markers that only segregate in one
241 parent (i.e. *nn* x *np*) only two phenotype by genotype averages are given, one for the homozygote and one
242 for the heterozygote offspring. Alternatively, for markers segregating in both parents (i.e. *hk* x *hk* or *ef* x
243 *eg*), three phenotype averages are given, two for the alternate homozygotes and one for the heterozygote
244 in *hk* x *hk* marker types and two for the alternate heterozygotes and one for the homozygote in *ef* x *eg*
245 marker types. Sex-specific averages were calculated when the QTL required sex as a covariate in the
246 model. RAD tags for all alleles and associated QTL results are available in File S2.
247 To identify the sex chromosome, offspring sex was coded as a binary trait to identify linkage to
248 any of the LG by QTL mapping as described above (Broman and Sen 2009). Furthermore, the effect of a

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249 QTL may vary depending on the sex of an individual in a non-additive manner (Broman and Sen 2009)
250 due to genetic variation in sexual dimorphism for the trait (e.g. loci that have a different effect in males
251 and females Mackay 2001). Therefore, QTL by sex interaction effects were inspected per trait by
252 subtracting an additive model (*genotype* and *sex*) from a full model (*genotype*, *sex* and a *sex-by-genotype*
253 interaction term) as described by Broman and Sen (2009) . If the additive model is largely driving the
254 effect, the model with only the interaction effect will not be significant, and in this case the interaction
255 effect would not be included in the model. As suggested by Broman and Sen (2009), interaction effects
256 were only tested in this way when a full model for a locus was significant.

257 Identities of sex chromosomes of other species were obtained from references listed in Table 1.
258 For Atlantic Salmon, Artieri *et al.* (2006) identify that the sex determining region is on the long (q) arm of
259 chromosome Ssa02, and Lien *et al.* identify that Ssa02q is homeologous to Ssa12q, indicating that the
260 chromosome arm holding the sex determining region corresponds to the ancestral chromosome 9.1
261 (Sutherland *et al.* 2016). Other species were directly obtained from references in Table 1. Correspondence
262 between Arctic Charr *S. alpinus* and Brook Charr were identified indirectly through other species shared
263 between Nugent *et al.* (2016) and Sutherland *et al.* (2016).

264

265 **Data availability**

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

270

271 ## RESULTS

272 **Sex-linked chromosome in Brook Charr**

273 Sex was highly associated with the majority of Brook Charr (BC) linkage group (LG) BC35, indicating
274 that this is the sex-linked chromosome in Brook Charr (Figure 1). Linkage across the entire LG until the
275 LOD score decreases at the distal end can be explained by male salmonid-specific low recombination rate
276 and male bias of crossovers towards telomeric regions (Sakamoto *et al.* 2000). The drop in LOD suggests
277 the far end of the chromosome is pseudoautosomal, which even occurs in the highly differentiated
278 mammalian X/Y chromosomes in a recombinogenic distal region of the Y chromosome (Lahn *et al.* 2001).
279 Many of the recombination events in BC35 were at a similar section of the LG (~90-110 cM; Figure S1B).

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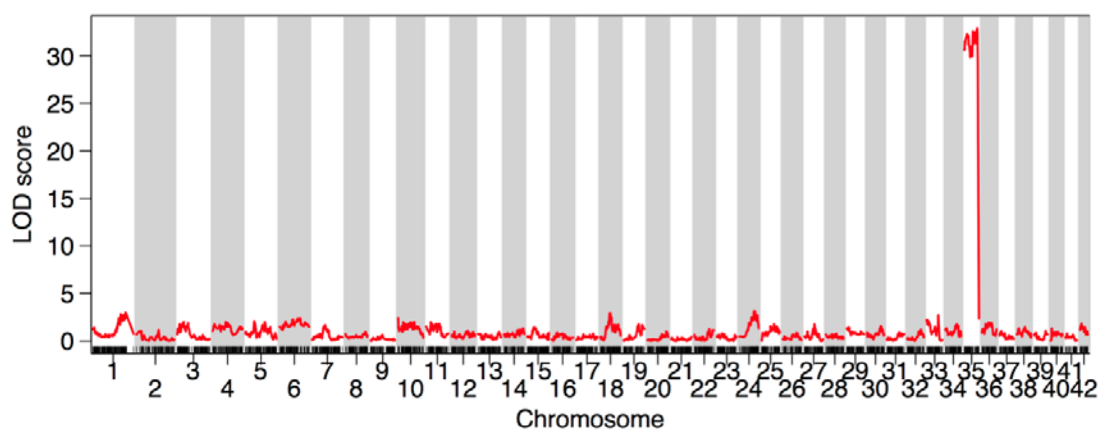
280 BC35 is an acrocentric chromosome homologous to the Northern Pike chromosome 15.1
281 (homeolog naming from Sutherland *et al.* 2016). There are no other sex chromosomes in the other
282 salmonid species with high-density genetic maps available with the chromosome arm containing the sex
283 determining region homologous to BC35 (Table 1). Arctic Charr has a sex chromosome that is comprised
284 of a triple fused chromosome (although this may vary across populations) that contains 15.1 in the fusion,
285 but in Arctic Charr this is not the chromosome arm that holds the sex determining region, which is held
286 within the arm on the other side of the chromosome AC04p (Nugent *et al.* 2016). Other salmonids have
287 different sex chromosomes, as shown in Table 1, including Lake Whitefish (3.1; Gagnaire *et al.* 2013),
288 Atlantic Salmon (9.1-20.2; Artieri *et al.* 2006; Lien *et al.* 2011), Rainbow Trout (14.2; Palti *et al.* 2015),
289 Coho Salmon *O. kisutch* (3.1; Phillips *et al.* 2005; Kodama *et al.* 2014), Chinook Salmon *O. tshawytscha*
290 (23.2; Phillips *et al.* 2005; Naish *et al.* 2013; Brieuc *et al.* 2014) or Sockeye Salmon (3.1-19.1; Larson *et*
291 *al.* 2016). This further refines previous observations of the general lack of homology in the sex
292 chromosomes of the salmonids (Woram *et al.* 2003). Some information on sex chromosomes identities
293 across *Salmo, Salvelinus* and *Oncorhynchus* have been previously reported (Phillips 2013) and most of
294 the results correspond with those here, with the exception of the Brook Charr sex chromosome, which the
295 two studies identify as corresponding to opposite arms of the Arctic charr sex chromosome. This is
296 possibly due to a population polymorphism, but more work would be needed to confirm this.
297



298

299 **Figure 1.** The acrocentric linkage group BC35 is highly associated with sex in Brook Charr. Due to low
300 recombination in males, high linkage is viewed across the majority of the linkage group.
301

302 Considering the importance of inversions to sex chromosome formation through the reduction of
303 recombination between X and Y (Lahn *et al.* 2001; van Doorn and Kirkpatrick 2007; Berset-Brandli *et al.*
304 2008), it is interesting to note that Brook Charr has a species-specific inversion in BC35 in the female
305 map (15.1; see Figure 5 in Sutherland *et al.* 2016). As is usual for salmonid linkage maps, the male-

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specific map was not produced as the low recombination frequency resulted in poorly placed male-specific markers (Sutherland *et al.* 2016), and so it is not possible to check whether this inversion is heterozygous within the species, but this will be valuable to investigate in future studies.

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

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

*neo-Y chromosome in Sockeye Salmon

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319 **Sex-specific recombination rate and positions of crossovers**
320 Crossovers occurred 2.7-fold more often in the maternal haplotypes (total = 3679) than in the paternal
321 (total = 1368; Figure 2) based on the phased haplotypes of 169 individual offspring (Wu *et al.* 2002;
322 Sutherland *et al.* 2016). The double recombinant correction (see Methods) in the autosomes removed 606
323 and 682 crossover events due to probable genotyping errors from the dam and sire, respectively,
324 providing a more accurate estimation of the heterochiasmy ratio, although the trends regarding the
325 crossover positions remained similar. Crossovers were biased towards the center of the linkage groups in
326 the dam and towards the external 20% of the linkage groups in the sire (Figure 2). This bias is similar to
327 that observed in Rainbow Trout (Sakamoto *et al.* 2000), and although reasons for it remain under
328 investigation, there are currently several hypotheses that may explain it including protection against
329 selfish genetic elements and meiotic drive in female meiosis (Brandvain and Coop 2012; Johnston *et al.*
330 2017), among others (see Introduction). When the distance between putative recombination events was
331 reduced to 25 cM or 10 cM for the double-recombinant correction method, the overall trend of more
332 recombination in the female remained (2.5 and 2.2-fold more recombination in the female, respectively),
333 and the spatial bias with male recombination near telomeres and female throughout the chromosome
334 remained.

335 Separating chromosomes into fused metacentric (n = 8) and acrocentric chromosomes (n = 34)
336 indicated a higher heterochiasmy ratio in fused metacentric than acrocentric chromosomes (5.6-fold and
337 2.2-fold, respectively). The male had fewer crossovers per chromosome in the fused metacentrics (mean =
338 26.5) than acrocentrics (mean = 32.9), even though fused metacentrics are comprised of two acrocentric
339 chromosomes combined and thus are longer. In contrast, the female had approximately twice as many
340 crossovers per fused metacentric chromosome (mean = 148.9) than acrocentric (mean = 70.5). The lower
341 recombination in the paternal fused metacentrics than the paternal acrocentrics is probably due to missing
342 regions of the genetic map that are residually tetraploid that were removed during marker filtering due to
343 quality filtering, as the map was produced using a diploid cross (Limborg *et al.* 2016). Inspection of
344 individual chromosomes indicates that the chromosomes expected to still exhibit residual tetraploidy
345 (Sutherland *et al.* 2016) all show a lack of crossovers in the male relative to the chromosomes expected to
346 have returned to a diploid state (Figure S1). The missing regions in the 16 (of 50) chromosome arms
347 expected to be residually tetraploid will result in an inflation of the heterochiasmy ratio, as male
348 crossovers will be specifically underestimated for these arms. This was confirmed by separating
349 chromosomes into those expected to exhibit residual tetraploidy or to be rediploidized (Sutherland *et al.*
350 2016) and recalculating the heterochiasmy ratio. For residually tetraploid chromosomes, including both
351 arms when present in a fused chromosome (total = 16 chromosomes), the female:male heterochiasmy
352 ratio is 6.4 (female = 1608; male = 251) and for expected rediploidized chromosomes (total = 26) the ratio

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353 is 1.85. The overall ratio is therefore probably inflated due to missing regions of the male map, and when
354 the residually tetraploid chromosomes are removed, the ratio remains female-biased at 1.85-fold more
355 than male. Furthermore, biased positions of crossovers remain regardless of residual tetraploid status
356 where the female is towards the center and the male towards the external sides of the chromosome (Figure
357 S1). In summary, the male has fewer recombination events than the female and the crossovers are biased
358 to the distal portions of the chromosomes.

359 The identified sex chromosome had more crossovers than the average male acrocentric
360 chromosomes (sex = 71, other acrocentrics average = 32.9), and all of the crossover events in the sex
361 chromosome occurred at one end of the chromosome and almost no crossovers occurred in the rest of the
362 chromosome (Figure S1). This bias to only a single end of each chromosome in the male map was
363 consistent throughout all of the chromosomes with crossovers present. Using the positions of centromeres
364 determined for Chinook Salmon (Brieuc *et al.* 2014), and placing them in the corresponding position on
365 the Brook Charr map using map correspondence (Sutherland *et al.* 2016), indicates that the end of the
366 acrocentric chromosomes where the crossovers occur is the opposite end to that containing the probable
367 centromere (see Figure S1). Using this information, the few occurrences of unknown centromere
368 positions can be easily determined as they are probably at the opposite side to where the crossovers occur.

369

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

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

376 Several traits showed sexual dimorphism and therefore required sex as a model covariate. These
377 included weight, length, liver weight, hepatosomatic index, hematocrit, change in osmolality and cortisol
378 from stressor, resting plasma chloride, hepatic glycogen, *insulin-like growth factor 1* and *igf receptor 1*
379 (Table S1). Specific growth rate, condition factor, change in chloride, resting plasma osmolality and
380 glucose, and *growth hormone receptor* gene expression did not show sexual dimorphism. Traits with high
381 phenotypic correlation included length and weight ($r = 0.90$ at T1), and liver weight and hepatosomatic
382 index ($r = 0.85$; Figure S2). Specific growth rate T1-T2 was negatively correlated with weight at T1 ($r = -$
383 0.64), suggesting that larger individuals measured at T1 subsequently grew slower than smaller
384 individuals. Other traits generally were not as highly correlated ($r < 0.35$). Even though the phenotypes
385 were not highly correlated, QTL were identified for condition factor and weight in the same region of

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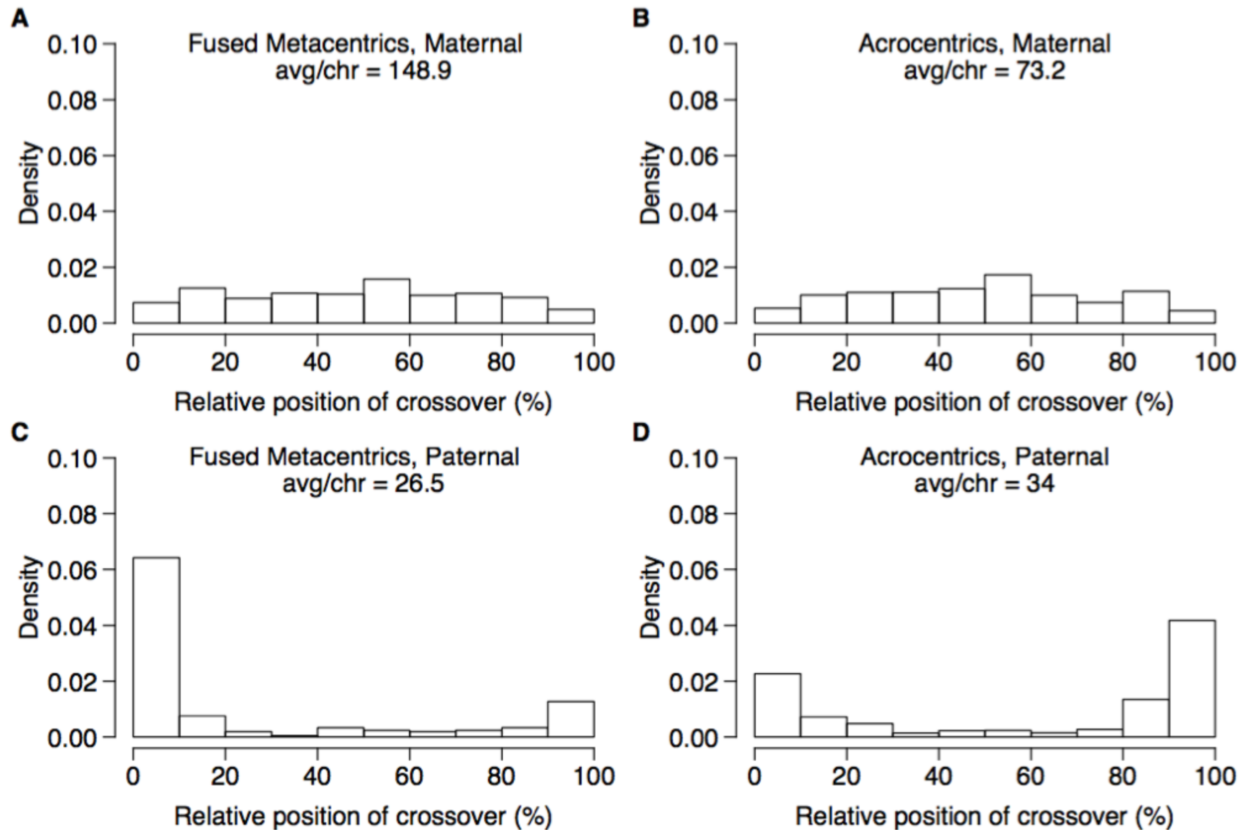
386 BC20, and QTL affecting hematocrit and weight (r = 0.24) were found in the same region on BC04
387 (Figure 3).
388



389

390 **Figure 2**. Maternal and paternal cumulative crossover positions across the chromosomes. The position of
391 each crossover is expressed as a percent of the total crossover length and cumulated for all crossovers
392 within each chromosome type, specifically fused metacentric chromosomes (A,C) and acrocentric
393 chromosomes (B,D) in the maternal and paternal haplotypes, respectively. Maternal haplotypes had 2.7-
394 fold more crossovers than paternal haplotypes, with the maternal crossovers occurring throughout the
395 chromosome and the paternal crossovers restricted mainly to the first and/or last 20% of the linkage
396 groups.
397

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

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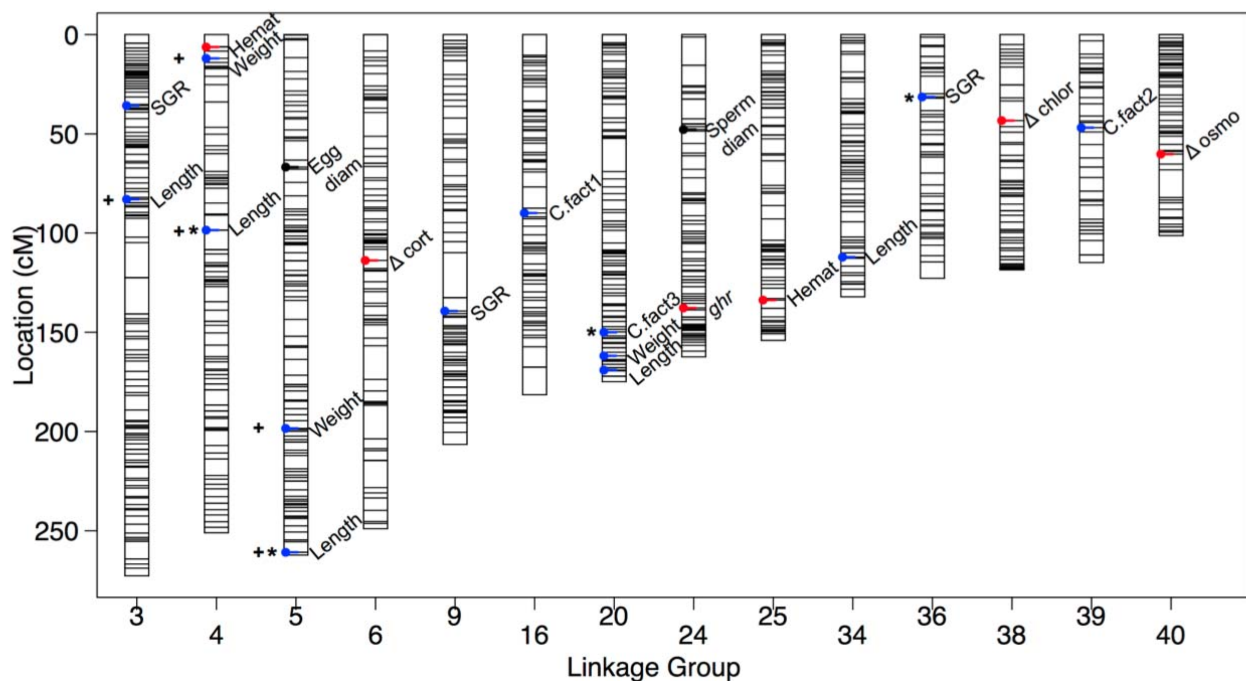
405 for these three traits, the full analysis includes these markers, but the exact locations within the LG of
406 these QTL cannot be determined without additional families or crosses (markers noted in Figure 3).

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

415



416

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

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

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

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

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

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456 **DISCUSSION**

457 Salmonids are a model system for studying the effects of whole genome duplication (WGD) on genome
458 evolution, sex determination and speciation. Specifically, the evolution of sex determination after WGD
459 and its interaction with heterochiasmy remain active areas of research. In this study we identify the sex-
460 linked chromosome and strong heterochiasmy using the high-density genetic map of Brook Charr. Female
461 recombination rates were 2.7-fold higher than those in the male, and male recombination was highly
462 biased to chromosome ends. When considering only chromosomes expected to be rediploidized this
463 female:male heterochiasmy ratio was 1.85, a lower ratio due to missing regions of the Brook Charr genetic
464 map in residually tetraploid regions. Using recently established correspondence among salmonid
465 chromosomes, we show that the Brook Charr sex chromosome is not the same chromosome arm linked to
466 sex in any other species characterized with high-density genetic maps. However, this chromosome arm
467 (ancestral 15.1) is contained in a fusion within a triple chromosome fused sex chromosome of the
468 congener Arctic Charr. In other salmonid species, some consistencies in sex chromosomes can be viewed,
469 even as distant as Lake Whitefish and members of the genus *Oncorhynchus* (discussed below). We
470 additionally evaluate linkage to 29 reproductive, stress and growth phenotypes (21 not including
471 phenotypes measured at multiple time points) and identify 29 genome- or chromosome-wide QTL on 14
472 of 42 linkage groups and compare these to known QTL in other salmonids. This work provides markers
473 for selective breeding of Brook Charr as well as insight into the role of heterochiasmy in sex
474 determination and genome evolution in a post-WGD salmonid genome.

475 **Sex determination post whole genome duplication**

476 In species with genetic sex determination, WGD generates multiple copies of sex chromosomes and this
477 may present challenges to the new lineage (Davidson *et al.* 2009) such as unbalanced gametes and
478 independent segregation of sex chromosomes (Muller 1925) or disruptions in dosage balance in species
479 with heteromorphic sex chromosomes (Orr 1990). However, polyploidization also poses other challenges
480 independent to the duplicated sex chromosome system (Mable 2004; Otto 2007). Nonetheless, ancestral
481 polyploidization resulting in paleopolyploid lineages has occurred throughout plant and animal evolution
482 (Allendorf and Thorgaard 1984; Taylor *et al.* 2003; Session *et al.* 2016), although debate still exists on the
483 effect of polyploidization on diversification (e.g. Clarke *et al.* 2016). Diversification may involve sex
484 determination systems, as paleopolyploids may develop a wide variety of sex determination systems, as
485 observed in the teleosts (Mank and Avise 2009). A wide variety of sex chromosomes are used in different
486 teleost groups including stickleback species (Ross *et al.* 2009; Kirkpatrick 2016) and Medaka (Kondo
487 2006; Myosho *et al.* 2015).. Ancestral allotetraploids can also develop new sex determination systems,
488 such as the African clawed frog *Xenopus laevis* (ZZ/ZW) having a probable translocated W-specific

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489 region (Session *et al.* 2016). However, variable sex determination systems are not unique to
490 paleopolyploid lineages. For example, *X. tropicalis* did not undergo the allotetraploid event but has a
491 unique sex determination system that involves W, Z, and Y chromosomes (Roco *et al.* 2015). The extent
492 of the involvement of polyploidization on the lability of sex determination systems remains to be
493 determined.

494 Taxa with high rates of turnover in sex chromosomes have indicated that some chromosomes are
495 more likely to become sex chromosomes. This is possibly due to favourable gene content, for example
496 when an autosome contains sexually antagonistic genes it can be repeatedly selected to become a sex
497 chromosome (Marshall Graves and Peichel 2010). A comparative analysis of teleosts indicates repeated
498 independent evolution of the same chromosomes as sex chromosomes throughout evolutionary history
499 (see Table 2 in Marshall Graves and Peichel 2010). Therefore it is not only the master sex determining
500 gene that can be repeatedly utilized by evolution, but also certain chromosomes due to the gene content,
501 which can occur over large evolutionary distance. For example, the teleost tongue sole *Cynoglossus*
502 *semilaevis* and the chicken *Gallus gallus* (both ZZ/ZW) independently evolved sex chromosomes in
503 homologous chromosomes (Chen *et al.* 2014). Similarly, species from three anuran genera that have
504 diverged for over 210 million years (*Bufo*, *Hyla* and *Rana* spp.) have sex-linked markers that map to the
505 same *X. tropicalis* chromosome with a large region homologous to the avian sex chromosome (Brelsford
506 *et al.* 2013), which the authors suggest is due to independent evolution to the same sex chromosome
507 across the different genera. The platypus *Ornithorhynchus anatinus* has five Y and five X chromosomes,
508 all of which are independent but form a chain at meiosis to co-segregate all together into sperm; this
509 system connects the two sex determination types, with the most degenerate sex chromosome as
510 homologous to the Z chromosome of birds and the least degenerate as that homologous to the X
511 chromosome of mammals (Grützner *et al.* 2004; Charlesworth and Charlesworth 2005). At least three
512 non-homologous sex chromosomes exist within *Xenopus*, and the sex determining region of *X. borealis*
513 shares orthologous genes to mammal sex determination pathways (Furman and Evans 2016). In summary,
514 repeated, independent evolution of the same sex chromosome or use of the same set of specific genes for
515 sex determination therefore has been documented across a variety of animal taxa.

516 Salmonids have genetically controlled sex determination with XX/XY systems (Thorgaard 1977;
517 Davidson *et al.* 2009). Putative translocation of the sex determining gene to different autosomes has
518 resulted in many different sex chromosomes in different lineages (Woram *et al.* 2003) and even within the
519 same species (Küttner *et al.* 2011; Eisbrenner *et al.* 2013). However, comparing across the phylogeny
520 indicates some noteworthy consistencies. The first consistency is that several species use 3.1 as the sex
521 chromosome, or have this chromosome fused with the sex chromosome, including the fused neo-Y of
522 Sockeye Salmon and the sex chromosome of Coho Salmon (Faber-Hammond *et al.* 2012), as well as the

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523 sex-linked LG in Lake Whitefish (Gagnaire *et al.* 2013), identified as 3.1 during map comparisons
524 (Sutherland *et al.* 2016). Relative to the variability seen in sex chromosomes in the salmonids, this is a
525 striking consistency considering that these species have diverged for approximately 50 million years
526 (Crête-Lafrenière *et al.* 2012). This consistency may indicate that either a) 3.1 is an ancestral sex
527 chromosome in the salmonids; or b) the different species converged on this chromosome independently as
528 it contains a gene complement that is highly beneficial to be present as a sex chromosome (Marshall
529 Graves and Peichel 2010; Chen *et al.* 2014; Furman and Evans 2016). The second consistency is that the
530 Brook Charr sex chromosome (15.1) is fused within the sex chromosome of Arctic Charr, but is not the
531 same arm that contains the sex marker in Arctic Charr (Nugent *et al.* 2016), indicating that one of these is
532 a neo-sex chromosome. Furthermore, the middle fused chromosome arm in Arctic Charr is 19.1, which is
533 the newly fused arm in the neo-Y of Sockeye Salmon (Table 1). These observations provide further
534 evidence for the fusion of specific chromosomes together that are beneficial for maintaining within the sex
535 chromosome environment. Finally, intraspecific polymorphism in sex chromosomes occurs in Arctic
536 Charr (Moghadam *et al.* 2007; Küttner *et al.* 2011), and Icelandic Arctic Charr were identified as having a
537 sex chromosome as one of the two homeologs AC01 or AC21 instead of AC04, and state that this is
538 homologous to the sex chromosome of Atlantic Salmon Ssa02 (Küttner *et al.* 2011), which is 9.1
539 (Sutherland et al 2016), again indicating the potential re-use of the same chromosome as the sex
540 chromosome.

541 The presence of both the Y chromosome of Brook Charr and the neo-Y of Sockeye Salmon as
542 putative fusions into the neo-Y chromosome of Arctic Charr is worth further investigation because neo-Y
543 chromosomes can influence phenotypic divergence and reproductive isolation, as observed in sympatric
544 Threespine Stickleback *Gasterosteus aculeatus* populations (Kitano *et al.* 2009; Kitano and Peichel 2011).
545 Consistencies across a phylogeny can provide insight into speciation. For example, the Threespine
546 Stickleback and Ninespine Stickleback *Pungitius pungitius* have two different sex chromosomes (LG19
547 and LG12, respectively), and the Blackspotted Stickleback *G. wheatlandi* has a fused Y-chromosome
548 made up of these two linkage groups (Ross *et al.* 2009), to which the authors suggest multiple independent
549 recruitment of LG12 as the sex or neo-Y chromosome. Furthermore, other sticklebacks have different sex
550 chromosomes (Ross *et al.* 2009). The salmonid diversity and consistencies identified here provide another
551 group for analyzing sex chromosome differences in relation to gene content and speciation, and in
552 salmonids also occurs with the salmonid-specific WGD. As more salmonid genomes are characterized, it
553 will become clearer whether certain sex chromosomes are ancestral or have independently evolved, and
554 whether there is a favourable gene content within often-viewed sex chromosomes.

555 In the context of sex chromosome fusions and residual tetraploidy, several additional observations
556 on the nature of salmonid sex chromosomes can be made from the present analysis (four genera; eight

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557 species; Table 1). The first observation is that sex chromosomes with fusions occur only in species
558 specific fusions rather than conserved fusions in the present data (Sutherland *et al.* 2016). Arctic Charr *S.*
559 *alpinus* has a sex chromosome that in some individuals involved three fused chromosomes (Nugent *et al.*
560 2016), and all available evidence suggests this is a species-specific fusion given that these fusions are not
561 present in the more basally diverging Atlantic Salmon nor the congener Brook Charr (Sutherland *et al.*
562 2016). Y fusions are the most common of sex chromosome fusions (Pennell *et al.* 2015) and can permit
563 other sexually antagonistic genes to be linked to the non-recombining regions (Charlesworth and
564 Charlesworth 1980; Charlesworth *et al.* 2005). Y fusions may also occur due to drift with only slightly
565 deleterious effects (Kirkpatrick 2016), as males have increased fusion prevalence in general and increased
566 repeat content (and thus fusion potential) in degenerating Y (Pennell *et al.* 2015). However, since the same
567 chromosomes that are involved in Y fusions in some species are the sex chromosomes in others (e.g. 15.1
568 or 19.1, discussed above), it suggests that these fusions could have an adaptive advantage, such as the
569 movement of an autosome with alleles under sexually antagonistic selection into the Y chromosome
570 environment, as discussed by Charlesworth and Charlesworth (1980) and Kirkpatrick (2016). When
571 recombination is low in males (i.e. heterochiasmy), this Y fusion holds an additional chromosome in a
572 constantly lower recombination environment, as it will always be present in males. The use of the same
573 chromosomes as sex chromosomes and as fusion partners within the salmonids merits further study. The
574 second observation is that chromosomes with regions of residual tetraploidy can become sex
575 chromosomes; two of the seven identified sex chromosomes (Chinook Ots17 (23.2) and Atlantic Salmon
576 Ssa02q (9.1)) are chromosomes known to exhibit residual tetraploidy (Brieuc *et al.* 2014; Allendorf *et al.*
577 2015; Lien *et al.* 2016; Sutherland *et al.* 2016), therefore suggesting that exhibiting residual tetraploidy
578 does not prevent a chromosome from becoming a sex chromosome.

579 Translocation of a sex determining gene to an autosome and the adoption of the autosome as a
580 new sex chromosome may be possible if the gene moves into linkage with a locus that is under sexually
581 antagonistic selection (van Doorn and Kirkpatrick 2007). The probability of this adoption is increased
582 with the occurrence of an inversion in the region by increased linkage through reduced recombination (van
583 Doorn and Kirkpatrick 2007). The retention still will require that the benefit of the new chromosome is
584 greater than that existing on the original sex chromosome. Interestingly, in the unique sex chromosome of
585 Brook Charr (15.1), there is a large inversion in relation to the other salmonids (Sutherland *et al.* 2016).
586 However, this is an interspecific inversion and has not yet been determined whether it is also heterozygous
587 within the species due to low recombination and resultant challenges of generating male maps. To further
588 characterize this, genome sequence for both the X and Y chromosomes of Brook Charr will be valuable.

589 The salmonids, being at an early stage of sex chromosome evolution (Phillips and Ihssen 1985)
590 provide a good system to study sex chromosome evolution (van Doorn and Kirkpatrick 2007). As we

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591 observed here, reduced recombination occurs consistently in male salmonids, being restricted to the
592 telomeric region opposite the centromere, resulting in a lack of recombination between X and Y in the
593 middle of the chromosome. This may facilitate sex chromosome formation, with tight linkage developing
594 across the entire Y chromosome (Haldane 1922; Nei 1969; Lenormand 2003). Heterochiasmy is not only
595 restricted to the sex chromosome, but rather occurs throughout the genome, as has been viewed in several
596 systems with developing sex chromosomes, such as the European tree frog *Hyla arborea* (Berset-Brandli
597 *et al.* 2008), Medaka (see Kondo *et al.* 2001; Kondo 2006), zebrafish *Danio rerio* (Singer *et al.* 2002), and
598 the salmonids of genera *Oncorhynchus* (Sakamoto *et al.* 2000), *Salmo* (Moen *et al.* 2004) and *Salvelinus*
599 (present study). However, heterochiasmy also occurs in systems with fully developed sex chromosomes,
600 such as humans, where females have ~1.6-fold higher rates than males, which recombine predominantly at
601 telomeric regions (Broman *et al.* 1998).

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

613 In the salmonids, sex chromosome turnover by *sdY* translocation may restart the process of Y
614 degeneration (Yano *et al.* 2012b). In species with heterochiasmy rather than achiasmy, occasional
615 crossover between X and Y would also reduce sex chromosome heteromorphism and Y degeneration. This
616 may be the reason for sex chromosomes remaining homomorphic in green toad species (*Bufo viridis*) all
617 which have the same sex chromosomes (Stöck *et al.* 2013), and in several members of the *Hyla* genus of
618 European tree frogs, which also all share the same sex chromosomes (Stöck *et al.* 2011). Regeneration of
619 Y chromosomes by occasional crossover is termed the ‘fountain-of-youth’ hypothesis, and is particularly
620 likely for species with the possibility of sex reversal, as recombination rate is based on phenotypic sex
621 rather than genetic sex (discussed in Perrin 2009). Some salmonid sex chromosomes are heteromorphic
622 (Davidson *et al.* 2009) and accumulate repeats (Devlin *et al.* 1998), this may suggest in some species this
623 regeneration is not occurring. Lack of recombination will be accentuated by inversion accumulation and
624 other differentiation between sex chromosomes reducing meiotic pairing and crossovers. Sex reversal is

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625 possible in salmonids (Johnstone *et al.* 1978) and has been observed in the wild, for example in Chinook
626 Salmon (Nagler *et al.* 2001), but the greater extent of this occurring in nature in other salmonids is yet to
627 be determined. Relative effects of sex chromosome turnovers, occasional X/Y crossovers, and large sex
628 chromosomal polymorphisms merits further investigation for which the salmonids are a good model
629 system. The extent of Y or neo-Y degeneration, gene migration, or other aspects of sex chromosome
630 evolution have not yet been explored comparatively in the salmonids. As these aspects may differ among
631 species depending on the length of time the chromosome has been used as the Y chromosome, further
632 investigation into interspecific differences (e.g. 3.1 sex chromosome in both Lake Whitefish and members
633 of *Oncorhynchus*), or intraspecific differences between populations having different sex chromosomes
634 (Eisbrenner *et al.* 2013), will be valuable to determine the history of the sex chromosome evolution in the
635 salmonids.

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

637 Knowledge on the genetic architecture of important traits in the salmonids is improving, for example for
638 aquaculture-related traits such as disease resistance (Yáñez *et al.* 2014) and stress tolerance (Rexroad *et al.*
639 2012), and ecologically-relevant traits such as age-at-sea (Barson *et al.* 2015) and body shape evolution
640 (Laporte *et al.* 2015). In the present study we improve the understanding of genetic architecture of growth,
641 reproductive and stress-response traits by identifying QTL on 14 of the 42 LGs in the Brook Charr linkage
642 map (four fused metacentric and 10 acrocentric chromosomes). This improves the previous analysis of
643 these traits on a low-density map (Sauvage *et al*. 2012a, 2012b) and brings the QTL for these phenotypes
644 into the context of the more characterized high-density Brook Charr map with information on
645 correspondence of arms with other salmonids, probable residual tetraploidy and centromere positions,
646 ancestral chromosomes, and identified sex chromosome. Furthermore, the present analysis was conducted
647 on the female map, which has improved positioning of markers relative to the sex-averaged map or the
648 male map (Sutherland *et al.* 2016), probably as a result of low recombination in the male (as viewed here).
649 Finally, in the present work, phenotypes were investigated for sexual dimorphism and QTL analysis used
650 sex as a covariate when necessary, improving resolution of QTL effects.

651 Several traits measured in the present study have shown significant heritability in the specific
652 Brook Charr strains used in this study. Stress response as measured by cortisol and glucose responses to
653 transport stress showed mean heritability of 0.60 and 0.61 (± 0.2 SE), respectively (Crespel *et al.* 2011).
654 Often heritability depended on strain, for example heritability of body mass showed mean (± standard
655 error) for domestic, Laval and Rupert strains of 0.61 (± 0.07), 0.37 (± 0.06), and 0.30 (± 0.08),
656 respectively (Crespel *et al.* 2013a). Further, condition factor showed mean heritability (± SE) of 0.09 (±
657 0.1), 0.032 (± 0.017) and 0.5 (± 0.31) for domestic, Laval and Rupert strains, respectively (Crespel *et al.*
658 2013b). The different estimates for heritability observed in different strains further indicates the

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659 importance of evaluating effects of QTL identified here in multiple strains to identify broader implications
660 of the QTL. Although correlated phenotypes clustered on the map as expected (e.g. length, weight), no
661 clustering was observed for blood and stress-related parameters (i.e. hematocrit, change in cortisol,
662 chloride and osmolality), with each trait having a QTL on a different chromosome. Pleiotropy can occur
663 with both positive and negative genetic correlations between traits with common underlying biology
664 (Mackay *et al.* 2009). This is important to consider in marker-assisted selection, to identify QTL useful for
665 simultaneous selective breeding of multiple traits and to avoid negative correlations between desirable
666 traits (Lv *et al.* 2016). Mapping multiple correlated traits simultaneously can help define regions (Jiang
667 and Zeng 1995). However, it can be difficult to determine whether two traits are truly pleiotropic or
668 whether causal variants for each trait are in in tight linkage, especially when a QTL region is wide
669 (Mackay *et al.* 2009) or when paternal inheritance occurs over long fragments due to low recombination
670 rate (as viewed here).

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

678 Weight and growth are expected to be highly polygenic traits, therefore requiring many
679 individuals to have sufficient power to identify loci of minor effect (Rockman 2012; Ashton *et al.* 2016).
680 For example, sample sizes of at least 500 individuals may be required to identify QTL accounting for less
681 than 5% of the total phenotypic variance (Mackay 2001). This means that often only large effect QTL are
682 identified, leading to the misconception that these are the norm and to an inflation of the actual percent
683 variance explained by the QTL (Beavis 1997; Xu 2003). A negative relationship between sample size and
684 overestimation of effect sizes occurs in QTL studies of outbred populations (Slate 2013). High powered
685 studies can identify more QTL, such as a recent study in Atlantic Salmon with 1695 offspring and 20 sires,
686 which identified four chromosomes harboring major effect growth QTL (Tsai *et al.* 2015). Similarly, a
687 study in Common Carp *Cyprinus carpio* with 522 offspring and eight families identified 10 genome-wide
688 and 28 chromosome-wide significant QTL for three growth traits, with 30/50 chromosomes containing
689 suggestive QTL (Lv *et al.* 2016). QTL can be detected with fewer individuals, although this may result in
690 overestimation of effect sizes for the QTL (Slate 2013). QTL for polygenic traits growth rate, behavior
691 and morphology were identified in Lake Whitefish with 102 individuals in the mapping family (Gagnaire
692 *et al.* 2013; Laporte *et al.* 2015), and in the present study we identified QTL for many of the traits with

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693 169 or fewer individuals. Since the effect of a QTL can differ in different genetic backgrounds due to
694 epistasis (Mackay 2001), it is therefore important to evaluate the effect of markers in different crosses
695 with different genetic backgrounds to better understand the broader use of the marker (Lv *et al.* 2016),
696 which also gives more confidence on true positives and estimated effect sizes of the QTL (Slate 2013).
697 The precision of mapping QTL within a family depends on recombination rate (Mackay 2001;
698 Mackay *et al.* 2009). Therefore the low number of crossovers in male salmonids will reduce the overall
699 precision of trait mapping. This effect of heterochiasmy has been used by recent salmonid studies to use a
700 two-stage approach by initially using a sire-based analysis with few markers per chromosome to identify
701 chromosomes of interest followed by a dam-based analysis to more finely resolve the QTL positions (Tsai
702 *et al.* 2015). Heterochiasmy is therefore important to consider when designing QTL experiments for
703 species exhibiting this trait. In the present study, several QTL with very broad regions of elevated LOD
704 were identified (e.g. for length on BC03, BC04, and BC05), which may be due to low recombination and
705 paternally associated haplotypes (see Results). In contrast, many of the other identified QTL in this study
706 have small confidence intervals and high percent variance explained, and therefore will be useful for
707 selective breeding (Table 2; Figure 3).
708 Although QTL mapping connects nucleotide sequence with trait variation, it generally ignores
709 intermediate phenotypes that can be very useful in determining underlying drivers of traits, and the use of
710 the expression levels of gene transcripts as traits to identify eQTL can provide information on the
711 intermediate steps to generate a phenotype (Mackay *et al.* 2009). Traits queried in eQTL experiments have
712 the additional information on gene location in the genome, providing information on cis or trans-eQTL
713 (Mackay *et al.* 2009). This will be an important next step in determining the underlying causes of the
714 genotype-phenotype interaction in Brook Charr.
715
716 **CONCLUSIONS**

717 The relationships between sex chromosomes, heterochiasmy and polyploidization have important
718 influences on genome architecture for key biological traits, but much remains unknown about these
719 interactions. Here we identify the sex-linked chromosome in Brook Charr and compare sex chromosome
720 identities across the salmonids to investigate consistencies. Although many different chromosomes are
721 used as sex chromosomes in salmonids, some consistencies can be identified, even in lineages that have
722 diverged for ~50 million years, *Coregonus* and *Oncorhynchus*. Sex chromosomes that are contained
723 within fused chromosomes thus far are only observed in species-specific fusions and not in conserved
724 fusions. Heterochiasmy, or differences in recombination rate between sexes, may play an important role in
725 the evolution of sex chromosomes. Heterochiasmy is viewed here in the *Salvelinus* genus, and in other
726 salmonid genera *Oncorhynchus* and *Salmo*, with male recombination less frequent than female, and with

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727 male crossovers restricted to telomeric regions. Inversions are also important for sex chromosome
728 evolution, and the Brook Charr sex chromosome from the female map exhibits a large interspecific
729 inversion, although the intraspecific polymorphism of this inversion has not yet been determined.
730 Additional analysis of salmonid genomes is needed to understand the effect of the mobile sex determining
731 gene on phenomena such as Y degeneration. To improve the characterization of important traits and
732 potential for selective breeding, we additionally identify 29 QTL across the genome for growth,
733 reproduction, and stress-response traits, several of which having high PVE and well-refined intervals.
734 Hotspots for multiple traits were not common, but we identify that an earlier identified hotspot in
735 *Oncorhynchus* also contains a length QTL in Brook Charr, further indicating the importance of this
736 chromosome region and the value of identifying orthologous QTL with comparative genomics.
737

738 ## ACKNOWLEDGEMENTS

739 This work was funded by a Fonds de Recherche du Québec Nature et Technologies (FRQNT) research
740 grant awarded to Céline Audet, Louis Bernatchez and Nadia Aubin-Horth, a grant from the Société de
741 Recherche et de Développement en Aquaculture Continentale (SORDAC) awarded to Louis Bernatchez
742 and Céline Audet, and a grant from the Spanish Ministry of Education (Grant PR2010-0601) awarded to
743 Ciro Rico. Thanks to G. Côté for laboratory assistance, M. Laporte for discussion on QTL and for
744 comments on the manuscript, M. Lamothe and T. Gosselin for discussion on double recombinants and
745 genotyping errors in RADseq data and to T. Gosselin for exporting the required files from STACKS for
746 QTL analysis. Thanks to C. Nugent for discussion on the sex chromosome of Arctic Charr. Thanks to S.
747 Johnston, an anonymous reviewer, and Editor Ross Houston for providing comments and suggestions that
748 improved the manuscript. During this work, BJGS was supported by an NSERC postdoctoral fellowship,
749 and then an FRQS postdoctoral fellowship.
750

751 ## REFERENCES

752 Allendorf F. W., Thorgaard G. H., 1984 Tetraploidy and the evolution of salmonid fishes. In:Turner BJ
753 (Ed.), *Evolutionary genetics of fishes*, Plenum Publishing Corporation, New York, pp. 1–53.
754 Allendorf F. W., Bassham S., Cresko W. A., Limborg M. T., Seeb L. W., Seeb J. E., 2015 Effects of
755 crossovers between homeologs on inheritance and population genomics in polyploid-derived
756 salmonid fishes. J. Hered. **106**: 217–227.
757 Andrews K. R., Good J. M., Miller M. R., Luikart G., Hohenlohe P. A., 2016 Harnessing the power of
758 RADseq for ecological and evolutionary genomics. Nat Rev Genet **17**: 81–92.
759 Artieri C. G., Mitchell L. A., Ng S. H. S., Parisotto S. E., Danzmann R. G., Hoyheim B., Phillips R. B.,
760 Morasch M., Koop B. F., Davidson W. S., 2006 Identification of the sex-determining locus of
761 Atlantic Salmon (*Salmo salar*) on chromosome 2. Cytogenet Genome Res **112**: 152–159.

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762 Ashton D. T., Ritchie P. A., Wellenreuther M., 2016 15 years of QTL studies in fish: challenges and
763 future directions. Mol. Ecol.
764 Bachtrog D., 2006 A dynamic view of sex chromosome evolution. Current Opinion in Genetics &
765 Development **16**: 578–585.
766 Barson N. J., Aykanat T., Hindar K., Baranski M., 2015 Sex-dependent dominance at a single locus
767 maintains variation in age at maturity in salmon. Nature **528**: 405–408.
768 Beavis W. D., 1997 QTL Analyses: Power, Precision, and Accuracy. In:Paterson AH (Ed.), *Molecular*
769 *Dissection of Complex Traits*, Boca Raton, pp. 145–159.
770 Berset-Brandli L., Jaquiery J., Broquet T., Ulrich Y., Perrin N., 2008 Extreme heterochiasmy and nascent
771 sex chromosomes in European tree frogs. Proceedings of the Royal Society B: Biological Sciences
772 **275**: 1577–1585.
773 Brandvain Y., Coop G., 2012 Scrambling eggs: Meiotic drive and the evolution of female recombination
774 rates. Genetics **190**: 709–723.
775 Brelsford A., Stöck M., Betto-Colliard C., Dubey S., Dufresnes C., Jourdan-Pineau H., Rodrigues N.,
776 Savary R., Sermier R., Perrin N., 2013 Homologous sex chromosomes in three deeply divergent
777 anuran species. Evolution **67**: 2434–2440.
778 Brieuc M. S. O., Brieuc M. S. O., Waters C. D., Waters C. D., Seeb J. E., Seeb J. E., Naish K. A., 2014 A
779 dense linkage map for Chinook Salmon (*Oncorhynchus tshawytscha*) reveals variable chromosomal
780 divergence after an ancestral whole genome duplication event. G3 - Genes|Genomes|Genetics **4**: 447–
781 460.
782 Broman K. W., Sen S., 2009 *A Guide to QTL Mapping with R/qtl*. Springer, New York.
783 Broman K. W., Murray J. C., Sheffield V. C., White R. L., Weber J. L., 1998 Comprehensive human
784 genetic maps: Individual and sex-specific variation in recombination. The American Journal of
785 Human Genetics **63**: 861–869.
786 Broman K. W., Wu H., Sen S., Churchill G. A., 2003 R/qtl: QTL mapping in experimental crosses.
787 Bioinformatics **19**: 889–890.
788 Brunelli J. P., Wertzler K. J., Sundin K., Thorgaard G. H., 2008 Y-specific sequences and polymorphisms
789 in Rainbow Trout and Chinook Salmon. Genome **51**: 739–748.
790 Bush W. S., Moore J. H., 2012 Chapter 11: Genome-Wide Association Studies. PLoS Comput Biol **8**:
791 e1002822.
792 Carvalho A., 2002 Origin and evolution of the *Drosophila* Y chromosome. Current Opinion in Genetics &
793 Development **12**: 664–668.
794 Catchen J. M., Amores A., Hohenlohe P., Cresko W., Postlethwait J. H., 2011 Stacks: building and
795 genotyping loci *de novo* from short-read sequences. G3 - Genes|Genomes|Genetics **1**: 171–182.
796 Charlesworth B., 1991 The evolution of sex chromosomes. Science **251**: 1030–1033.
797 Charlesworth D., Charlesworth B., 1980 Sex differences in fitness and selection for centric fusions
798 between sex-chromosomes and autosomes. Genet. Res. **35**: 205–214.
799 Charlesworth D., Charlesworth B., 2005 Sex chromosomes: Evolution of the weird and wonderful.
800 Current Biology **15**: R129–R131.
801 Charlesworth D., Charlesworth B., Marais G., 2005 Steps in the evolution of heteromorphic sex
802 chromosomes. Heredity **95**: 118–128.
803 Chen S., Zhang G., Shao C., Huang Q., Liu G., Zhang P., Song W., An N., Chalopin D., Volff J.-N., Hong
804 Y., Li Q., Sha Z., Zhou H., Xie M., Yu Q., Liu Y., Xiang H., Wang N., Wu K., Yang C., Zhou Q.,
805 Liao X., Yang L., Hu Q., Zhang J., Meng L., Jin L., Tian Y., Lian J., Yang J., Miao G., Liu S., Liang
806 Z., Yan F., Li Y., Sun B., Zhang H., Zhang J., Zhu Y., Du M., Zhao Y., Schartl M., Tang Q., Wang J.,
807 2014 Whole-genome sequence of a flatfish provides insights into ZW sex chromosome evolution and
808 adaptation to a benthic lifestyle. Nat Genet **46**: 253–260.
809 Clarke J. T., Lloyd G. T., Friedman M., 2016 Little evidence for enhanced phenotypic evolution in early
810 teleosts relative to their living fossil sister group. Proc. Natl. Acad. Sci. U.S.A.
811 Crespel A., Bernatchez L., Audet C., 2013a Strain Specific Genotype– Environment Interactions and
812 Evolutionary Potential for Body Mass in Brook Charr (Salvelinus fontinalis). G3: Genes| Genomes|

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813 ….
814 Crespel A., Bernatchez L., Garant D., Audet C., 2011 Quantitative genetic analysis of the physiological
815 stress response in three strains of brook charr Salvelinus fontinalis and their hybrids. Journal of Fish
816 Biology **79**: 2019–2033.
817 Crespel A., Bernatchez L., Garant D., Audet C., 2013b Genetically based population divergence in
818 overwintering energy mobilization in brook charr (Salvelinus fontinalis). Genetica **141**: 51–64.
819 Crête-Lafrenière A., Weir L. K., Bernatchez L., 2012 Framing the Salmonidae Family phylogenetic
820 portrait: a more complete picture from increased taxon sampling. PLoS ONE **7**: e46662.
821 Davidson W. S., Huang T.-K., Fujiki K., Schalburg von K. R., Koop B. F., 2009 The sex determining loci
822 and sex chromosomes in the Family Salmonidae. Sexual Development **3**: 78–87.
823 Davidson W. S., Koop B. F., Jones S. J. M., Iturra P., Vidal R., Maass A., Jonassen I., Lien S., Omholt S.
824 W., 2010 Sequencing the genome of the Atlantic Salmon (*Salmo salar*). Genome Biol. **11**: 403.
825 Devlin R. H., Stone G. W., Smailus D. E., 1998 Extensive direct-tandem organization of a long repeat
826 DNA sequence on the Y chromosome of Chinook Salmon (*Oncorhynchus tshawytscha*). J Mol Evol
827 **46**: 277–287.
828 Eisbrenner W. D., Botwright N., Cook M., Davidson E. A., Dominik S., Elliott N. G., Henshall J., Jones S.
829 L., Kube P. D., Lubieniecki K. P., Peng S., Davidson W. S., 2013 Evidence for multiple sex-
830 determining loci in Tasmanian Atlantic Salmon (*Salmo salar*). **113**: 86–92.
831 Faber-Hammond J. J., Phillips R. B., Brown K. H., 2015 Comparative analysis of the shared sex-
832 determination region (SDR) among salmonid fishes. Genome Biology and Evolution **7**: 1972–1987.
833 Faber-Hammond J., Phillips R. B., Park L. K., 2012 The Sockeye Salmon neo-Y chromosome is a fusion
834 between linkage groups orthologous to the Coho Y chromosome and the long arm of Rainbow Trout
835 chromosome 2. Cytogenet Genome Res **136**: 69–74.
836 Furman B., Evans B. J., 2016 Sequential turnovers of sex chromosomes in African clawed frogs
837 (*Xenopus*) suggest some genomic regions are good at sex determination. G3 -
838 Genes|Genomes|Genetics.
839 Gagnaire P.-A., Normandeau E., Pavey S. A., Bernatchez L., 2013 Mapping phenotypic, expression and
840 transmission ratio distortion QTL using RAD markers in the Lake Whitefish (*Coregonus*
841 *clupeaformis*). Mol. Ecol. **22**: 3036–3048.
842 Grützner F., Rens W., Tsend-Ayush E., El-Mogharbel N., O'Brien P. C. M., Jones R. C., Ferguson-Smith
843 M. A., Marshall Graves J. A., 2004 In the platypus a meiotic chain of ten sex chromosomes shares
844 genes with the bird Z and mammal X chromosomes. Nature **432**: 913–917.
845 Hackett C. A., Broadfoot L. B., 2003 Effects of genotyping errors, missing values and segregation
846 distortion in molecular marker data on the construction of linkage maps. **90**: 33–38.
847 Haldane J., 1922 Sex ratio and unisexual sterility in hybrid animals. Journal of Genetics **12**: 101–109.
848 Hecht B. C., Thrower F. P., Hale M. C., Miller M. R., Nichols K. M., 2012 Genetic architecture of
849 migration-related traits in rainbow and steelhead trout, *Oncorhynchus mykiss*. G3 -
850 Genes|Genomes|Genetics **2**: 1113–1127.
851 Henning F., Lee H. J., Franchini P., Meyer A., 2014 Genetic mapping of horizontal stripes in Lake
852 Victoria cichlid fishes: benefits and pitfalls of using RAD markers for dense linkage mapping. Mol.
853 Ecol. **23**: 5224–5240.
854 Jiang C., Zeng Z. B., 1995 Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics
855 **140**: 1111–1127.
856 Johnston S. E., Berenos C., Slate J., Pemberton J. M., 2016 Conserved genetic architecture underlying
857 individual recombination rate variation in a wild population of Soay sheep (*Ovis aries*). Genetics **203**:
858 583–598.
859 Johnston S. E., Huisman J., Ellis P. A., Pemberton J. M., 2017 Sexual dimorphism in recombination
860 landscape in red deer (*Cervus elaphus*): a role for meiotic drive? bioRxiv.
861 Johnstone R., Simpson T. H., Youngson A. F., 1978 Sex reversal in salmonid culture. Aquaculture **13**:
862 115–134.
863 Kirkpatrick M., 2016 The evolution of genome structure by natural and sexual selection. J. Hered **108**: 3–

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864 11.
865 Kitano J., Peichel C. L., 2011 Turnover of sex chromosomes and speciation in fishes. Environ Biol Fish
866 **94**: 549–558.
867 Kitano J., Ross J. A., Mori S., Kume M., Jones F. C., Chan Y. F., Absher D. M., Grimwood J., Schmutz J.,
868 Myers R. M., Kingsley D. M., Peichel C. L., 2009 A role for a neo-sex chromosome in stickleback
869 speciation. Nature **461**: 1079–1083.
870 Kodama M., Brieuc M. S. O., Devlin R. H., Hard J. J., Naish K. A., 2014 Comparative mapping between
871 Coho Salmon (*Oncorhynchus kisutch*) and three other salmonids suggests a role for chromosomal
872 rearrangements in the retention of duplicated regions following a whole genome duplication event.
873 G3 - Genes|Genomes|Genetics **4**: 1717–1730.
874 Kondo M., 2006 Genomic organization of the sex-determining and adjacent regions of the sex
875 chromosomes of medaka. Genome Research **16**: 815–826.
876 Kondo M., Nagao E., Mitani H., Shima A., 2001 Differences in recombination frequencies during female
877 and male meioses of the sex chromosomes of the medaka, *Oryzias latipes*. Genet Res **78**.
878 Küttner E., Nilsson J., Skúlason S., Gunnarsson S., Ferguson M. M., Danzmann R. G., Civetta A., 2011
879 Sex chromosome polymorphisms in Arctic Charr and their evolutionary origins. Genome **54**: 852–
880 861.
881 Lahn B. T., Pearson N. M., Jegalian K., 2001 The human Y chromosome, in the light of evolution. Nat
882 Rev Genet **2**: 207–216.
883 Laporte M., Rogers S. M., Dion-Côté A.-M., Normandeau E., Gagnaire P.-A., Dalziel A. C., Chebib J.,
884 Bernatchez L., 2015 RAD-QTL mapping reveals both genome-level parallelism and different genetic
885 architecture underlying the evolution of body shape in Lake Whitefish (*Coregonus clupeaformis*)
886 species pairs. G3 - Genes|Genomes|Genetics **5**: 1481–1491.
887 Larson W. A., McKinney G. J., Limborg M. T., Everett M. V., Seeb L. W., Seeb J. E., 2015 Identification
888 of multiple QTL hotspots in Sockeye Salmon (*Oncorhynchus nerka*) using Genotyping-by-
889 Sequencing and a dense linkage map. J. Hered. **107**: 122–133.
890 Larson W. A., McKinney G. J., Seeb J. E., Seeb L. W., 2016 Identification and characterization of sex-
891 associated loci in Sockeye Salmon using genotyping-by-sequencing and comparison with a sex-
892 determining assay based on the *sdY* gene. J. Hered. **107**: 559–566.
893 Lenormand T., 2003 The evolution of sex dimorphism in recombination. Genetics **163**: 811–822.
894 Lenormand T., Dutheil J., 2005 Recombination difference between sexes: a role for haploid selection. Plos
895 Biol **3**: e63.
896 Lenormand T., Engelstädter J., Johnston S. E., Wijnker E., Haag C. R., 2016 Evolutionary mysteries in
897 meiosis. Phil. Trans. R. Soc. B **371**: 20160001.
898 Lien S., Gidskehaug L., Moen T., Ben J Hayes, Berg P. R., Davidson W. S., Omholt S. W., Kent M. P.,
899 2011 A dense SNP-based linkage map for Atlantic Salmon (*Salmo salar*) reveals extended
900 chromosome homeologies and striking differences in sex-specific recombination patterns. BMC
901 Genomics **12**: 615.
902 Lien S., Koop B. F., Sandve S. R., Miller J. R., Kent M. P., Nome T., Hvidsten T. R., Leong J. S.,
903 Minkley D. R., Zimin A., Grammes F., Grove H., Gjuvsland A., Walenz B., Hermansen R. A.,
904 Schalburg von K., Rondeau E. B., Di Genova A., Samy J. K. A., Olav Vik J., Vigeland M. D., Caler
905 L., Grimholt U., Jentoft S., Våge D. I., de Jong P., Moen T., Baranski M., Palti Y., Smith D. R.,
906 Yorke J. A., Nederbragt A. J., Tooming-Klunderud A., Jakobsen K. S., Jiang X., Fan D., Hu Y.,
907 Liberles D. A., Vidal R., Iturra P., Jones S. J. M., Jonassen I., Maass A., Omholt S. W., Davidson W.
908 S., 2016 The Atlantic Salmon genome provides insights into rediploidization. Nature **533**: 200–205.
909 Limborg M. T., Seeb L. W., Seeb J. E., 2016 Sorting duplicated loci disentangles complexities of
910 polyploid genomes masked by genotyping by sequencing. Mol. Ecol. **25**: 2117–2129.
911 Lubieniecki K. P., Lin S., Cabana E. I., Li J., Lai Y. Y. Y., Davidson W. S., 2015 Genomic instability of
912 the sex-determining locus in Atlantic Salmon (*Salmo salar*). G3 - Genes|Genomes|Genetics **5**: 2513–
913 2522.
914 Lv W., Zheng X., Kuang Y., Cao D., Yan Y., Sun X., 2016 QTL variations for growth-related traits in

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915 eight distinct families of Common Carp (*Cyprinus carpio*). BMC Genet **17**: 65.
916 Mable B. K., 2004 'Why polyploidy is rarer in animals than in plants': myths and mechanisms. Biological
917 Journal of the Linnean Society **82**: 453–466.
918 Mackay T. F., 2001 The genetic architecture of quantitative traits. Annu. Rev. Genet. **35**: 303–339.
919 Mackay T. F. C., Stone E. A., Ayroles J. F., 2009 The genetics of quantitative traits: challenges and
920 prospects. Nat Rev Genet **10**: 565–577.
921 Mank J. E., Avise J. C., 2009 Evolutionary diversity and turn-over of sex determination in teleost fishes.
922 Sex Dev **3**: 60–67.
923 Marshall Graves J. A., Peichel C. L., 2010 Are homologies in vertebrate sex determination due to shared
924 ancestry or to limited options? Genome Biol. **11**: 205.
925 May B., Delany M. E., 2015 Meiotic models to explain classical linkage, pseudolinkage, and
926 chromosomal pairing in tetraploid derivative salmonid genomes: II. Wright is still right. J. Hered.
927 **106**: 762–766.
928 McKinney G. J., Seeb L. W., Larson W. A., Gomez Uchida D., Limborg M. T., Brieuc M. S. O., Everett
929 M. V., Naish K. A., Waples R. K., Seeb J. E., 2016 An integrated linkage map reveals candidate
930 genes underlying adaptive variation in Chinook Salmon (*Oncorhynchus tshawytscha*). Mol. Ecol.
931 Resour. **16**: 769–783.
932 Moen T., Hoyheim B., Munck H., Gomez-Raya L., 2004 A linkage map of Atlantic Salmon (*Salmo salar*)
933 reveals an uncommonly large difference in recombination rate between the sexes. Anim. Genet. **35**:
934 81–92.
935 Moghadam H. K., Ferguson M. M., Danzmann R. G., 2007 Linkage variation at the sex-determining locus
936 within Fraser strain Arctic charr *Salvelinus alpinus*. Journal of Fish Biology **71**: 294–301.
937 Muller H. J., 1925 Why polyploidy is rarer in animals than in plants. The American Naturalist **59**: 346–
938 353.
939 Myosho T., Takehana Y., Hamaguchi S., 2015 Turnover of sex chromosomes in Celebensis group
940 Medaka fishes. G3 - Genes|Genomes|Genetics **5**: 2685–2691.
941 Nagler J. J., Bouma J., Thorgaard G. H., Dauble D. D., 2001 High incidence of a male-specific genetic
942 marker in phenotypic female Chinook Salmon from the Columbia River. Environ. Health Perspect.
943 **109**: 67–69.
944 Naish K. A., Phillips R. B., Brieuc M. S. O., Newton L. R., Elz A. E., Park L. K., 2013 Comparative
945 genome mapping between Chinook Salmon (*Oncorhynchus tshawytscha*) and Rainbow Trout (*O.*
946 *mykiss*) based on homologous microsatellite loci. G3 - Genes|Genomes|Genetics **3**: 2281–2288.
947 Nei M., 1969 Linkage modification and sex difference in recombination. Genetics **63**: 681–699.
948 Nugent C. M., Easton A. A., Norman J. D., Ferguson M. M., Danzmann R. G., 2016 A SNP based linkage
949 map of the Arctic Charr (*Salvelinus alpinus*) genome provides insights into the diploidization process
950 after whole genome duplication. G3 - Genes|Genomes|Genetics.
951 Ohno S., 1970 *Evolution by Gene Duplication*. Springer Berlin Heidelberg, Berlin, Heidelberg.
952 Orr H. A., 1990 “Why polyploidy is rarer in animals than in plants” revisited. The American Naturalist
953 **136**: 759–770.
954 Otto S. P., 2007 The Evolutionary Consequences of Polyploidy. Cell **131**: 452–462.
955 Palti Y., Vallejo R. L., Gao G., Liu S., Hernandez A. G., Rexroad C. E. III, Wiens G. D., 2015 Detection
956 and validation of QTL affecting bacterial cold water disease resistance in Rainbow Trout using
957 restriction-site associated DNA sequencing. PLoS ONE **10**: e0138435.
958 Pennell M. W., Kirkpatrick M., Otto S. P., Vamosi J. C., Peichel C. L., Valenzuela N., Kitano J., 2015 Y
959 fuse? Sex chromosome fusions in fishes and reptiles. PLoS Genet **11**: e1005237.
960 Perrin N., 2009 Sex reversal: a fountain of youth for sex chromosomes? Evolution **63**: 3043–3049.
961 Phillips R., Ráb P., 2001 Chromosome evolution in the Salmonidae (Pisces): an update. Biol Rev Camb
962 Philos Soc **76**: 1–25.
963 Phillips R. B., 2013 Evolution of the sex chromosomes in salmonid fishes. Cytogenet Genome Res **141**:
964 177–185.
965 Phillips R. B., Ihssen P. E., 1985 Identification of sex chromosomes in lake trout (*Salvelinus namaycush*).

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966 Cytogenet. Cell Genet. **39**: 14–18.
967 Phillips R. B., Keatley K. A., Morasch M. R., Ventura A. B., Lubieniecki K. P., Ben F Koop, Danzmann
968 R. G., Davidson W. S., 2009 Assignment of Atlantic Salmon (*Salmo salar*) linkage groups to specific
969 chromosomes: Conservation of large syntenic blocks corresponding to whole chromosome arms in
970 Rainbow Trout (*Oncorhynchus mykiss*). BMC Genet **10**: 46.
971 Phillips R. B., Morasch M. R., Park L. K., Naish K. A., Devlin R. H., 2005 Identification of the sex
972 chromosome pair in Coho Salmon (*Oncorhynchus kisutch*): lack of conservation of the sex linkage
973 group with Chinook Salmon (*Oncorhynchus tshawytscha*). Cytogenet Genome Res **111**: 166–170.
974 Phillips R. B., Nichols K. M., DeKoning J. J., Morasch M. R., Keatley K. A., Rexroad C., Gahr S. A.,
975 Danzmann R. G., Drew R. E., Thorgaard G. H., 2006 Assignment of Rainbow Trout linkage groups
976 to specific chromosomes. Genetics **174**: 1661–1670.
977 R Development Core Team, 2017 R: A language and environment for statistical computing.
978 Rexroad C. E., Palti Y., Gahr S. A., Vallejo R. L., 2008 A second generation genetic map for Rainbow
979 Trout (*Oncorhynchus mykiss*). BMC Genet **9**: 74.
980 Rexroad C. E., Vallejo R. L., Liu S., Palti Y., Weber G. M., 2012 QTL affecting stress response to
981 crowding in a Rainbow Trout broodstock population. BMC Genet **13**: 97.
982 Rockman M. V., 2012 The QTN program and the alleles that matter for evolution: all that's gold does not
983 glitter. Evolution **66**: 1–17.
984 Roco Á. S., Olmstead A. W., Degitz S. J., Amano T., Zimmerman L. B., Bullejos M., 2015 Coexistence of
985 Y, W, and Z sex chromosomes in *Xenopus tropicalis*. Proceedings of the National Academy of
986 Sciences **112**: E4752–E4761.
987 Rogers S. M., Bernatchez L., 2007 The genetic architecture of ecological speciation and the association
988 with signatures of selection in natural Lake Whitefish (*Coregonus* sp. *Salmonidae*) species pairs.
989 Molecular Biology and Evolution **24**: 1423–1438.
990 Rondeau E. B., Minkley D. R., Leong J. S., Messmer A. M., Jantzen J. R., Schalburg von K. R., Lemon C.,
991 Bird N. H., Koop B. F., 2014 The genome and linkage map of the Northern Pike (*Esox lucius*):
992 conserved synteny revealed between the salmonid sister group and the Neoteleostei. PLoS ONE **9**:
993 e102089.
994 Ross J. A., Urton J. R., Boland J., Shapiro M. D., Peichel C. L., 2009 Turnover of sex chromosomes in the
995 stickleback fishes (*Gasterosteidae*). PLoS Genet **5**: e1000391–12.
996 Sakamoto T., Danzmann R. G., Gharbi K., Howard P., Ozaki A., Khoo S. K., Woram R. A., Okamoto N.,
997 Ferguson M. M., Holm L. E., Guyomard R., Hoyheim B., 2000 A microsatellite linkage map of
998 Rainbow Trout (*Oncorhynchus mykiss*) characterized by large sex-specific differences in
999 recombination rates. Genetics **155**: 1331–1345.
1000 Santure A. W., Poissant J., De Cauwer I., Oers K., Robinson M. R., Quinn J. L., Groenen M. A. M.,
1001 Visser M. E., Sheldon B. C., Slate J., 2015 Replicated analysis of the genetic architecture of
1002 quantitative traits in two wild great tit populations. Mol. Ecol. **24**: 6148–6162.
1003 Sauvage C., Vagner M., Derôme N., Audet C., Bernatchez L., 2012a Coding gene single nucleotide
1004 polymorphism mapping and quantitative trait loci detection for physiological reproductive traits in
1005 Brook Charr, *Salvelinus fontinalis*. G3 - Genes|Genomes|Genetics **2**: 379–392.
1006 Sauvage C., Vagner M., Derôme N., Audet C., Bernatchez L., 2012b Coding gene SNP mapping reveals
1007 QTL linked to growth and stress response in Brook Charr (*Salvelinus fontinalis*). G3 -
1008 Genes|Genomes|Genetics **2**: 707–720.
1009 Session A. M., Uno Y., Kwon T., Chapman J. A., Toyoda A., Takahashi S., Fukui A., Hikosaka A.,
1010 Suzuki A., Kondo M., van Heeringen S. J., Quigley I., Heinz S., Ogino H., Ochi H., Hellsten U.,
1011 Lyons J. B., Simakov O., Putnam N., Stites J., Kuroki Y., Tanaka T., Michiue T., Watanabe M.,
1012 Bogdanovic O., Lister R., Georgiou G., Paranjpe S. S., van Kruijsbergen I., Shu S., Carlson J.,
1013 Kinoshita T., Ohta Y., Mawaribuchi S., Jenkins J., Grimwood J., Schmutz J., Mitros T., Mozaffari S.
1014 V., Suzuki Y., Haramoto Y., Yamamoto T. S., Takagi C., Heald R., Miller K., Haudenschild C.,
1015 Kitzman J., Nakayama T., Izutsu Y., Robert J., Fortriede J., Burns K., Lotay V., Karimi K., Yasuoka
1016 Y., Dichmann D. S., Flajnik M. F., Houston D. W., Shendure J., DuPasquier L., Vize P. D., Zorn A.

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1017 M., Ito M., Marcotte E. M., Wallingford J. B., Ito Y., Asashima M., Ueno N., Matsuda Y., Veenstra
1018 G. J. C., Fujiyama A., Harland R. M., Taira M., Rokhsar D. S., 2016 Genome evolution in the
1019 allotetraploid frog *Xenopus laevis*. Nature **538**: 336–343.
1020 Singer A., Perlman H., Yan Y., Walker C., Corley-Smith G., Brandhorst B., Postlethwait J., 2002 Sex-
1021 specific recombination rates in zebrafish (*Danio rerio*). Genetics **160**: 649–657.
1022 Slate J., 2005 Quantitative trait locus mapping in natural populations: progress, caveats and future
1023 directions. Mol. Ecol. **14**: 363–379.
1024 Slate J., 2008 Robustness of linkage maps in natural populations: a simulation study. Proceedings of the
1025 Royal Society B: Biological Sciences **275**: 695–702.
1026 Slate J., 2013 From beavis to beak color: a simulation study to examine how much qtl mapping can reveal
1027 about the genetic architecture of quantitative traits. Evolution **67**: 1251–1262.
1028 Steinemann M., Steinemann S., 1998 Enigma of Y chromosome degeneration: neo-Y and neo-X
1029 chromosomes of *Drosophila miranda* a model for sex chromosome evolution. Genetica **102-103**:
1030 409–420.
1031 Stöck M., Horn A., Grossen C., Lindtke D., Sermier R., Betto-Colliard C., Dufresnes C., Bonjour E.,
1032 Dumas Z., Luquet E., Maddalena T., Sousa H. C., Martinez-Solano I., Perrin N., 2011 Ever-young
1033 sex chromosomes in European tree frogs. Plos Biol **9**: e1001062–9.
1034 Stöck M., Savary R., Betto-Colliard C., Biollay S., Jourdan-Pineau H., Perrin N., 2013 Low rates of X-Y
1035 recombination, not turnovers, account for homomorphic sex chromosomes in several diploid species
1036 of Palearctic green toads (*Bufo viridis* subgroup). Journal of Evolutionary Biology **26**: 674–682.
1037 Sutherland B. J. G., Gosselin T., Normandeau E., Lamothe M., Isabel N., Audet C., Bernatchez L., 2016
1038 Salmonid chromosome evolution as revealed by a novel method for comparing RADseq linkage maps.
1039 Genome Biology and Evolution **8**: 3600–3617.
1040 Taylor J. S., Braasch I., Frickey T., Meyer A., Van de Peer Y., 2003 Genome duplication, a trait shared by
1041 22,000 species of ray-finned fish. Genome Research **13**: 382–390.
1042 Thorgaard G. H., 1977 Heteromorphic sex chromosomes in male Rainbow Trout. Science **196**: 900–902.
1043 Trivers R., 1998 Sex differences in rates of recombination and sexual selection. In:Michod R, Levin D
1044 (Eds.), *The evolution of sex*,, pp. 270–286.
1045 Tsai H. Y., Hamilton A., Guy D. R., Tinch A. E., Bishop S. C., Houston R. D., 2015 The genetic
1046 architecture of growth and fillet traits in farmed Atlantic Salmon (*Salmo salar*). BMC Genet **16**: 117–
1047 11.
1048 van Doorn G. S., Kirkpatrick M., 2007 Turnover of sex chromosomes induced by sexual conflict. **449**:
1049 909–912.
1050 van Ooijen J. W., 2006 JoinMap4: Software for the calculation of genetic linkage maps in experimental
1051 populations.
1052 Wei T., Simko V., 2017 corrplot: Visualization of a correlation matrix.
1053 Woram R. A., Gharbi K., Sakamoto T., Høyheim B., Holm L.-E., Naish K., McGowan C., Ferguson M.
1054 M., Phillips R. B., Stein J., Guyomard R., Cairney M., Taggart J. B., Powell R., Davidson W.,
1055 Danzmann R. G., 2003 Comparative genome analysis of the primary sex-determining locus in
1056 salmonid fishes. Genome Research **13**: 272–280.
1057 Wu R., Ma C.-X., Painter I., Zeng Z.-B., 2002 Simultaneous maximum likelihood estimation of linkage
1058 and linkage phases in outcrossing species. Theor Popul Biol **61**: 349–363.
1059 Xu S., 2003 Theoretical basis of the Beavis effect. Genetics **165**: 2259–2268.
1060 Yano A., Guyomard R., Nicol B., Jouanno E., Quillet E., Klopp C., Cabau C., Bouchez O., Fostier A.,
1061 Guiguen Y., 2012a An immune-related gene evolved into the master sex-determining gene in
1062 Rainbow Trout, *Oncorhynchus mykiss*. Curr. Biol. **22**: 1423–1428.
1063 Yano A., Nicol B., Jouanno E., Quillet E., Fostier A., Guyomard R., Guiguen Y., 2012b The sexually
1064 dimorphic on the Y-chromosome gene (*sdY*) is a conserved male-specific Y-chromosome sequence in
1065 many salmonids. Evol Appl **6**: 486–496.
1066 Yáñez J. M., Houston R. D., Newman S., 2014 Genetics and genomics of disease resistance in salmonid
1067 species. Front. Genet. **5**: 415.

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1068 Yoshida K., Makino T., Yamaguchi K., Shigenobu S., Hasebe M., Kawata M., Kume M., Mori S., Peichel
1069 C. L., Toyoda A., Fujiyama A., Kitano J., 2014 Sex chromosome turnover contributes to genomic
1070 divergence between incipient stickleback species. PLoS Genet **10**: e1004223–16.

1071
1072
1073 **SUPPLEMENTAL MATERIAL**

1074 **Table S1.** Phenotype average, standard deviation and sample size in males and females. Phenotypes
1075 showing any differences between males and females ($p \leq 0.2$) included sex as a covariate in the model.
1076 Sex-specific phenotypes were only tested within the one sex and therefore had smaller sample sizes.

1077 **Table S2.** Complete QTL table with all identified genome- and chromosome-wide QTLs and associated
1078 values, including marker sequence and SNP, and effect size of different genotypes.

1079 **Figure S1.** Heterochiasmy plots for individual chromosomes in the maternal (a) and paternal (b)
1080 haplotypes. Grey boxes above the plot indicate probable residually tetraploid chromosome arms, and
1081 centromere positions transferred from Chinook Salmon are indicated by stars. When Chinook Salmon
1082 chromosomes were not fusions but were metacentrics (n = 2), this is denoted by a '?' to denote the
1083 uncertainty as to the centromere position in Brook Charr. However, given the clear pattern of
1084 recombination at the opposite end of centromeres in the male, the location for these unknown centromeres
1085 is most likely to the opposite side where the recombination events occurred.

1086 **Figure S2.** Correlation plot of phenotypes used in QTL analysis. Phenotype pairs that do not share any
1087 individuals for correlation are shown with '?'.

1088 **File S1.** Required files for running Rqtl analysis (phenotype (.qua), map (.map) and genotype (.loc)). The
1089 map file corresponds to the female map from Sutherland *et al.* 2016. See the Data Availability for code for
1090 performing complete analysis with these files.

1091 **File S2.** Fasta file with RAD-seq tags output using the STACKs population module for alleles from all
1092 individual offspring and parents.