SEXUAL CONFLICT OVER SURVIVAL IN TRINIDADIAN GUPPIES

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ABSTRACT

Sexual conflict over survival produces two distinct population genetic signatures. Fluctuating selection in males and females produces balancing selection. Additionally, at conception, allele frequencies are the same in males and females. However, loci with alleles that benefit the survival of one sex at some survival cost to the other should diverge over the course of a generation. We therefore expect that sexual conflict over survival would produce both signatures of allelic differentiation between the sexes and balancing selection. However, given the substantial mortality costs required to produce allelic differences between males and females, it remains unclear how many loci within the genome, if any at all, experience significant sexual conflict over survival. We assessed the genomes of 120 wild-caught guppies, which are expected to experience substantial predation- and pathogen-induced mortality. We identified a core list of 15 high confidence genes that show allelic differences between male and female adults. However, eight of these show evidence of having duplicated copies on the Y chromosome, suggesting that the male-specific region of the guppy Y chromosome may act as a hotspot for the resolution of conflict. We recovered just seven genes with significant male-female allelic differentiation without evidence of Y duplication, and these show elevated Tajima's D, consistent with balancing selection from sexual conflict. Only one of these seven genes, Puf60b, shows substantial intersexual F_{ST}. Puf60b has roles in cognition and the immune system, suggesting substantial ongoing, unresolved sexual conflict related to predator and pathogen avoidance strategies.

Intra-locus sexual conflict, where an allele increases the fitness of one sex at a cost to the other, leaves distinct genomic signatures (Mank 2017). All forms of intra-locus sexual conflict are expected to result in balancing selection, as alleles are selected for or against depending on whether they are present in males or females (Foerster et al. 2007; Johnston et al. 2013; Connallon and Clark 2014; Barson et al. 2015; Hawkes et al. 2016; 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 and 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.

However, many factors can produce balancing selection in addition to sexual conflict, and a signature of balancing selection alone is not sufficient to identify conflict loci within the genome. 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 and Kirkpatrick 2016; Lucotte et al. 2016; Flanagan and Jones 2017; Dutoit et al. 2018; Wright et al. 2018; Wright et al. 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. We would expect that sexual conflict over survival would produce both a signature of allelic differentiation between the sexes and balancing selection, and these two measures together offer a powerful approach to identify potentially sexually antagonistic loci within the genome.

Intra-locus conflict over survival implies a significant mortality cost each generation. Modelling and simulation methods (Bissegger et al. 2019; Kasimatis et al. 2019; Ruzicka et al. 2020) 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. 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 actually are the result of sequencing homology

between autosomal and sex-linked loci (Bissegger et al. 2019; Kasimatis et al. 2019; Ruzicka et al. 2020). The Y chromosome may preferentially retain duplicates that play an important role in male development or fitness (Bachtrog 2013; Carvalho et al. 2015), and the process of Y duplication ironically offers a route to sexual conflict resolution even though it may lead to the mistaken perception of unresolved conflict acting on autosomal loci.

It therefore remains unclear how common and pervasive sexual conflict over survival is in natural populations, or what types of loci, if any, it is expected to target. Here we assess the potential for intersexual F_{ST} based on a sample of 120 wild-caught adult guppies, which would be expected to experience substantial predation- and pathogen-induced mortality. We identified a core list of 15 high confidence genes that show evidence of allelic differences between adult males and females. Of these, eight show evidence of Y duplicates, suggesting that the Y chromosome in guppies may indeed act as a hotspot for the resolution of conflict. We recovered just seven genes with evidence of male-female allelic differentiation without evidence of Y duplication, suggesting the number of loci subject to sex-specific mortality selection in wild guppies is relatively small. Importantly, these genes show elevated Tajima's D, consistent with expectations for balancing selection due to sexual conflict. Six of the seven genes exhibit modest levels of allelic differentiation, and only one, Puf60b, shows F_{ST} levels consistent with high selection coefficients. Importantly, all high F_{ST} SNPs in this gene are localized within one exon. Puf60b is broadly expressed, and has both neurological and immune system functions (Low et al. 2017; Kew et al. 2020). This, in combination with the differences between female and male guppies in predation risk and predator avoidance (Magurran and Nowak 1991; Croft et al. 2006) as well as pathogen infection and avoidance (Stephenson et al. 2015), suggests that sexual conflict over survival may primarily target loci that play a role in behavior, cognition and the immune system.

Results

We individually sequenced 60 male and 60 female wild-caught adults from three rivers in Trinidad to ~30X coverage in males and ~20X coverage in females after filtering, trimming and

113 quality control (see Materials and Methods for further details). The greater read depth in males
114 was designed to aid detection of the Y chromosome, which is present in only one copy in each
115 genome.

116 Because the size of the non-recombining region of the sex chromosome varies across guppy
117 populations (Wright et al. 2017; Almeida et al. 2020), and sex chromosome divergence results
118 in allelic differences on the X and Y between males and females, we confined all our analyses to
119 autosomal sequence, although we present the X chromosome (Chromosome 12) here in figures
120 for comparison. From our sequencing data, we obtained 8,054,872 biallelic high quality filtered
121 autosomal SNPs. We further filtered these to 268,416 SNPs present in annotated autosomal
122 coding sequence. We focused on coding sequence for three reasons. First, non-coding
123 sequence is enriched for repetitive elements, and the accumulation of repetitive elements on
124 the ancestral guppy Y chromosome (Almeida et al. 2020) would lead to male-specific SNPs in
125 repetitive elements that could bias our estimates of Y duplications. Second, previous work has
126 suggested that unresolved sexual conflict occurs primarily in coding sequence (Ruzicka et al.
127 2019), and this fits with the substantial evidence that sexual conflict over gene regulation can
128 be relatively quickly resolved through sex-specific gene regulation (Kopp et al. 2000; Mank
129 2017; Wright et al. 2019). Finally, by focusing on functional coding sequence, we are able to
130 determine the potential functional role of genes that are subject to sexual conflict.

131 ***Intersexual F_{ST}***

132 Due to the limited number of loci expected, and the low level of allelic divergence that is
133 tolerable due to the required mortality loads (Kasimatis et al. 2019), it is critical to minimize
134 false positives and to avoid potential genomic biases in allele frequency estimation. In order to
135 reduce the possibility of false positives, we used three independent methods to estimate
136 intersexual F_{ST}. We identified SNPs that were 1) in the top 1% of the autosomal F_{ST} distribution
137 2) were significant after permutation testing of samples (1000 replicates, $P < 0.001$) and 3)
138 showed significant differences in male and female allele frequency based on Fisher's exact test
139 ($P < 0.001$) (Supplementary Fig. 1). We identified 504 autosomal coding sequence SNPs that
140 were significant by all three of these measures (Supplementary Figs. 1 and 2), designated as
141 high intersexual F_{ST} SNPs.

142 **Table 1.** Summary statistics of sexually differentiated genes. Those values that are statistically
143 significant are in bold.

Gene Name	Chrom	Mean F_{ST}[1]	M:F read depth[2]	M:F SNP density[2]	Gene functions[3]
Puf60b	LG20	**0.1386**	0.9759	1.00	Poly-U binding factor, mutations result in neurological conditions
Spata6	LG4	**0.0645**	1.0186	1.00	Spermiogenesis
Syngap1a	LG11	**0.0363**	1.0558	1.00	development of cognition & proper synapse function
MARK4	LG13	**0.0259**	1.0911	1.00	microtubule organization in neuronal cells
ENSPREG13206	LG11	**0.0113**	0.9338	1.00	N/A
ENSPREG21185	LG2	**0.0108**	0.9848	1.00	N/A
ENSPREG12132	LG2	**0.0096**	1.0652	1.00	N/A
Olr1496	LG7	**0.1802**	**1.3854**	1.23	immune response & germline differentiation
Atat1	LG11	**0.1203**	**1.1219**	1.00	sperm flagellar function, microtubules, neuronal migration and maturation, embryogenesis
si:rp71-17i16.5	LG7	**0.0889**	**1.1817**	**3.35**	Phosphatidylinositol 3-kinase signalling, involved in immune, inflammatory and allergic responses
ENSPREG16391	LG14	**0.0462**	**2.0197**	0.96	N/A
Fezf1	LG6	**0.0407**	**1.5379**	1.01	Nervous system development, migration of gonadotropin-releasing hormone neurons
ENSPREG05754	LG15	**0.0244**	**1.6949**	0.99	N/A
ENSPREG18780	LG9	**0.0236**	**1.5771**	0.96	N/A
ENSPREG12441	LG7	**0.0176**	**1.1185**	1.04	N/A

144 1 Significance based on Wilcoxon's rank-sum test ($P < 0.01$), compared to autosomal average.
145 2 Significance based on Fisher's Exact Test ($P < 0.05$), relative to genome-wide M:F SNP density.
146 3 From Gene Ontology (Ashburner et al. 2000; Anon 2021) and Gene Cards (Stelzer et al. 2016).
147

148

149 To further reduce false positives, we identified autosomal coding sequence with ≥ 5 high
150 intersexual F_{ST} SNPs (Tobler et al. 2017; Bissegger et al. 2019), resulting in 15 sexually
151 differentiated genes. For each of the 15 sexually differentiated genes, average intersexual F_{ST}
152 significantly greater than the autosomal average (Table 1, Supplementary Fig. 3).

153 Estimates of intersexual F_{ST} and Tajima's D can be biased due to relatedness of individuals
154 within groups. Although our sampling design was balanced, with 10 females and 10 males
155 collected at two separate sites for each of three rivers across the island of Trinidad (see
156 Almeida et al. 2020 for details), we could not account for relatedness in wild-caught samples.
157 We therefore assessed pairwise kinship coefficients among all our samples using both KING
158 (Manichaikul et al. 2010) and NgsRelate (Korneliussen and Moltke 2015), as implemented in
159 ANGSD (Korneliussen et al. 2014). Neither method identified greater relatedness among male
160 or female samples (Supplementary Fig. 4).

161 ***Y duplications***

162 When mapping whole-genome data to a female reference genome, sequence similarity
163 between the male-specific Y chromosome and the autosomes can lead to the perception of
164 allelic differences between males and females. Recent work has shown that that many genes
165 with allelic sex differences are in fact autosomal loci that are either have recent Y duplicates
166 (Bissegger et al. 2019; Mank et al. 2020) or otherwise display sequence homology to the Y
167 (Kasimatis et al. 2020) . We can use differences in M:F read depth to identify these genes (Hall
168 et al. 2013). Duplication of the complete coding sequence for an autosomal gene on the Y
169 chromosome would produce an average M:F read depth of 1.5 (three copies in males, two in
170 females), and subsequent tandem duplications on the Y would result in M:F read depth > 1.5.
171 Partial Y duplications, or a full duplication followed by significant differentiation, would result in
172 average M:F read depth > 1 and < 1.5.

173 Eight of our 15 sexually differentiated genes showed average M:F read depth significantly > 1
174 (Table 1, Supplemental Fig. 5) consistent with at least a partial duplication of the coding
175 sequence on the Y chromosome. Four of our eight Y-duplicated genes have annotated function,

176 three of which (Olr1496, Atat1 and Fezf1) have a known role in sexual differentiation, and two
177 (Olr1496 and si:rp71-17i16.5) with immune functions (Table 1).

178 Given sufficient evolutionary time, Y duplications will also accumulate male-specific SNPs
179 (Tobler et al. 2017), leading to a signal of elevated M:F SNP density. Of the eight sexually
180 differentiated genes with greater read depth in males, one showed significantly higher M:F SNP
181 density (Table 1), suggesting it is older than the remaining seven Y duplicated genes. This high
182 number of male-specific SNPs in this gene, si:rp71-17i16.5, accounts for the very high spike in
183 significant F_{ST} at the proximal end of Chromosome 7 (Supplemental Fig. 1), illustrating the
184 potential for duplications on the Y chromosome to affect M:F F_{ST} estimates of autosomal
185 paralogs.
186 Across our 15 sexually differentiated genes, we did not observe evidence of duplications to the
187 X chromosome, which would result in three copies in males and four in females and therefore
188 an average M:F read depth of 0.75
189 ***Sexual conflict over survival for autosomal genes***

190 We identified seven sexually differentiated genes without evidence of Y duplication (Table 1),
191 six of which displayed relatively modest levels of intersexual F_{ST},(0.009-0.0645), consistent with
192 the expectation that large numbers of high intersexual F_{ST} loci would require unsustainable
193 levels of sex-specific mortality (Bissegger et al. 2019; Kasimatis et al. 2019; Ruzicka et al. 2020).
194 Genes subject to ongoing sexual conflict are expected to experience balancing selection, which
195 is often measured with Tajima's D (Cheng and Kirkpatrick 2016; Mank 2017; Wright et al. 2018;
196 Bissegger et al. 2019). We compared Tajima's D for these seven genes to the autosomal
197 average, as well as average Tajima's D for genes with known immune function, which are
198 known to be subject to high levels of balancing selection (Ferrer-Admetlla et al. 2008; Andrés et
199 al. 2009; Van Oosterhout 2009; Weedall and Conway 2010). Tajima's D for the seven sexually
200 differentiated genes without evidence of Y duplicates is significantly higher than the autosomal
201 average (Fig. 1), and is also significantly higher than that for immune genes. The combined
202 signatures of both significantly elevated intersexual F_{ST} and Tajima's D are consistent with these
203 genes experiencing sexual conflict over survival, with alleles benefiting the survival of one sex at
204 a survival cost to the other (Mank 2017; Wright et al. 2018).

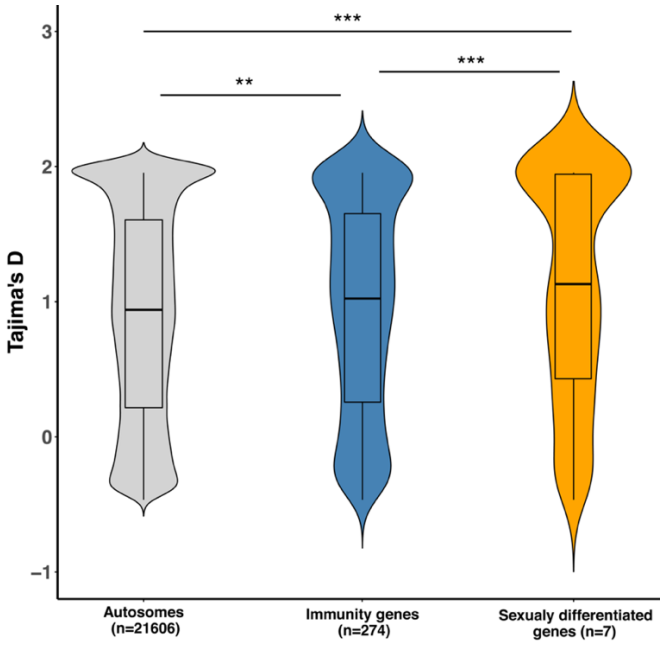

Fig. 1. Distribution of Tajima's D among autosomal genes, immunity genes, and seven sexually differentiated genes without significantly elevated M:F read depth. * indicates pair-wise significantly elevated Tajima's D (Wilcoxon rank-sum test). *P < 0.01, **P < 0.001, ***P < 0.0001

One of these genes, Puf60b, exhibits substantially higher M:F F_{ST} (0.1386). In order to understand the nature of sexual conflict at this locus, and to validate our results, we mapped our intersexual F_{ST} SNPs to this coding sequence, identifying 8 high F_{ST} SNPs, all localized in exon 6 of this gene (Fig. 2A). We also verified the M:F read depth for these SNPs (Fig. 2B), which do not show elevated read depth in males and is therefore inconsistent with Y duplication. Of the eight high intersexual F_{ST} SNPs, seven are synonymous, and just one is non-synonymous (Fig. 2A). The single non-synonymous SNP results in an amino acid change from aspartic acid to glutamic acid, two amino acids with broadly similar biochemical properties.

We also examined sex-differences in haplotype structure for this exon (Fig. 2C), recovering two major haplotypes. Haplotype 1 includes the non-synonymous SNP for aspartic acid (Asp), and Haplotype 2 contains the glutamic acid SNP (Glu). All rare haplotypes include Glu, and were included as Haplotype 2. There are significantly more Haplotype 1 homozygotes among females than expected, and Haplotype 1/Haplotype 2 heterozygotes are significantly under-represented ($P < 0.008$ in both cases, based on χ^2, 1.d.f, Fig. 2D, Supplementary Table 1). The relative

survival for female heterozygotes is 0.315 (Fig. 2D, Supplementary Table 2). In contrast, there are significantly less Haplotype 1 homozygotes among males and significantly more Haplotype 1/Haplotype 2 heterozygotes ($P < 0.0005$ in both cases, χ^2, 1.d.f.). The relative inferred survival for Haplotype 1 homozygotes in males is just 0.158. In both sexes, we observed no Haplotype 2 homozygotes, although this is marginally non-significant when assessing the sexes separately ($P = 0.061$, χ^2, 1.d.f.), if we assume concordant selection against Haplotype 2 homozygotes, this becomes significant ($P = 0.008$, χ^2, 1.d.f.) due to the increased sample size.

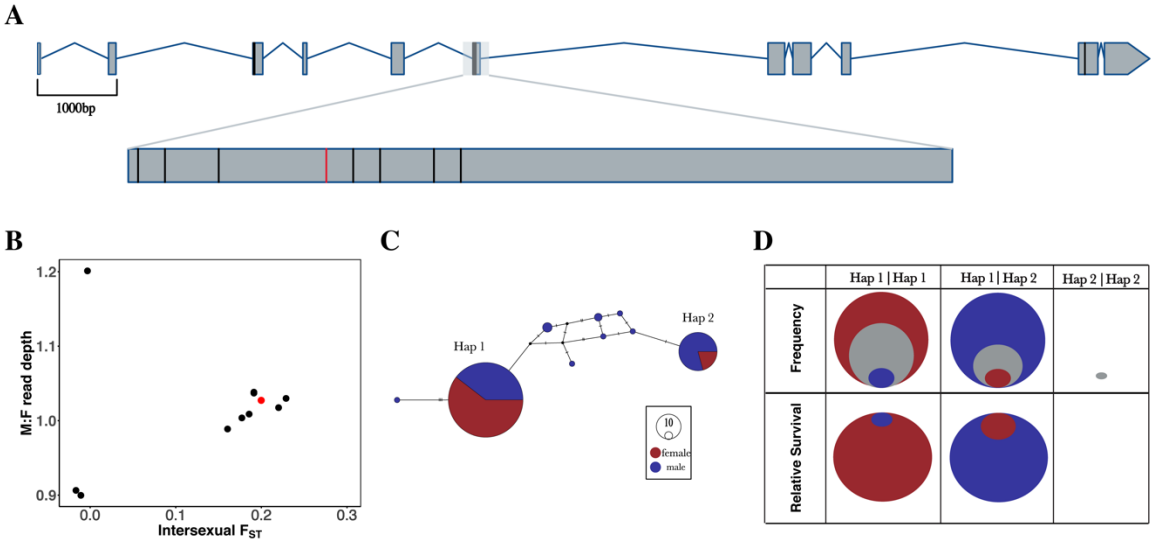

Fig. 2. (A) All high F_{ST} SNPs localize in Exon 6 of Puf60b. Gene structure shown, with exons (grey boxes) and introns (blue lines). Within Exon 6, vertical black lines represent synonymous mutations, vertical red line represents non-synonymous mutation (Asp→ Glu). (B) F_{ST} and M:F read depth for all SNPs in Puf60b. Of 11 SNPs in Puf60b coding sequence, 8 high intersexual SNPs do not show evidence of elevated M:F read depth. Synonymous SNPs in black, non-synonymous SNP in red. (C) Haplotype network recovers two main haplotypes of Puf60b, with Haplotype 1 includes the aspartic acid, and Haplotype 2 contains glutamic acid SNP variant. Each circle represents one haplotype, and rare haplotypes all contain glutamic acid (Haplotype 2). The size of circle is proportion to the number of individuals belonging to that haplotype. Red and blue of each circle represent the number of female and male individuals of each haplotype, respectively. Bars show mutation steps between haplotypes. (D) Genotype frequencies and relative survival for males (blue) and females (red), compared to expected (grey). See Supplementary Tables 1 and 2 for further details.

In order to understand the mortality load these allele frequencies might impose, we calculated the proportion of individuals of each sex that would be expected to be lost due to selection

249 over survival based on Pub60b genotypes (Fig. 2D, Supplementary Table 2). Selection at Pub60b
250 would remove 39% of females and 54% of males at each generation. The frequencies of
251 Haplotype 1 and Haplotype 2 did not differ between High and Low Predation populations
252 (Supplementary Tables 3 and 4, Fisher's Exact Test, P = 0.36).

253 We next assessed whether we observe the evidence of sexual conflict over survival in Puf60b in
254 our lab population, which experiences no predation, as well as greatly reduced parasite load
255 and pathogens due to our biosafety protocols. To do this, we identified the Puf60b coding
256 sequence from previously collected RNA-Seq data for three families (Darolti et al. 2020).
257 Haplotype 2 was not present in any of our lab samples. We also used this data to quantify
258 expression in males and females, and found that Puf60b is expressed at a significantly higher
259 level in males (Supplementary Fig. 6).

260 ***Functional annotations***

261 Of the seven genes without evidence of Y duplications, four of the seven genes have functional
262 annotations in the *Danio rerio* Gene Ontology (Ashburner et al. 2000; Anon 2021) or GeneCards
263 (Stelzer et al. 2016) databases. Three, Puf60b, Syngap1a and MARK4 have neurological
264 functions (Table 1). Puf60b is broadly expressed, and has additional immunological functions
265 (Kew et al. 2020).

266

267 **Discussion**

268 Using a conservative approach, we identified 504 coding sequence SNPs in the guppy
269 autosomal genome which showed significant differences in allele frequency between males and
270 females. We used these to identify 15 autosomal genes with significant average intersexual F_{ST}.
271 Our approach, based on the intersection of three statistical methods, reduces the likelihood of
272 false positives and results in a high confidence gene list of intersexual F_{ST} (Table 1,
273 Supplementary Figs. 1, 2, and 3). This list of genes can be used to assess the role of Y
274 duplication in resolving conflict and driving intersexual F_{ST}, and the role of sex differences in
275 mortality in sexual conflict of autosomal genes.

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

277 Over half (8 out of 15) of our high-confidence sexually differentiated genes showed evidence of
278 Y duplications based on elevated male-to-female read depth ratios (Table 1, Supplementary Fig.
279 4). Four of these genes have sex-specific functions based on Gene Ontology and Gene Cards
280 annotations, consistent with theory and empirical studies showing that Y chromosomes can
281 accumulate gene duplicates with male-specific functions (Carvalho et al. 2015; Mahajan and
282 Bachtrog 2017). Specifically, Olr1496 (Olfactory factor 1496-like gene) plays a role in multiple
283 aspects of germline development in *C. elegans* (Cho et al. 2007), Atat1 (Alpha-tubulin N-
284 acetyltransferase 1) plays a role in sperm flagellar functions (Yanai et al. 2020), and Fezf1 (FEZ
285 Family Zink Finger 1) plays an important role in the migration of gonadotropin-releasing
286 neurons (Damla Kotan et al. 2014).

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

296 However, it remains unclear how common Y duplications of autosomal genes occur in younger
297 systems with a far smaller male-specific region. Recent work has suggested that the ancestral
298 non-recombining region of the Y chromosome in guppies is relatively small, spanning at most 5
299 Mb (Darolti et al. 2019; Almeida et al. 2020). Others have failed to recover evidence for even
300 this limited region of Y degeneration (Charlesworth et al. 2020; Fraser et al. 2020). Our findings
301 add further support for a region of recombination suppression across populations on Trinidad,
302 as only a male-specific region of the Y can explain the M:F read depth differences we observe.
303 Moreover, our work suggests that the guppy Y chromosome is dynamic with regard to gene
304 content, and acts as a hotspot for gene duplications with male-specific functions despite its

305 recent origin and small size. It is also possible that Y genes have duplicated to the autosomes,
306 which would also produce a pattern of increased male read depth, although this is arguably less
307 likely. Taken together, our work suggests that even homomorphic sex chromosomes may act as
308 a hotspot of sexual conflict resolution. Moreover, our results further emphasize the importance
309 of accounting for Y gene duplications in scans for M:F F_{ST}, as over half of our sexually
310 differentiated genes show evidence of Y duplication, rather than sex-specific mortality, as the
311 cause of allelic differences between the sexes.

312 These eight Y-duplicated genes are not present in our previous list of Y-linked genes based on
313 male-specific sequence (Almeida et al. 2020), or in other similar analyses (Fraser et al. 2020).
314 This is not surprising, as duplications, particularly if recent, will still retain substantial homology
315 to the autosomal copy and will not be detected when bioinformatically identifying sequence
316 that is unique to male genomes. Consistent with recent duplications and limited divergence,
317 seven of our eight Y-duplicated genes do not exhibit elevated average M:F SNP density (Table
318 1).

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

327 ***Sexual conflict over survival targets neurological and immune functions***

328 Intra-locus sexual selection over survival or viability leads to allele frequency differences
329 between the sexes over the course of a generation, as an allele increases the survival of one sex
330 at a mortality cost to the other (Mank 2017; Wright et al. 2018; Kasimatis et al. 2019). The
331 significant mortality costs required each generation to generate allele frequency differences
332 between the sexes preclude large numbers of genes with significant M:F F_{ST} (Bissegger et al.

333 2019; Kasimatis et al. 2019; Ruzicka et al. 2020). At most, we might expect a limited number of
334 loci with significant allelic differentiation between males and females, and this would be most
335 evidence in wild species where males and females experience differences in predation, parasite
336 or pathogen loads. Consistent with this, we observe just seven loci in the genome with high
337 confidence evidence of sexual conflict over survival based on M:F F_{ST}. Additionally, these seven
338 genes exhibit elevated levels of balancing selection (Fig. 1) as expected under sexual conflict
339 over survival. Six of these seven genes have quite modest F_{ST} levels (ranging from 0.0096 to
340 0.0645).

341 Puf60b F_{ST} is substantially higher (0.1386), and requires a mortality load of 39% in females and
342 54% in males. Although high, it is worth noting that survival is significantly greater in females,
343 which would allow for significant population growth despite the strong levels of selection.
344 These results were similar for both High and Low Predation populations, and suggest similar
345 selection coefficients across all populations in Trinidad. However, we did not detect Haplotype
346 2 in our lab colony, originally collected from the Quare River. This colony has been maintained
347 in order to maximize genetic diversity, and it may be that the loss of predation, and the
348 reduction in pathogen and parasite loads associated with our biosecurity protocols have
349 eliminated sexual conflict and therefore the selection for heterozygotes in males. However, we
350 also cannot rule out the possibility that Haplotype 2 has been lost due to genetic drift.

351 Recent work in salmon (Barson et al. 2015) has suggested that some sexually antagonistic loci
352 experience sex-specific dominance effects. This reduces the mortality load associated with
353 sexual conflict over survival. Our genotype data (Supplemental Tables 1 & 2) suggests that this
354 is not the case for Puf60b, as male heterozygotes have the highest survival relative to both
355 homozygous genotypes. This is inconsistent with sex-specific dominance.

356 Four of the seven fully autosomal sexually differentiated genes have cognitive or neurological
357 functions. Puf60b (Poly(U) binding splicing factor 60) is related to RNA-binding, mRNA
358 processing and RNA-splicing and mutations can result in neurological conditions (El Chehadeh
359 et al. 2016). Syngap1 (Synaptic Ras GTPase-activating protein 1), encodes a Ras GTPase
360 activating protein, regulating synaptic plasticity and neuronal homeostasis, and mutations
361 affect forebrain and cognitive development (Ozkan et al. 2014). Mark4 (microtubule affinity

362 regulating kinase 4) has been shown to be involved in microtubule organization in neuronal
363 cells (Trinczek et al. 2004). Puf60b has also been recently implicated as well in immune system
364 functions (Kew et al. 2020).

365 At first it may seem counterintuitive that sexual conflict over survival would preferentially
366 target neurological genes. However, in light of the differences between females and males in
367 predation risk and predator avoidance (Magurran and Nowak 1991; Croft et al. 2006) as well as
368 pathogen infection and avoidance (Stephenson et al. 2015). Our results suggest that sexual
369 conflict over survival may primarily target genes that play a role in behavior, cognition and the
370 immune system.

371

372 ***Concluding remarks***

373 We found profound sexual conflict over survival at one locus in the genome, Puf60b, and
374 limited evidence at six additional loci in wild-caught guppy samples from across the island of
375 Trinidad. Our results highlight the limited but important role of sexual conflict over survival in
376 shaping patterns of genomic diversity. Additionally, we identified eight loci that have at least
377 partial Y homology, indicating the potential of the guppy Y chromosome to act as a hotspot of
378 sexual conflict resolution.

379

380 **Materials and Methods**

381 ***Data Collection and Genotyping***

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

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

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

395 We called genotypes across all the samples using the `mpileup` function from SAMtools-1.9
396 with the following parameters: --min-MQ 20 --min-BQ 20 --skip-indels -a FORMAT/AD,
397 FORMAT/DP. After genotyping, we used VCFtools v0.1.16 (Danecek et al. 2011) to exclude SNPs
398 that had either : (1) genotype quality < 20; (2) sequencing depth <0.5x or >10x of average
399 depth; (3) missing data in > 10% of individuals or (4) minor allele frequency < 0.05. In total, the
400 autosomal filtered SNP dataset consisted of 8,054,872 biallelic SNPs. We extracted 268,416
401 SNPs in annotated coding sequences (Ensembl build Guppy Female 1.0) for downstream
402 analysis. Finally, we confined our analysis to autosomal genes, but included the X chromosome
403 (Chromosome 12) in figures as a means of comparison.

404 ***Intersexual F_{ST}***

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

415 Using approaches to further limit false positives (Tobler et al. 2017; Bissegger et al. 2019), we
416 identified 15 genes with ≥ 5 high intersexual F_{ST} SNPs, which we designated as sexually

417 differentiated genes. We calculated average intersexual F_{ST} for all genes using VCFtools v0.1.16,
418 respectively. We used Wilcoxon rank-sum test to indicate statistical difference in intersexual F_{ST}
419 between autosomal genes and other categorical genes (sexually differentiated genes and genes
420 on the sex chromosome).

421 ***Haplotype network***

422 To further investigate allelic differences of Puf60b, we used SHAPEIT4 v4.2.0 (Delaneau et al.
423 2019) with default parameters to phase all the samples. We then reconstructed the haplotype
424 sequence of Puf60b for all samples. We used MEGAX (Kumar et al. 2018) for multiple sequence
425 alignment, and the inferred haplotype network using PopART v1.7 (Leigh JW and Bryant D
426 2015).

427 ***Relatedness Inference***

428 In order to avoid biases in calculating intersexual allele frequency differences due to
429 relatedness, we used KING (Manichaikul et al. 2010) to infer the pairwise degree of relatedness
430 between individuals from estimated kinship coefficients. We first converted genotype data
431 from the raw, unfiltered SNPs dataset to plink binary format using PLINK (Purcell et al. 2007). In
432 order to avoid potential biases from KING software (Ramstetter et al. 2017) and validations, we
433 also used NgsRelate (Korneliussen and Moltke 2015), implemented in ANGSD (Korneliussen et
434 al. 2014) to infer genetic relatedness coefficients for each pair of individuals.

435 ***Assessing Y duplications of sexually differentiated genes***

436 When using a female reference genome, reads from genes which have duplicates to the male-
437 specific region of the Y chromosome will map back to original autosomal or X chromosome
438 regions, resulting in elevated M:F coverage ratio (Bissegger et al. 2019; Mank et al. 2020). For
439 example, if an autosomal gene has one Y duplication, we would expect three copies in males
440 (two autosomal and one Y-linked) and two copies in females, and therefore an average M:F
441 read depth of 1.5. We first calculated M:F read depth for each coding sequence SNP from
442 genotype data, as male coverage/female coverage, correcting for differences in average
443 genomic read depth between males and females.

444 Genes on the Y chromosome will accumulate male-specific mutations over time, leading to an
445 increased number of male-specific mutations as well as elevated M:F SNP density (Bissegger et
446 al. 2019; Mank et al. 2020). M:F SNP density for each gene was calculated as number of male
447 SNPs /number of female SNPs.

448 In order to validate the M:F read depth ratio of sexually differentiated genes, we first extracted
449 coding sequence of our 15 sexually differentiated genes. We then calculated male and female
450 normalized read depth for each gene, based on male and female pooled reads. Additionally, we
451 calculated M:F read depth from our pools for each high FST SNP identified in reads mapping to
452 Puf60b.

453 ***Tajima's D***

454 Based on the filtered genotype data, we calculated Tajima's D for the coding sequence of all
455 autosomal genes using VCFtools v1.19. We compared mean Tajima's D for autosomal genes
456 (minus those with immune function), genes with immune function (defined following Wright et
457 al. 2017, Wright et al. 2018), and our seven sexually differentiated genes.

458 ***Expression and diversity in lab colony***

459 We used the *Poecilia reticulata* RNA-Seq data from Darolti et al., (2020) to first determine
460 differences in male and female expression for Puf60b. We first BLASTed the reference sequence
461 for Puf60b against our *de novo* transcriptome (see Darolti et al. 2019 for details), and
462 determined FPKM for male and female samples by mapping reads back to this contig. We also
463 mapped reads back to the Ensembl reference genome to determine isoform variation, and
464 examined this RNA-Seq data to determine whether we observe Haplotype 2 in our lab colony.

465 ***Functional Annotations***

466 The small number of sexually differentiated genes precludes Gene Ontology enrichment
467 analysis. We therefore cataloged functional annotations from the Danio rerio Gene Ontology
468 (Ashburner et al. 2000; Anon 2021) and Gene Cards (Stelzer et al. 2016).

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References Cited:

Almeida P, Sandkam BA, Morris J, Darolti I, Breden F, Mank JE. 2020. Divergence and Remarkable Diversity of the Y Chromosome in Guppies. *Mol. Biol. Evol.*:1–15.

Andrés AM, Hubisz MJ, Indap A, Torgerson DG, Degenhardt JD, Boyko AR, Gutenkunst RN, White TJ, Green ED, Bustamante CD, et al. 2009. Targets of balancing selection in the human genome. *Mol. Biol. Evol.*

Anon. 2021. The Gene Ontology resource: enriching a GOld mine. *Nucleic Acids Res.*

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. 2000. Gene ontology: Tool for the unification of biology. *Nat. Genet.*

Bachtrog D. 2013. Y-chromosome evolution: Emerging insights into processes of Y-chromosome degeneration. *Nat. Rev. Genet.* 14:113–124.

Barson NJ, Aykanat T, Hindar K, Baranski M, Bolstad GH, Fiske P, Jacq C, Jensen AJ, Johnston SE, Karlsson S, et al. 2015. Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. *Nature* 528:405–408.

Bissegger M, Laurentino TG, Roesti M, Berner D. 2019. Widespread intersex differentiation across the stickleback genome – The signature of sexually antagonistic selection? *Mol. Ecol.*:1–10.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120.

Carvalho AB, Vicoso B, Russo CAM, Swenor B, Clark AG. 2015. Birth of a new gene on the Y chromosome of Drosophila melanogaster. *Proc. Natl. Acad. Sci. U. S. A.*

Charlesworth D, Bergero R, Graham C, Gardner J, Yong L. 2020. Locating the sex determining region of linkage group 12 of guppy (Poecilia reticulata). *G3 Genes, Genomes, Genet.*

El Chehadeh S, Kerstjens-Frederikse WS, Thevenon J, Kuentz P, Bruel AL, Thauvin-Robinet C, Bensignor C, Dollfus H, Laugel V, Rivière JB, et al. 2016. Dominant variants in the splicing factor PUF60 cause a recognizable syndrome with intellectual disability, heart defects and short stature. In: European Journal of Human Genetics.

Cheng C, Kirkpatrick M. 2016. Sex-Specific Selection and Sex-Biased Gene Expression in Humans and Flies. *PLoS Genet.*

Cho S, Rogers KW, Fay DSS. 2007. The C. elegans Glycopeptide Hormone Receptor Ortholog, FSHR-1, Regulates Germline Differentiation and Survival. *Curr. Biol.*

Connallon T, Clark AG. 2014. Balancing selection in species with separate sexes: Insights from

fisher's geometric model. *Genetics*.
Croft DP, Morrell LJ, Wade AS, Piyapong C, Ioannou CC, Dyer JRG, Chapman BB, Wong Y, Krause J. 2006. Predation risk as a driving force for sexual segregation: A cross-population comparison. *Am. Nat.*
Damla Kotan L, Ian Hutchins B, Ozkan Y, Demirel F, Stoner H, Cheng PJ, Esen I, Gurbuz F, Kenan Bicakci Y, Mengen E, et al. 2014. Mutations in FEZF1 cause kallmann syndrome. *Am. J. Hum. Genet.*
Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, et al. 2011. The variant call format and VCFtools. *Bioinformatics*.
Darolti I, Wright AE, Mank JE. 2020. Guppy Y Chromosome Integrity Maintained by Incomplete Recombination Suppression. *Genome Biol. Evol.* 12:965–977.
Darolti I, Wright AE, Sandkam BA, Morris J, Bloch NI, Farré M, Fuller RC, Bourne GR, Larkin DM, Breden F, et al. 2019. Extreme heterogeneity in sex chromosome differentiation and dosage compensation in livebearers. *Proc. Natl. Acad. Sci. U. S. A.* 116:19031–19036.
Delaneau O, Zagury JF, Robinson MR, Marchini JL, Dermitzakis ET. 2019. Accurate, scalable and integrative haplotype estimation. *Nat. Commun.*
Dutoit L, Mugal CF, Bolívar P, Wang M, Nadachowska-Brzyska K, Smeds L, Yazdi HP, Gustafsson L, Ellegren H. 2018. Sex-biased gene expression, sexual antagonism and levels of genetic diversity in the collared flycatcher (Ficedula albicollis) genome. *Mol. Ecol.*
Ferrer-Admetlla A, Bosch E, Sikora M, Marquès-Bonet T, Ramírez-Soriano A, Muntasell A, Navarro A, Lazarus R, Calafell F, Bertranpetit J, et al. 2008. Balancing Selection Is the Main Force Shaping the Evolution of Innate Immunity Genes. *J. Immunol.*
Flanagan SP, Jones AG. 2017. Genome-wide selection components analysis in a fish with male pregnancy. *Evolution (N. Y).*
Foerster K, Coulson T, Sheldon BC, Pemberton JM, Clutton-Brock TH, Kruuk LEB. 2007. Sexually antagonistic genetic variation for fitness in red deer. *Nature*.
Fraser BA, Whiting JR, Paris JR, Weadick CJ, Parsons PJ, Charlesworth D, Bergero R, Bemm F, Hoffmann M, Kottler VA, et al. 2020. Improved Reference Genome Uncovers Novel Sex-Linked Regions in the Guppy (Poecilia reticulata). *Genome Biol. Evol.* 12:1789–1805.
Hall AB, Qi Y, Timoshevskiy V, Sharakhova M V., Sharakhov I V., Tu Z. 2013. Six novel Y chromosome genes in Anopheles mosquitoes discovered by independently sequencing males and females. *BMC Genomics*.
Hawkes MF, Gamble CE, Turner ECR, Carey MR, Wedell N, Hosken DJ. 2016. Intralocus sexual conflict and insecticide resistance. *Proc. R. Soc. B Biol. Sci.*
Johnston SE, Gratten J, Berenos C, Pilkington JG, Clutton-Brock TH, Pemberton JM, Slate J. 2013. Life history trade-offs at a single locus maintain sexually selected genetic variation. *Nature*.
Kasimatis KR, Abraham A, Ralph PL, Kern AD, Capra JA, Phillips PC. 2020. Evaluating Human Autosomal Loci for Sexually Antagonistic Viability Selection in Two Large Biobanks. *Genetics*.
Kasimatis KR, Ralph PL, Phillips PC. 2019. Limits to genomic divergence under sexually antagonistic selection. *G3 Genes, Genomes, Genet.* 9:3813–3824.
Kew C, Huang W, Fischer J, Ganesan R, Robinson N, Antebi A. 2020. Evolutionarily conserved regulation of immunity by the splicing factor RNP-6/PUF60. *Elife* 9:1–23.
Koerich LB, Wang X, Clark AG, Carvalho AB. 2008. Low conservation of gene content in the Drosophila Y chromosome. *Nature*.

557 Kopp A, Duncan I, Carroll SB. 2000. Genetic control and evolution of sexually dimorphic
558 characters in Drosophilia. *Nature*.
559 Korneliussen TS, Albrechtsen A, Nielsen R. 2014. ANGSD: Analysis of Next Generation
560 Sequencing Data. *BMC Bioinformatics* 15:1–13.
561 Korneliussen TS, Moltke I. 2015. NgsRelate: A software tool for estimating pairwise relatedness
562 from next-generation sequencing data. *Bioinformatics*.
563 Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary
564 genetics analysis across computing platforms. *Mol. Biol. Evol.*
565 Künstner A, Hoffmann M, Fraser BA, Kottler VA, Sharma E, Weigel D, Dreyer C. 2016. The
566 genome of the trinidadian guppy, Poecilia reticulata, and variation in the Guanapo
567 population. *PLoS One* 11:1–25.
568 Leigh JW, Bryant D. 2015. POPART: full-featrure software for haplotype network construction.
569 *Methods Ecol. Evol.*
570 Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform.
571 *Bioinformatics*.
572 Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R.
573 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*.
574 Lonn E, Koskela E, Mappes T, Mokkonen M, Sims AM, Watts PC. 2017. Balancing selection
575 maintains polymorphisms at neurogenetic loci in field experiments. *Proc. Natl. Acad. Sci.*
576 *U. S. A.*
577 Low KJ, Ansari M, Abou Jamra R, Clarke A, El Chehadeh S, Fitzpatrick DR, Greenslade M,
578 Henderson A, Hurst J, Keller K, et al. 2017. PUF60 variants cause a syndrome of ID, short
579 stature, microcephaly, coloboma, craniofacial, cardiac, renal and spinal features. *Eur. J.*
580 *Hum. Genet.*
581 Lucotte EA, Laurent R, Heyer E, Ségurel L, Toupance B. 2016. Detection of allelic frequency
582 differences between the sexes in humans: A signature of sexually antagonistic selection.
583 *Genome Biol. Evol.* 8:1489–1500.
584 Magurran AE, Nowak MA. 1991. Another battle of the sexes: The consequences of sexual
585 asymmetry in mating costs and predation risk in the guppy, Poecilia reticulata. *Proc. R. Soc.*
586 *B Biol. Sci.*
587 Mahajan S, Bachtrog D. 2017. Convergent evolution of y chromosome gene content in flies. *Nat.*
588 *Commun.*8
589 Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen WM. 2010. Robust
590 relationship inference in genome-wide association studies. *Bioinformatics*.
591 Mank JE. 2017. Population genetics of sexual conflict in the genomic era. *Nat. Rev. Genet.*
592 18:721–730.
593 Mank JE, Shu JJ, Wright AE. 2020. Signature of sexual conflict is actually conflict resolved.
594 *Mol. Ecol.*
595 Van Oosterhout C. 2009. A new theory of MHC evolution: Beyond selection on the immune
596 genes. *Proc. R. Soc. B Biol. Sci.*
597 Ozkan ED, Creson TK, Kramár EA, Rojas C, Seese RR, Babyan AH, Shi Y, Lucero R, Xu X,
598 Noebels JL, et al. 2014. Reduced cognition in Syngap1 mutants is caused by isolated
599 damage within developing forebrain excitatory neurons. *Neuron*.
600 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, De
601 Bakker PIW, Daly MJ, et al. 2007. PLINK: A tool set for whole-genome association and
602 population-based linkage analyses. *Am. J. Hum. Genet.*

603 Ramstetter MD, Dyer TD, Lehman DM, Curran JE, Duggirala R, Blangero J, Mezey JG,
604 Williams AL. 2017. Benchmarking relatedness inference methods with genome-wide data
605 from thousands of relatives. *Genetics*.
606 Ruzicka F, Dutoit L, Czuppon P, Jordan CY, Li X, Olito C, Runemark A, Svensson EI, Yazdi
607 HP, Connallon T. 2020. The search for sexually antagonistic genes: Practical insights from
608 studies of local adaptation and statistical genomics. *Evol. Lett.* 4:398–415.
609 Ruzicka F, Hill MS, Pennell TM, Flis I, Ingleby FC, Mott R, Fowler K, Morrow EH, Reuter M.
610 2019. Genome-wide sexually antagonistic variants reveal long-standing constraints on
611 sexual dimorphism in fruit flies.
612 Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Iny Stein T, Nudel R,
613 Lieder I, Mazor Y, et al. 2016. The GeneCards suite: From gene data mining to disease
614 genome sequence analyses. *Curr. Protoc. Bioinforma.*
615 Stephenson JF, Van Oosterhout C, Mohammed RS, Cable J. 2015. Parasites of Trinidadian
616 guppies: Evidence for sex- and age-specific trait-mediated indirect effects of predators.
617 *Ecology*.
618 Tobler R, Nolte V, Schlötterer C. 2017. High rate of translocation-based gene birth on the
619 Drosophila Y chromosome. *Proc. Natl. Acad. Sci. U. S. A.* 114:11721–11726.
620 Trinczek B, Brajenovic M, Ebneth A, Drewes G. 2004. MARK4 Is a Novel Microtubule-
621 associated Proteins/Microtubule Affinity-regulating Kinase That Binds to the Cellular
622 Microtubule Network and to Centrosomes. *J. Biol. Chem.*
623 Weedall GD, Conway DJ. 2010. Detecting signatures of balancing selection to identify targets of
624 anti-parasite immunity. *Trends Parasitol.*
625 Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure.
626 *Evolution (N. Y).*
627 Wright AE, Darolti I, Bloch NI, Oostra V, Sandkam B, Buechel SD, Kolm N, Breden F, Vicoso
628 B, Mank JE. 2017. Convergent recombination suppression suggests role of sexual selection
629 in guppy sex chromosome formation. *Nat. Commun.* 8.
630 Wright AE, Fumagalli M, Cooney CR, Bloch NI, Vieira FG, Buechel SD, Kolm N, Mank JE.
631 2018. Male-biased gene expression resolves sexual conflict through the evolution of sex-
632 specific genetic architecture. *Evol. Lett.* 2:52–61.
633 Wright AE, Rogers TF, Fumagalli M, Cooney CR, Mank JE. 2019. Phenotypic sexual
634 dimorphism is associated with genomic signatures of resolved sexual conflict. *Mol. Ecol.*
635 Yanai R, Yamashita Y, Umezu K, Hiradate Y, Hara K, Tanemura K. 2020. Expression and
636 localization of alpha-tubulin N-acetyltransferase 1 in the reproductive system of male mice.
637 *J. Reprod. Dev*
638