GENE DUPLICATION TO THE Y CHROMOSOME IN TRINIDADIAN GUPPIES

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ABSTRACT

Differences in allele frequencies at autosomal genes between males and females in a population can result from two scenarios. Unresolved sexual conflict over survival produces allelic differentiation between the sexes. However, given the substantial mortality costs required to produce allelic differences between males and females at each generation, it remains unclear how many loci within the genome experience significant sexual conflict over survival. Alternatively, recent studies have shown that similarity between autosomal and Y sequence, arising from duplication onto the Y, can create perceived allelic differences, and this represents potentially resolved sexual conflict. However, Y duplications are most likely in species with large non-recombining regions, in part because they simply represent larger targets for duplications. We assessed the genomes of 120 wild-caught guppies, which experience extensive predation- and pathogen-induced mortality and have a relatively small ancestral Y chromosome. We identified seven autosomal genes that show allelic differences between male and female adults. Five of these genes show clear evidence of whole or partial gene duplication to the Y chromosome, suggesting that the male-specific region of the guppy Y chromosome, although relatively small, may nonetheless act as a hotspot for the resolution of sexual conflict. The remaining two genes show evidence of partial homology to the Y. Overall, our findings suggest that the guppy genome experiences a very low level of unresolved sexual conflict over survival, and instead the Y chromosome, despite its small ancestral size and recent origin, acts as a major mechanism of conflict resolution.

Keywords: gene duplications, Y chromosome, sexual conflict, *Poecilia reticulata*

Introduction

Differences in allele frequency between males and females at autosomal loci can result from two alternative sources. Numerous recent studies have used allelic differences between males and females within a population (F_{ST}, D_{xy}, etc) as a way to infer sex-differences in viability or survival, and therefore sexual conflict over mortality (Cheng & Kirkpatrick, 2016; Dutoit et al., 2018; Flanagan & Jones, 2017; Kasimatis et al., 2021; Lucotte et al., 2016; Wright et al., 2018, 2019).This approach assumes that allele frequencies are the same in males and females at conception, but diverge over the course of a generation for loci with alleles that benefit the survival of one sex at some survival cost to the other (intra-locus sexual conflict). We expect that sexual conflict over survival would produce a signature of allelic differentiation between the sexes as well as balancing selection (Mank, 2017). The latter results from all forms of intra-locus sexual conflict, not just that over survival, as alleles are selected for or against depending on whether they are present in males or females (Barson et al. 2015; Connallon & Clark 2014; Foerster et al. 2007; Hawkes et al. 2016; Johnston et al. 2013; Lonn et al. 2017; Ruzicka et al. 2019). Balancing selection resulting from sexual conflict may be a major factor in the maintenance of genetic polymorphisms within populations (Connallon & Clark 2014), therefore, the proportion of loci subject to unresolved sexual conflict within the genome, as well as the types of loci affected, may have important implications for a range of evolutionary factors, such as the speed and genetic basis of adaptation.

Intra-locus conflict over survival implies a significant mortality cost each generation. Modelling and simulation methods suggest that the sex-specific mortality rates necessary to generate significant allelic differences between the sexes within each generation are quite high for any one locus (Bissegger et al. 2019; Kasimatis, Ralph, & Phillips 2019; Ruzicka et al. 2020). This precludes the presence of large numbers of sites subject to sexual conflict due to survival, as the associated mortality load would simply be too great. Moreover, recent work has highlighted the potential that many perceived allelic sex differences between the sexes actually are the result of sequence homology between autosomal and sex-linked loci (Bissegger et al. 2019; Kasimatis et al. 2019; Mank, Shu, & Wright 2020; Ruzicka et al. 2020). The Y chromosome may preferentially retain duplicates that play an important role in male development or fitness

86 (Bachtrog, 2013; Carvalho et al., 2015), and the process of Y duplication ironically offers a route
87 to sexual conflict resolution even though it may lead to the mistaken perception of unresolved
88 conflict acting on autosomal loci.

89 It therefore remains unclear how common and pervasive sexual conflict over survival is in
90 natural populations, or what types of loci, if any, it is expected to target. Here we assess the
91 potential for intersexual F_{ST} based on a sample of 120 wild-caught adult guppies, which are
92 expected to experience substantial predation- and pathogen-induced mortality. We identified
93 seven genes that show evidence of allelic differences between adult males and females. Of
94 those seven genes, five show evidence of Y duplicates, and two have sex-specific functions,
95 including germline differentiation and sex hormone signaling. This is surprising, as although the
96 size of the guppy Y varies substantially across populations and related species (Wright et al.
97 2017; Darolti et al. 2019; Ameida et al. 2021), the conserved, ancestral part of the Y is very
98 small, spanning at most 5 Mb (Almeida et al., 2020; Darolti et al., 2019; Fraser et al., 2020).
99 Given this small size, we might expect that the Y chromosome in guppies does not represent a
100 large enough genomic target for sexual conflict resolution through gene duplication. Instead,
101 our results show that the guppy Y, although a very small region of the genome, may indeed act
102 as a hotspot for the resolution of conflict.

103

104 **Results**

105 We individually sequenced 60 male and 60 female wild-caught adults from three rivers in
106 Trinidad to an average of ~30X coverage in males and ~20X coverage in females after filtering,
107 trimming and quality control (see Materials and Methods for further details). The greater read
108 depth in males was designed to aid detection of the Y chromosome, which is present in only
109 one copy in each genome. Because the size of the non-recombining region of the sex
110 chromosome varies across guppy populations (Almeida et al., 2020; Wright et al., 2017), and
111 sex chromosome divergence results in allelic differences on the X and Y between males and
112 females, we confined all our analyses to autosomal sequence, although we present the X
113 chromosome (Chromosome 12) in figures for comparison.

114 From our sequencing data, we obtained 7,889,657 biallelic high quality filtered autosomal SNPs.
115 We further filtered these to 253,375 SNPs present in annotated autosomal coding sequence.
116 We focused on coding sequence for three reasons. First, non-coding sequence is enriched for
117 repetitive elements, and the accumulation of repetitive elements on the ancestral guppy Y
118 chromosome (Almeida et al., 2020) would lead to male-specific SNPs in repetitive elements that
119 could bias our estimates of Y duplications. Second, previous work has suggested that
120 unresolved sexual conflict on the autosomes occurs primarily in coding sequence (Ruzicka et al.,
121 2019), and this fits with the substantial evidence that sexual conflict over gene regulation can
122 be relatively quickly resolved through sex-specific gene regulation (Kopp, Duncan, & Carroll
123 2000; Mank 2017; Wright et al. 2019). Finally, by focusing on functional coding sequence, we
124 are able to determine the potential functional role of genes that are subject to sexual conflict.

125

126 ***Intersexual F_{ST}***

127 Given the low level of allelic divergence expected between males and females, it is critical to
128 minimize false positives (Kasimatis et al. 2019). We therefore used three independent methods
129 to estimate SNPs with elevated intersexual F_{ST}. We identified SNPs that were 1) in the top 1% of
130 the autosomal F_{ST} distribution 2) were significant after permutation testing of samples (1000
131 replicates, $P < 0.001$) and 3) showed significant differences in male and female allele frequency
132 based on Fisher's exact test ($P < 0.001$) (Supplementary Fig. 1). We identified 504 autosomal
133 coding sequence SNPs that were significant by all three of these measures (Supplementary Figs.
134 1 and 2), designated as high intersexual F_{ST} SNPs.

135 We then identified autosomal coding genes with ≥ 3 high intersexual F_{ST} SNPs (Bissegger et al.
136 2019; Tobler, Nolte, & Schlötterer 2017), resulting in seven loci with significant sexual
137 differentiation. For each of these genes, average intersexual F_{ST} was significantly greater than
138 the autosomal average (Table 1, Supplementary Fig. 3).

139 Estimates of intersexual F_{ST} and Tajima's D can be biased due to relatedness of individuals
140 within groups. Although our sampling design was balanced, with 10 females and 10 males
141 collected at two separate sites for each of three rivers across the island of Trinidad (see

142 Almeida et al. 2020 for details), we could not account for relatedness in wild-caught samples at
143 the time of collection. We therefore assessed pairwise kinship coefficients among all our
144 samples using both KING (Manichaikul et al., 2010) and NgsRelate (Korneliussen & Moltke
145 2015), as implemented in ANGSD (Korneliussen, Albrechtsen, & Nielsen 2014). Neither method
146 identified greater relatedness among male compared to female samples (Supplementary Fig. 4).

147

148 ***Y duplications***

149 When mapping whole-genome data to a female reference genome, sequence similarity
150 between the male-specific Y chromosome and the autosomes can lead to the perception of
151 allelic differences between males and females. Recent work has shown that many genes with
152 allelic sex differences are in fact autosomal loci that either have recent Y duplicates (Bissegger
153 et al. 2019; Mank et al. 2020) or otherwise display sequence homology to the Y (Kasimatis et al.,
154 2021) . We can use differences in M:F read depth to identify these genes (Hall et al., 2013).
155 Duplication of the complete coding sequence for an autosomal gene on the Y chromosome
156 would produce an average M:F read depth of 1.5 (three copies in males, two in females), and
157 subsequent tandem duplications on the Y would result in M:F read depth > 1.5. Partial Y
158 duplications, or a full duplication followed by significant differentiation, would result in average
159 M:F read depth > 1 and < 1.5 when mapping to a female genome after correcting for
160 differences in library size.

161 Five of our seven sexually differentiated genes showed average M:F read depth significantly > 1
162 (Table 1, Supplemental Fig. 5) consistent with at least a partial duplication of the coding
163 sequence on the Y chromosome (Fig. 1). Four of these Y-duplicated genes have annotated
164 functions, two of which (Olr1496-like and Fezf1) have a known role in sexual differentiation
165 (Table 1), as is expected for genes on the Y chromosome.

166 In order to differentiate whether the pattern of elevated M:F F_{ST} is due to limited sequence
167 homology between the autosomal genes and the Y chromosome, or results from at least partial
168 gene duplication, we mapped the pattern of M:F read depth across each of these 5 genes (Fig.
169 1). The high M:F read depth for Olr1492-like, consistent across the full length of the single exon

170 of this gene, suggests a complete duplication, possibly in two copies (Fig. 1A).Similarly, the M:F
171 read depth ratio for si:rp71-17i16.5 (Fig. 1C), Bcl11a (Fig. 1E), and Fezf1 (Fig. 1G) suggests a
172 single or double copy duplication, of just the exons, possibly through retrotransposition. The
173 high M:F read depth in ENSPRG05754 (Fig. 1I) suggests a partial Y duplication of the last exon of
174 the gene, likely present in several Y copies.
175 Given sufficient evolutionary time, Y duplications will also accumulate male-specific SNPs
176 (Tobler et al. 2017), leading to a signal of elevated M:F SNP density. Of the five sexually
177 differentiated genes with greater read depth in males, none showed significantly higher M:F
178 SNP density (Table 1), although all values were >1, suggesting that duplication to the Y is
179 relatively recent, and consistent with the recent origin of this chromosome (Darolti et al. 2019).
180 Across our seven sexually differentiated genes, we did not observe evidence of duplications to
181 the X chromosome, which would result in three copies in males and four in females and
182 therefore an average M:F read depth significantly <1.
183
184 ***Sexual conflict over survival for autosomal genes***

185 We identified just two sexually differentiated genes without significantly higher M:F read depth
186 (Table 1), both of which displayed relatively modest levels of intersexual F_{ST} (0.0237 and
187 0.0098). At first consideration, this small number of loci with modest F_{ST} is consistent with
188 limited unresolved sexual conflict and the expectation that large numbers of high intersexual
189 F_{ST} loci would require unsustainable levels of sex-specific mortality each generation (Bissegger
190 et al. 2019; Kasimatis et al. 2019; Ruzicka et al. 2020).

191 Genes subject to ongoing sexual conflict are expected to experience balancing selection, which
192 is often measured with Tajima's D (Bissegger et al. 2019; Cheng & Kirkpatrick 2016; Mank 2017;
193 Wright et al. 2018). We therefore compared Tajima's D for these two genes to the autosomal
194 average, genes with known immune function, which are known to be subject to high levels of
195 balancing selection (Andrés et al. 2009; Ferrer-Admetlla et al. 2008; Van Oosterhout 2009;
196 Weedall & Conway 2010), and to other genomic categories (Fig. 2). Contrary to our expectation
197 of elevated Tajima's D for these two sexually differentiated genes, neither showed a Tajima's D

198 value significantly greater than the autosomal average, and Tajima's D for ENSPREG15023 is
199 significantly lower than the autosomal average (Fig. 2).

200 Many things can influence Tajima's D estimates, and the lack of an elevated signature of
201 balancing selection relative to the remainder of the genome is not necessarily indicative of a
202 lack of sexual conflict. In order to understand the dynamics of these two genes in more detail,
203 we mapped M:F read depth as a function of genomic location and intersexual F_{ST} (Fig. 3).
204 Indeed, we found evidence of elevated M:F read depth for ENSPREG15023 across most of the
205 exon, consistent with either partial duplication or full exonic duplication to the Y followed by
206 partial degeneration. Many of the high F_{ST} SNPs in the gene have a high M:F read depth,
207 consistent with Y duplication or significant Y homology. The pattern for ENSPREG12439 is more
208 consistent with significant Y homology given the highly variable M:F read depth across the
209 gene. Importantly, all SNPs with high M:F F_{ST} values for this gene also show high M:F read
210 depth. These results, coupled with the lack of elevated Tajima's D, suggest that these two genes
211 also exhibit significant homology to the Y, and that this is driving the elevated intersexual F_{ST}
212 instead of unresolved sexual conflict over survival.

213

214 **Discussion**

215 We identified 504 coding sequence SNPs in the guppy autosomal genome which showed
216 significant differences in allele frequency between males and females. We used these to
217 identify seven autosomal genes with significant average intersexual F_{ST}. Our approach, based on
218 the intersection of three statistical methods, reduces the likelihood of false positives and
219 results in a high confidence gene list of intersexual F_{ST} (Table 1, Supplementary Figs. 1, 2, and
220 3). This list of genes can be used to assess the relative role of Y duplication in resolving conflict
221 and driving intersexual F_{ST}, versus the role of sex differences in mortality in sexual conflict of
222 autosomal genes.

223 ***The Y chromosome in guppies as a locus for conflict resolution.***

224 Five of the seven high-confidence sexually differentiated genes showed evidence of Y
225 duplications based on elevated male-to-female read depth ratios (Table 1, Supplementary Fig.

226 4). Two of these (Olr1496-like and Fezf1) have sex-specific functions based on Gene Ontology
227 and Gene Cards annotations, consistent with theory and empirical studies showing that Y
228 chromosomes can accumulate gene duplicates with male-specific functions (Carvalho et al.
229 2015; Mahajan & Bachtrog 2017). Specifically, Olr1496 (Olfactory factor 1496-like gene) plays a
230 role in multiple aspects of germline development in *C. elegans* (Cho, Rogers, & Fay 2007), and
231 Fezf1 (FEZ Family Zink Finger 1) plays an important role in the migration of gonadotropin-
232 releasing neurons (Damla Kotan et al., 2014).

233 Y chromosomes in general represent a unique genomic environment. Although Y chromosome
234 gene content degrades quickly following recombination suppression (Bachtrog, 2013), several
235 recent studies indicate that older, large Y chromosomes, such as that in *Drosophila* (Carvalho et
236 al. 2015; Koerich et al. 2008; Tobler et al. 2017), sticklebacks (Bissegger et al. 2019) and humans
237 (Kasimatis et al. 2020), contain substantial numbers of genes duplicated from autosomes.
238 Duplications may represent an important mechanism of sex chromosome divergence, and the Y
239 chromosome may preferentially retain duplicates that play an important role in male
240 development or fitness (Bachtrog, 2013; Carvalho et al., 2015), offering a hotspot for sexual
241 conflict resolution within the genome.

242 However, it remains unclear how common Y duplications of autosomal genes occur in younger
243 systems with a far smaller male-specific region. Although the size varies by population, the
244 conserved non-recombining region of the Y chromosome in guppies is relatively small, spanning
245 at most 5 Mb (Almeida et al., 2020; Darolti et al., 2019). Others have failed to recover evidence
246 for even this limited region of Y degeneration (Charlesworth et al., 2020; Fraser et al., 2020).
247 Our findings add further support for a region of recombination suppression across populations
248 on Trinidad, as only a male-specific region of the Y can explain the M:F read depth differences
249 we observe. Moreover, our work suggests that the guppy Y chromosome is dynamic with
250 regard to gene content, and acts as a hotspot for gene duplications with male-specific functions
251 despite its recent origin and small size. It is also possible that Y genes have duplicated to the
252 autosomes, which would also produce a pattern of increased male read depth, although this is
253 arguably less likely. Taken together, our work suggests that even homomorphic sex
254 chromosomes may act as a hotspot of sexual conflict resolution. Moreover, our results further

255 emphasize the importance of accounting for Y gene duplications in scans for M:F F_{ST}, as the all
256 of our sexually differentiated genes show evidence of Y duplication.

257 The Y-duplicated genes we identify here are not present in our previous list of Y-linked genes
258 based on male-specific sequence (Almeida et al., 2020), or in other similar analyses (Fraser et
259 al., 2020). This is not surprising, as duplications, particularly if recent, will still retain substantial
260 homology to the autosomal copy and will not be detected when bioinformatically identifying
261 sequence that is unique to male genomes. Consistent with recent duplications and limited
262 divergence, none of our Y-duplicated genes exhibit significantly elevated average M:F SNP
263 density (Table 1, Fig. 2), although the values are all >1.

264 We have previously (Wright et al., 2018) noted evidence of intersexual F_{ST} in guppies (*Poecilia*
265 *reticulata*). This finding was curious given that the data from this earlier work derived from a
266 lab population, free of most of the pathogens and all the predators that would exacerbate sex-
267 differences in mortality and predation (Wright et al., 2018). It was not clear how much of this
268 signal, if any, was due to Y duplications. However, it is worth noting that elevated intersexual
269 F_{ST} was highest for genes with male-biased expression, as would be expected for genes with Y
270 duplicates. We also did not observe a concomitant pattern of elevated Tajima's D for these
271 genes, which is inconsistent with sexual conflict over survival.

272 ***Sexual conflict over survival targets few genes in the guppy autosomal genome***

273 Intra-locus sexual selection over survival or viability leads to allele frequency differences
274 between the sexes over the course of a generation, as an allele increases the survival of one sex
275 at a mortality cost to the other (Kasimatis et al. 2019; Mank 2017; Wright et al. 2018). The
276 significant mortality costs required each generation to generate allele frequency differences
277 between the sexes preclude large numbers of genes with significant M:F F_{ST} (Bissegger et al.
278 2019; Kasimatis et al. 2019; Ruzicka et al. 2020). At most, we might expect a limited number of
279 loci with significant allelic differentiation between males and females, and this would be most
280 evident in wild species where males and females experience differences in predation, parasite
281 or pathogen loads. Despite the fact that we sampled wild individuals of a classic prey species
282 with very high potential for sexual conflict over survival, we observe just two loci in the genome

283 that show evidence of sexual conflict over survival based on M:F F_{ST}. Both of these loci exhibit
284 very low M:F F_{ST}.

285 We expect that if these genes are indeed subject to sexual conflict over mortality, we would
286 observe elevated Tajima's D (Fig. 2), however this was not the case for either locus. Moreover,
287 both of these loci exhibit patterns of M:F read depth consistent with significant Y homology
288 (Fig. 3). This suggests that the potential for sexual conflict over survival is quite low, even in a
289 species where we most expect to observe it.

290

291 ***Concluding remarks***

292 Here we use a high-stringency filtering method to detect genes within the guppy genome that
293 exhibit population genetic signatures expected from sexual conflict over survival. Although wild
294 guppies are expected to have high potential for sexual conflict over survival, we in fact found no
295 genes within the genome with patterns consistent with this. Instead, despite the small size of
296 the conserved non-recombining region of the guppy Y, we observe five loci that show patterns
297 consistent with autosome-to-Y duplications, and two more that are suggestive of Y homology of
298 without duplication. This highlights the potential of even young, small Y chromosomes as
299 regions of conflict resolution.

300

301 **Materials and Methods**

302 ***Data Collection and Genotyping***

303 Samples were collected from three rivers, Aripo, Yarra, and Quare, in Trinidad in December
304 2016, in accordance with national collecting guidelines. In total, 10 males and 10 females were
305 collected from one high predation and one low predation population in each river, resulting in
306 120 samples, which were sequenced individually with Illumina HISEQX. Further sequencing
307 details are available in Almeida et al. (2020).

308 We used FastQC v0.11 (www.bioinformatics. babraham.ac.uk/projects/fastqc) and
309 Trimmomatic 0.36 (Bolger et al., 2014) to remove adapter sequences and low-quality reads.

310 After quality control, we recovered ~30X average sequencing depth for males and ~20X
311 sequencing depth for females. High quality reads were aligned against the *Poecilia reticulata*
312 female reference genome (Ensembl GCA_000633615.2) (Künstner et al., 2016), using BWA
313 0.7.15 MEM algorithm (Li & Durbin 2009) with default parameters. We filled in mate
314 coordinates and mate related flags, sorted alignment by coordinates, and marked PCR
315 duplications with SAMtools-1.9 (Li et al., 2009).

316 We called genotypes across all the samples using the `mpileup` function from bcftools with the
317 following parameters: --min-MQ 20 --min-BQ 20 --skip-indels -a FORMAT/AD, FORMAT/DP.
318 After genotyping, we used VCFtools v0.1.16 (Danecek et al., 2011) to exclude SNPs that had
319 either : (1) genotype quality < 25; (2) sequencing depth <0.5x or >10x of average depth; (3)
320 missing data in > 10% of individuals; (4) minor allele frequency < 0.05 or (5) relative read depth
321 between reference allele and alternative allele < 0.2 or > 0.8. In total, the autosomal filtered
322 SNP dataset consisted of 7,889,657 biallelic SNPs. We extracted 253,375 SNPs in annotated
323 coding sequences (Ensembl build Guppy Female 1.0) for downstream analysis. Finally, we
324 confined our analysis to autosomal genes, but included the X chromosome (Chromosome 12) in
325 figures as a means of comparison.

326 ***Intersexual F_{ST}***

327 In order to estimate intersexual allele frequency differences, we implemented Weir &
328 Cockerham's estimator of F_{ST} (Weir & Cockerham 1984) between males and females using
329 VCFtools v0.1.16 for each SNP in genome-wide coding sequence regions. We employed three
330 methods jointly to identify SNPs exhibiting high F_{ST}. First, we used a cut-off method, retaining
331 SNPs in only the top 1% of autosomal F_{ST} values. Second, we performed permutation tests by
332 randomly assigning individuals to one of two sex groups to generate a null distribution of F_{ST}
333 across the genome. We determined significance for each SNP from 1000 replicates, using a $P <$
334 0.001 threshold. Finally, we performed Fisher's exact test on SNPs to determine significance of
335 allele frequency differences between males and females ($P < 0.001$). We denoted SNPs that
336 were significant in all three of these measures as high F_{ST} SNPs.

337 Using approaches to further limit false positives (Bissegger et al., 2019; Tobler et al., 2017), we
338 identified 7 genes with ≥ 3 high intersexual F_{ST} SNPs, which we designated as sexually
339 differentiated genes. We calculated average intersexual F_{ST} for all genes using VCFtools v0.1.16,
340 respectively. We used Wilcoxon rank-sum test to indicate statistical difference in intersexual F_{ST}
341 between autosomal genes and other gene categories (sexually differentiated genes and genes
342 on the sex chromosome).

343 ***Relatedness Inference***

344 In order to avoid biases in calculating intersexual allele frequency differences due to
345 relatedness among individuals, we used KING 2.2.7 (Manichaikul et al., 2010) to infer the
346 pairwise degree of relatedness between individuals from estimated kinship coefficients. We
347 first converted genotype data from the raw, unfiltered SNPs dataset to plink binary format
348 using PLINK 1.9 (Purcell et al., 2007). In order to avoid potential biases from KING software
349 (Ramstetter et al., 2017) and validations, we also used NgsRelate v2 (Korneliussen & Moltke
350 2015), implemented in ANGSD (Korneliussen et al. 2014) to infer genetic relatedness
351 coefficients for each pair of individuals.

352 ***Assessing Y Duplications of Sexually Differentiated Genes***

353 When using a female reference genome, reads from genes which have duplicated to the male-
354 specific region of the Y chromosome will map back to original autosomal or X chromosome
355 regions, resulting in elevated M:F coverage (Bissegger et al. 2019; Mank et al. 2020). For
356 example, if an autosomal gene has one Y duplication, we would expect three copies in males
357 (two autosomal and one Y-linked) and two copies in females, and therefore an average M:F
358 read depth of 1.5. We first calculated M:F read depth for each coding sequence SNP from
359 genotype data, as male coverage/female coverage, correcting for differences in average
360 genomic read depth between males and females. In order to validate the M:F read depth ratio
361 of sexually differentiated genes, we calculated male and female normalized read depth for 7
362 genes for each base pair, based on male and female pooled reads.

363 Genes on the Y chromosome will accumulate male-specific mutations over time, leading to an
364 increased number of male-specific mutations as well as elevated M:F SNP density (Bissegger et

365 al. 2019; Mank et al. 2020). M:F SNP density for each gene was calculated as number of male
366 SNPs/number of female SNPs.

367 ***Tajima's D***

368 Based on the filtered genotype data, we calculated Tajima's D for the coding sequence of all
369 autosomal genes using VCFtools v0.1.16. We compared mean Tajima's D for autosomal genes
370 (excluding those with immune function), genes with immune function (defined following Wright
371 et al. 2017, Wright et al. 2018), and our seven sexually differentiated genes.

372 ***Functional Annotations***

373 The small number of sexually differentiated genes precludes Gene Ontology enrichment
374 analysis. We therefore cataloged functional annotations from the *Danio rerio* Gene Ontology
375 (Ashburner et al., 2000; "The Gene Ontology resource: enriching a GOld mine," 2021) and Gene
376 Cards (Stelzer et al., 2016).

377

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384

385 **Data Accessibility Statement**

386 DNA-seq data are publicly available in the NCBI SRA (BioProject ID PRJEB39998). Scripts of all
387 the analysis are available on GitHub XXXXX.

388

389 **Author Contributions**

390 J.E.M. and Y.L. designed the study. B.S., F.B. and J.E.M. collected samples. P.A. collected raw
391 sequencing data. Y.L., I.D., B.L.S.F., P.A., B.S., A.E.W. and J.E.M. performed the data analysis.
392 J.E.M., Y.L., I.D., B.L.S.F., B.S., F.B. and A.E.W. contributed to the writing of the manuscript.

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562 **Tables and Figures**

563 **Table 1.** Summary statistics of sexually differentiated genes. Statistically significant values are
564 shown in bold.

ENSPREG[1] (Gene Name if known)	Chrom	Mean F_{ST}[2]	M:F read depth[3]	M:F SNP density[3]	Gene functions[4]
03921 (Olr1496-like)	LG7	**0.1645**	**1.3809**	1.3000	Immune response & germline differentiation
03854 (si:rp71-17i16.5)	LG7	**0.0892**	**1.1580**	1.1200	Phosphatidylinositol 3-kinase signalling, involved in immune, inflammatory and allergic responses
09757 (BCL11A)	LG15	**0.0419**	**1.3280**	1.0000	Hematopoietic cell differentiation & brain development
07572 (Fezf1)	LG6	**0.0412**	**1.5082**	1.0077	Nervous system development, migration of gonadotropin-releasing hormone neurons
05754	LG15	**0.0238**	**1.8887**	0.9445	N/A
15023	LG18	**0.0237**	1.0829	1.2143	N/A
12439	LG22	**0.0098**	1.0802	1.0417	N/A

565 1 Ensembl guppy gene number
566 2 Significance based on Wilcoxon's rank-sum test ($P < 0.01$), compared to autosomal average.
567 3 Significance based on Fisher's Exact Test ($P < 0.05$), relative to genome-wide M:F SNP density.
568 4 From Gene Ontology (Ashburner et al., 2000; "The Gene Ontology resource: enriching a GOld
569 mine," 2021) and Gene Cards (Stelzer et al., 2016).
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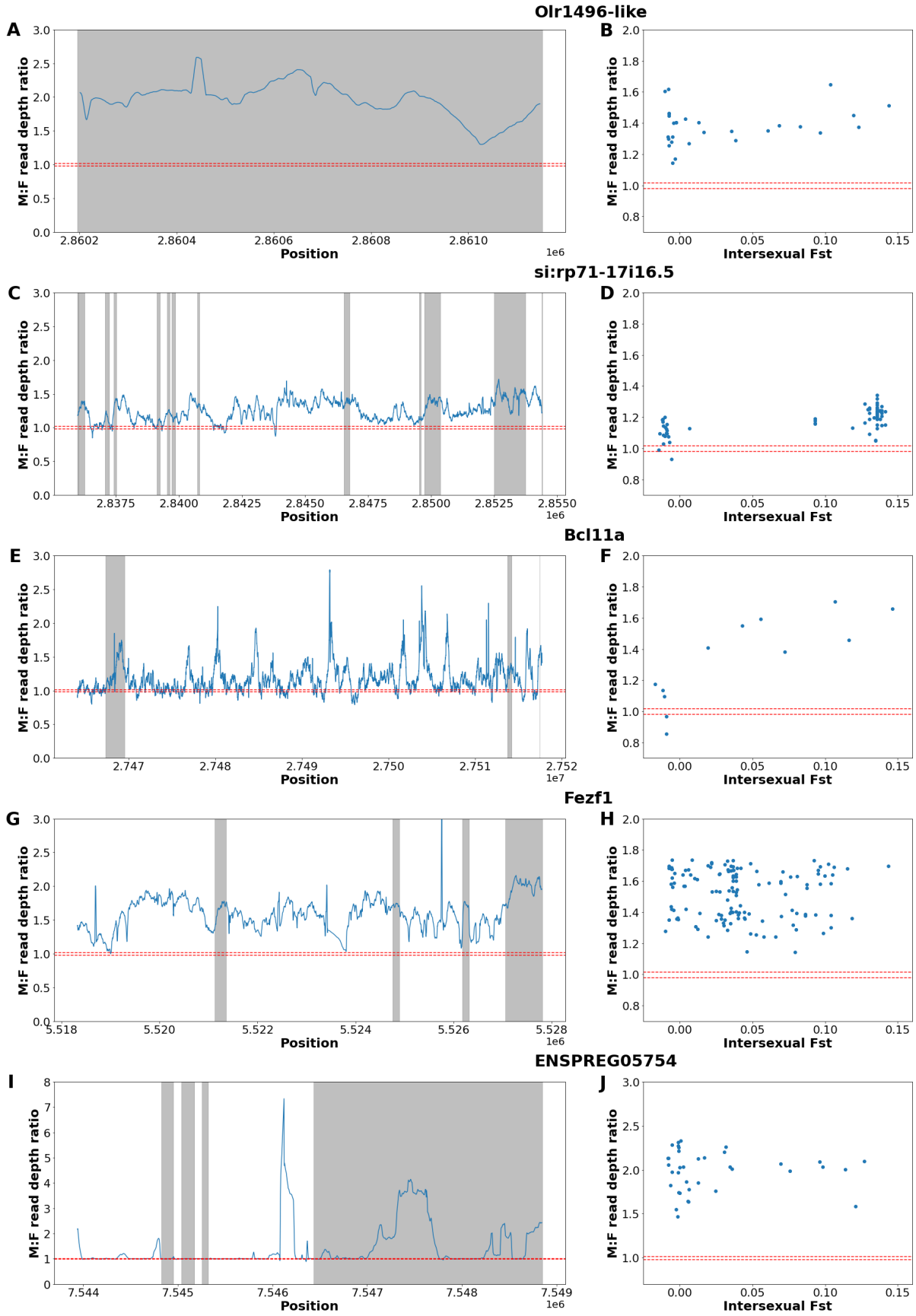
Olr1496-like
A
B
si:rp71-17i16.5
C
D
Bcl11a
E
F
Fezf1
G
H
ENSPREG05754
I
J
M:F read depth ratio
Position
Intersexual Fst
1e6
1e7

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572 **Fig. 1.** Elevated M:F read depth ratio indicates partial gene duplication of Olr1496-like (Panels A
573 and B), si:rp71-17i16.5 (Panels C and D), BCL11A (Panels E and F), Fezf1 (Panels G and H),
574 ENSPREG05754 (Panels I and J). Left panels show M:F read depth as a function of physical
575 position (in Mb), with coding regions shaded in grey. Panels on the right show intersexual F_{ST}
576 and M:F read depth ratio for each SNP. 95% autosomal confidence intervals for M:F read depth
577 are indicated by horizontal red lines.

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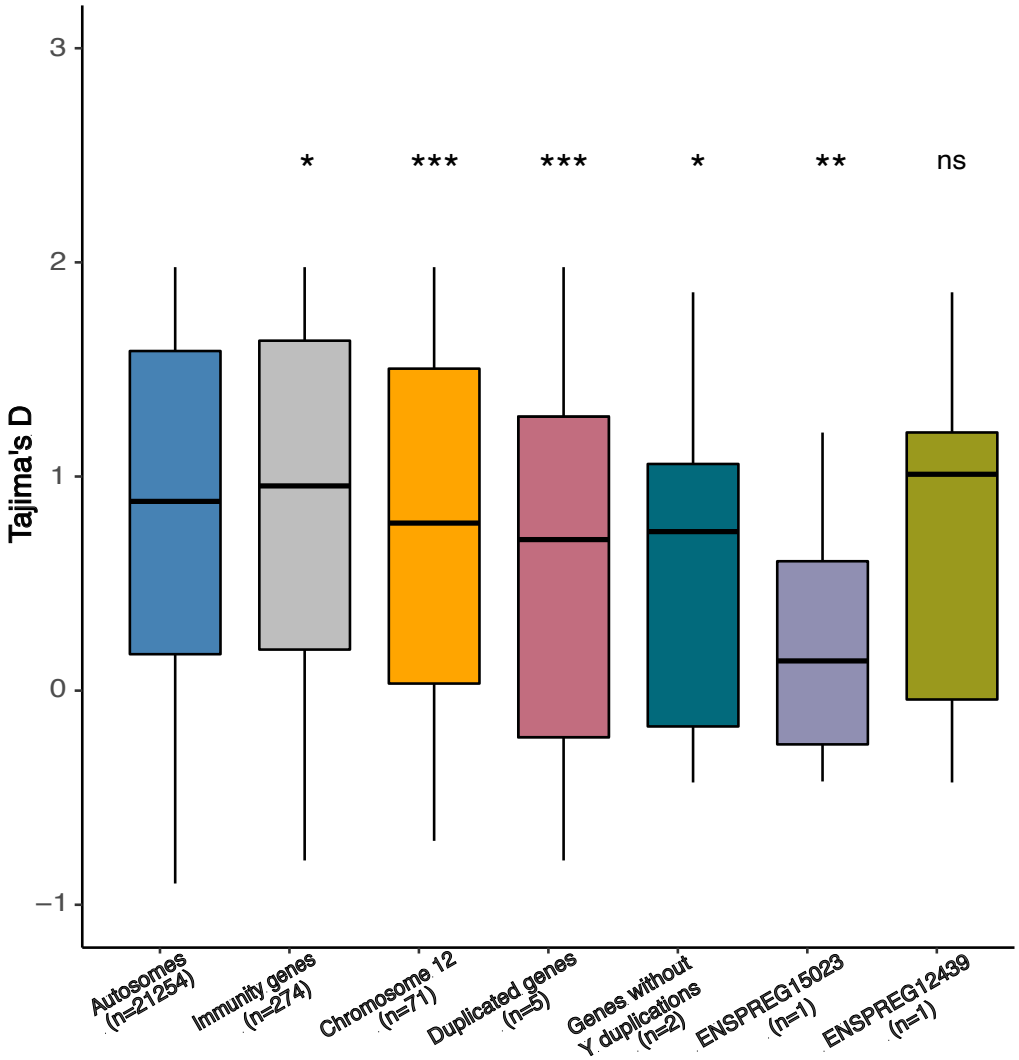

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580 **Fig. 2.** Distribution of Tajima's D among different gene categories. Significant differences to
581 autosomal distribution are indicated (Wilcoxon rank-sum test, *P < 0.01, **P < 0.001, ***P <
582 0.0001, ns: non-significant).

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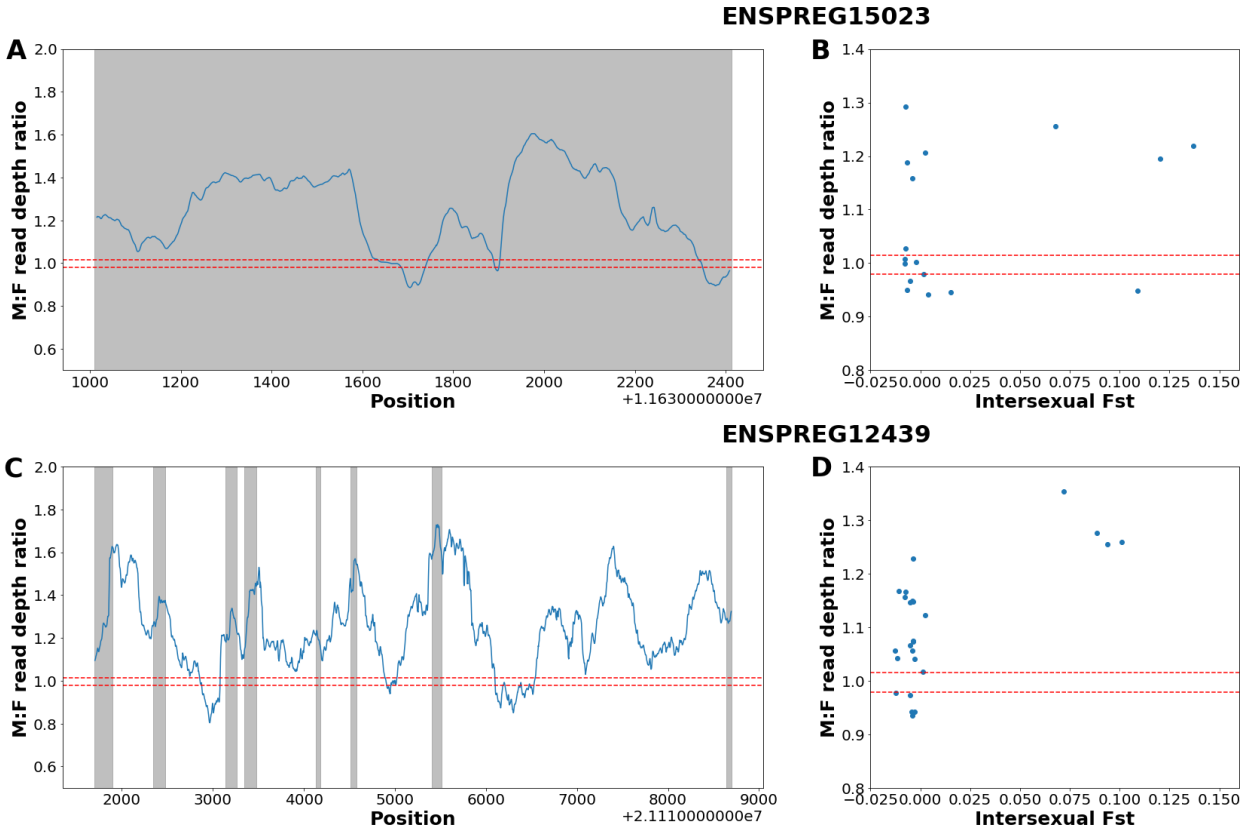

585
586
587 **Fig. 3.** Normalized M:F read depth ratio for ENSPREG15023 (panels A and B) and
588 ENSPREG12439 (panels C and D). Left panels show M:F read depth as a function of physical
589 position (in Mb), with coding regions shaded in grey. Panels on the right show intersexual F_{ST}
590 and M:F read depth ratio for each SNP. 95% autosomal confidence intervals for M:F read depth
591 are indicated by horizontal red lines.

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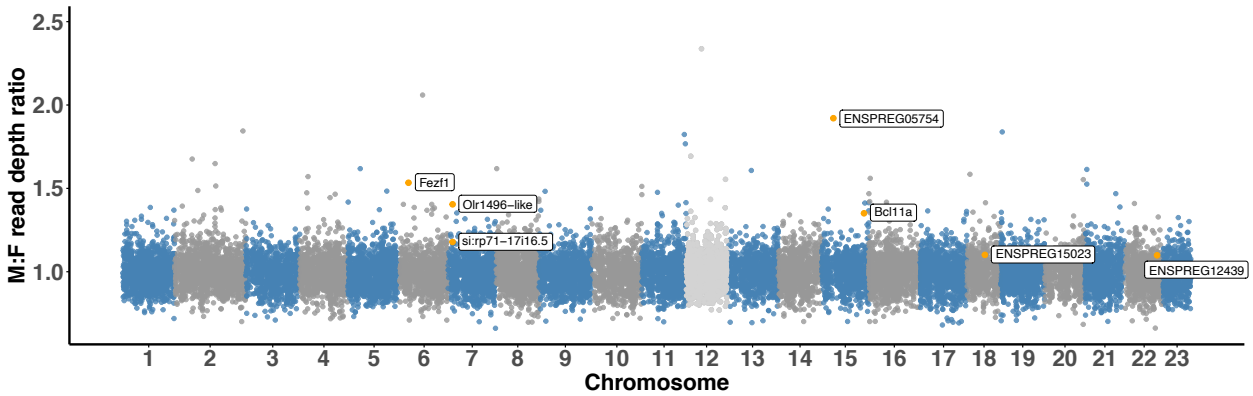

Supplementary Fig. 5. Normalized M:F read depth ratio, with seven sexually differentiated genes indicated in orange. Steel blue and grey: autosomes; Light grey: sex chromosome (Chromosome 12).