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Check for updates

DR. YUYING LIN (Orcid ID : 0000-0001-7057-5588)
MISS IULIA DAROLTI (Orcid ID : 0000-0002-5865-4969)
DR. BENJAMIN A SANDKAM (Orcid ID : 0000-0002-5043-9295)
DR. ALISON WRIGHT (Orcid ID : 0000-0003-2479-5250)
PROF. JUDITH MANK (Orcid ID : 0000-0002-2450-513X)

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GENE DUPLICATION TO THE Y CHROMOSOME IN TRINIDADIAN GUPPIES

Yuying Lin[1*], Iulia Darolti[1], Benjamin L. S. Furman[1], Pedro Almeida[2], Benjamin A. Sandkam[1], Felix Breden[3], Alison E. Wright[4], Judith E. Mank[1,5]

1 Department of Zoology and Biodiversity Research Centre, University of British Columbia, Canada

2 Department of Genetics, Evolution and Environment, University College London, United Kingdom

3 Department of Biological Sciences, Simon Fraser University, Canada

4 Ecology and Evolutionary Biology, School of Biosciences, University of Sheffield

5 Biosciences, University of Exeter, Penryn Campus, United Kingdom

***Corresponding author** E-mail: linyuying@zoology.ubc.ca

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ABSTRACT

Differences in allele frequencies at autosomal genes between males and females in a population can result from two scenarios. First, unresolved sexual conflict over survival can produce 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 sequences can create perceived allelic differences between the sexes. 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 between the Y chromosome and the

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autosomes. 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, may nonetheless accumulate genes with male-specific functions.

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

Introduction

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. In addition to producing a signature of allelic differentiation between the sexes, we expect sexual conflict over survival to result in balancing selection in many scenarios (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.,

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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; Wright et al., 2019). 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 with significant allelic differentiation between the sexes, 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 a bioinformatic artefact resulting from sequence homology between autosomal and sex-linked loci (Bissegger et al., 2019; Kasimatis et al., 2019; Mank, Shu, & Wright, 2020; Ruzicka et al., 2020). This could result from two phenomenon. First, the Y chromosome may preferentially retain autosomal duplicates that play an important role in male development or fitness (Bachtrog, 2013; Carvalho et al., 2015). Ironically, this means that the process of Y duplication offers a potential route to sexual conflict resolution even though it may lead to the mistaken perception of unresolved conflict acting on autosomal loci. Alternatively, Y chromosome gene duplicates to the autosomes may be favored to counter the non-adaptive decay of genes in non-recombining regions.

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 are expected to experience substantial predation- and pathogen-induced mortality, some of which may vary between the sexes (Richards et al., 2010; Stephenson et al., 2016). We identified seven genes that show evidence of allelic differences between adult males and females. Of those seven genes, five show evidence of duplicates between the autosomes and the Y, and two have sex-specific functions,

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including germline differentiation and sex hormone signaling. This is surprising, as although the size of the guppy Y varies substantially across populations and related species (Almeida et al., 2021; Darolti et al., 2019; Wright et al., 2017), the conserved, ancestral part of the Y is very small, spanning at most 5 Mb (Almeida et al., 2021; Darolti et al., 2019; Fraser et al., 2020). Despite this, our results suggest that the gene content of the Y chromosome is dynamic.

Results

We individually sequenced 60 male and 60 female wild-caught adults from three rivers in Trinidad to an average of ~30X coverage in males and ~20X coverage in females after filtering, trimming and quality control (see Materials and Methods for further details). The greater read depth in males was designed to aid detection of the Y chromosome, which is present in only one copy in each genome. Because the size of the non-recombining region of the sex chromosome varies across guppy populations (Almeida et al., 2021; Wright et al., 2017), and sex chromosome divergence results in allelic differences on the X and Y between males and females, we confined all of our analyses to autosomal sequence, although we present the X chromosome (Chromosome 12) in figures for comparison.

From our sequencing data, we obtained 7,889,657 biallelic high-quality filtered autosomal SNPs. We further filtered these to 253,375 SNPs present in annotated autosomal coding sequence. We focused on coding sequence for three reasons. First, non-coding sequence is enriched for repetitive elements, and the accumulation of repetitive elements on the ancestral guppy Y chromosome (Almeida et al., 2021) would lead to male-specific SNPs in repetitive elements that could bias our estimates of Y duplications. Second, previous work has suggested that unresolved sexual conflict on the autosomes occurs primarily in coding sequence (Ruzicka et al., 2019), and this fits with the substantial evidence that sexual conflict over gene regulation can be relatively quickly resolved through sex-specific gene regulation (Kopp, Duncan, & Carroll, 2000; Mank, 2017; Wright et al., 2018). Finally, by focusing on functional coding sequence, we are able to determine the potential functional role of genes that are subject to sexual conflict.

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Intersexual F_{ST}

We observed a genome-wide average intersexual F_{ST} of -0.0060. Given the low level of allelic divergence expected between males and females, it is critical to minimize false positives (Kasimatis et al., 2019). We therefore used three independent methods to estimate SNPs with elevated intersexual F_{ST}. We identified SNPs that were 1) in the top 1% of the autosomal F_{ST} distribution 2) were significant after permutation testing of samples (1000 replicates, $P < 0.001$) and 3) showed significant differences in male and female allele frequency based on Fisher's exact test ($P < 0.001$) (Supplementary Fig. 1). We expect false positives to occur across all methods, but true positives to be detected with multiple methods. We identified 504 autosomal coding sequence SNPs that were significant by all three of these measures (Supplementary Figs. 1 and 2), designated as high intersexual F_{ST} SNPs.

In order to reduce the potential for false positives, we then identified autosomal coding genes with ≥ 3 high intersexual F_{ST} SNPs (Bissegger et al., 2019; Tobler, Nolte, & Schlötterer, 2017), resulting in seven loci with significant sexual differentiation. For each of these genes, average intersexual F_{ST} was significantly greater than the autosomal average (Table 1, Supplementary Fig. 3).

Estimates of intersexual F_{ST} and Tajima's D can be biased due to relatedness of individuals within groups. Although our sampling design was balanced, with 10 females and 10 males collected at two separate sites for each of three rivers across the island of Trinidad (see Almeida et al., 2021 for details), we could not account for relatedness in wild-caught samples at the time of collection. We therefore assessed pairwise kinship coefficients among all our samples using both KING (Manichaikul et al., 2010) and NgsRelate (Korneliussen & Moltke 2015), as implemented in ANGSD (Korneliussen, Albrechtsen, & Nielsen, 2014). Neither method identified greater relatedness among male compared to female samples (Supplementary Fig. 4).

Y-autosome duplications

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

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

To differentiate whether the pattern of elevated M:F F_{ST} is due to limited sequence homology between autosomal genes and the Y chromosome, or results from at least partial gene duplication, we mapped the pattern of M:F read depth across each of these five genes and compared intersexual F_{ST} and M:F read depth for each SNP in a single gene (Fig. 1). The high M:F read depth for Olr1492-like was consistent across the full length of the single exon of this gene. This suggests a complete duplication, possibly in two copies (Fig. 1A). Similarly, the M:F read depth ratio for si:rp71-17i16.5 (Fig. 1C), Bcl11a (Fig. 1E), and Fezf1 (Fig. 1G) suggests a single or double copy duplication, of just the exons, possibly through retrotransposition. The high M:F read depth in ENSPRG05754 (Fig. 1I) suggests a partial duplication of the last exon of the gene, likely present in several Y copies. Within each gene, many of the high F_{ST} SNPs in these genes with high M:F read depth further suggests complete or partial gene duplication between the Y chromosome and autosomes (Fig 1B, D,

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F, H & J). Given sufficient evolutionary time, Y duplications will accumulate male-specific SNPs (Tobler et al., 2017), leading to a signal of elevated M:F SNP density. Of the five sexually differentiated genes with greater read depth in males, none showed significantly higher M:F SNP density (Table 1), although all values were >1, suggesting that the duplication events were relatively recent and the Y copies have not significantly differentiated. This is consistent with the recent origin of this chromosome (Darolti et al., 2019). Across our seven sexually differentiated genes, we did not observe evidence of duplications between the X chromosome and autosomes, which would result in three copies in males and four in females and therefore an average M:F read depth significantly <1. Gene duplications between the Y and autosomes can originate from either location. To define the direction of gene duplication, we calculated the relative expected probability of Y to autosome and autosome to Y duplications based on the size and gene content of each target and source area (Betrán et al., 2002, see Materials and Methods). The relative expected probability of gene duplication is 1.0727, suggesting an equal probability of movement from the Y or autosomes.

Sexual conflict over survival for autosomal genes

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

Genes subject to ongoing sexual conflict may be expected to experience balancing selection under some scenarios, which is often measured with Tajima's D (Bissegger et al., 2019; Cheng & Kirkpatrick, 2016; Mank, 2017; Wright et al., 2018). We therefore compared Tajima's D for these two genes to the autosomal average, as well as genes with known immune function, which are known to be subject to high levels of balancing selection (Andrés et al., 2009; Ferrer-Admetlla et al., 2008; Van Oosterhout, 2009; Weedall & Conway, 2010), and to other genomic categories (Fig. 2). Neither

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

Many things can influence Tajima's D estimates, and the lack of an elevated signature of balancing selection relative to the remainder of the genome is not necessarily indicative of a lack of sexual conflict. In order to understand the dynamics of these two genes in more detail, we mapped M:F read depth as a function of genomic location and intersexual F_{ST} (Fig. 3). Indeed, we found evidence of elevated M:F read depth for ENSPREG15023 across mos t of the exon (Fig. 3A), consistent with either partial duplication or full exonic duplication to the Y followed by partial degeneration. Many of the high F_{ST} SNPs in this gene have a high M:F read depth (Fig. 3B), consistent with Y duplication or significant Y sequence similarity.

Sequence similarity between the Y and autosomal sequence without gene duplication can also result in false intersexual F_{ST} signals (Kasimatis et al., 2021). In these cases, we expect that a limited number of Y reads mapping erroneously to autosomal coding sequence would result in similar overall male and female read depth across the entire coding sequence, with limited regions of increased male read depth (where the Y reads are mapping) in regions of high intersexual F_{ST}. This pattern is consistent with what we observe for ENSPREG12439, with highly variable M:F read depth across the gene (Fig. 3C). Importantly, all SNPs with high M:F F_{ST} values for this gene also show high M:F read depth (Fig. 3D). These results, coupled with the lack of elevated Tajima's D, suggest that these two genes also exhibit significant sequence similarity to the Y but not necessarily because of gene duplication.

Discussion

We identified 504 coding sequence SNPs in the guppy autosomal genome which showed significant differences in allele frequency between males and females. We used these to identify seven autosomal genes with significant average intersexual F_{ST}. Our approach, based on the intersection of three statistical methods, reduces the likelihood of false positives and results in a high confidence

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gene list of intersexual F_{ST} (Table 1, Supplementary Figs. 1, 2, and 3). This list of genes, either implicated in male-specific functions or essential developmental processes, can be used to assess the relative role of duplications onto or off of the Y chromosome in driving intersexual F_{ST} versus the role of sex differences in mortality in sexual conflict of autosomal genes.

The Y chromosome in guppies and sequence similarity

Five of the seven high-confidence sexually differentiated genes showed evidence of duplications between the Y chromosome and autosomes based on elevated male-to-female read depth ratios (Table 1, Supplementary Fig. 4). Two of these (Olr1496-like and Fezf1) have sex-specific functions based on Gene Ontology and Gene Cards annotations, consistent with theory and empirical studies showing that Y chromosomes can accumulate gene duplicates with male-specific functions (Carvalho et al., 2015; Mahajan & Bachtrog, 2017). Specifically, Olr1496 (Olfactory factor 1496-like gene) plays a role in multiple aspects of germline development in *C. elegans* (Cho, Rogers, & Fay, 2007), and Fezf1 (FEZ Family Zink Finger 1) plays an important role in the migration of gonadotropin-releasing neurons (Damla Kotan et al., 2014).

Y chromosomes in general represent a unique genomic environment. Although Y chromosome gene content degrades quickly following recombination suppression (Bachtrog, 2013), several recent studies indicate that older, large Y chromosomes, such as that in *Drosophila* (Carvalho et al., 2015; Koerich et al., 2008; Tobler et al., 2017), sticklebacks (Bissegger et al., 2019) and humans (Kasimatis et al., 2021), contain substantial numbers of genes duplicated from autosomes. Duplications may represent an important mechanism of sex chromosome divergence, and the Y chromosome may preferentially retain duplicates that play an important role in male development or fitness (Bachtrog, 2013; Carvalho et al., 2015). Gene duplicates that assume sex-specific functions offer a route to the resolution of sexual conflict (Perry, 2018; Vankuren & Long, 2018), and the accumulation of genes with male-specific function on the Y offers a potential path to conflict resolution within the genome.

However, it remains unclear how common Y duplications of autosomal genes occur in younger systems with a far smaller male-specific region. Although the size varies by population, the conserved non-recombining region of the Y chromosome in guppies is relatively small, spanning at

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most 5 Mb (Almeida et al., 2021; Darolti et al., 2019), and our sampling strategy means that we are only able to assess duplications in this region. Others have failed to recover evidence for even this limited region of Y degeneration (Charlesworth et al., 2020; Fraser et al., 2020). Our findings add further support for a region of recombination suppression across populations on Trinidad, as only a male-specific region of the Y can explain the M:F read depth differences we observe. Moreover, our work suggests that the guppy Y chromosome is dynamic with regard to gene content, and may act as a hotspot for gene duplications with male-specific functions despite its recent origin and small size.

It is also possible that Y genes have duplicated to the autosomes, which would also produce a pattern of increased male read depth. We expect duplications from the Y to the autosomes to be favoured as a function of increased expression in the diploid state or to protect the gene from Y degeneration (Dyer et al., 2011). Unfortunately, lack of high-quality genomes in close relatives precludes a phylogenetic determination of the direction of gene duplication, and our estimates of relative probability based on the size of the target region and the gene content of the source reveal roughly equivalent probabilities of Y→autosome versus autosome→Y duplications assuming equal selection pressures. However, although movement to the Y chromosome presents a clear advantage for sexually antagonistic or male-specific genes, the low level of degeneration of the guppy Y chromosome coupled with the fact that the Y chromosome appears to have retained all corresponding X genes (Almeida et al., 2021) suggests that there is little evolutionary pressure for gene movement from the Y to the autosomes due to Y degeneration or loss of expression. Therefore, Y to autosomal duplications are more likely to be evolutionarily advantageous than the reverse.

Regardless of direction of gene movement, our results further emphasize the importance of accounting for Y gene duplications in scans of M:F F_{ST}, as all of our sexually differentiated genes show evidence of Y duplication. The Y-duplicated genes we identify here are not present in our previous list of Y-linked genes based on male-specific sequence (Almeida et al., 2021), or in other similar analyses (Fraser et al., 2020). This is not surprising, as duplications, particularly if recent, will still retain substantial homology and will not be detected when bioinformatically identifying sequence that is unique to male genomes. Consistent with recent duplications and limited

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divergence, none of our Y-duplicated genes exhibit significantly elevated average M:F SNP density (Table 1, Fig. 2), although the values are all >1.

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

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

Intra-locus sexual selection over survival or viability leads to allele frequency differences between the sexes over the course of a generation, as an allele increases the survival of one sex at a mortality cost to the other (Kasimatis et al., 2019; Mank, 2017; Wright et al., 2018). The significant mortality costs required each generation to generate allele frequency differences between the sexes preclude large numbers of genes with significant M:F F_{ST} (Bissegger et al., 2019; Kasimatis et al., 2019; Ruzicka et al., 2020). Given the very high selection coefficients needed to produce a significant pattern of intersexual F_{ST}, at most, we might expect a limited number of loci with significant allelic differentiation between males and females, and this would be most evident in wild species where males and females experience differences in predation, parasite or pathogen loads. Despite the fact that we sampled wild individuals of a classic prey species with very high potential for sexual conflict over survival, we observe just two loci in the genome that show evidence of sexual conflict over survival based on M:F F_{ST}. Both of these loci exhibit very low M:F F_{ST}. It is worth noting that our strategy of analyzing samples collected across the island of Trinidad, done to increase sample size, does not necessarily assume that the genetic architecture of sexual conflict is consistent across populations as selection coefficient may be different across populations.

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

Concluding remarks

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

Materials and Methods

Data Collection and Genotyping

Samples were collected from three rivers, Aripo, Yarra, and Quare, in Trinidad in December 2016, in accordance with national collecting guidelines. In total, 10 males and 10 females were collected from one high predation and one low predation population in each river, resulting in 120 samples, which were sequenced individually with Illumina HISEQX. Further sequencing details are available in Almeida et al., (2021).

We used FastQC v0.11 (www.bioinformatics. babraham.ac.uk/projects/fastqc) and Trimmomatic 0.36 (Bolger et al., 2014) to remove adapter sequences and low-quality reads. After quality control, we recovered ~30X average sequencing depth for males and ~20X sequencing depth for females.

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High-quality reads were aligned against the *Poecilia reticulata* female reference genome (Ensembl GCA_000633615.2) (Künstner et al., 2016), using BWA 0.7.15 MEM algorithm (Li & Durbin, 2009) with default parameters. We filled in mate coordinates and mate related flags, sorted alignment by coordinates, and marked PCR duplications with SAMtools-1.9 (Li et al., 2009).

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

Intersexual F_{ST}

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

Using approaches to further limit false positives (Bissegger et al., 2019; Tobler et al., 2017), we identified 7 genes with ≥ 3 high intersexual F_{ST} SNPs, which we designated as sexually differentiated genes. We calculated average intersexual F_{ST} for all genes using VCFtools v0.1.16, respectively. We

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used Wilcoxon rank-sum test to indicate statistical difference in intersexual F_{ST} between autosomal genes and other gene categories (sexually differentiated genes and genes on the sex chromosome).

Relatedness Inference

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

Assessing Y Duplications of Sexually Differentiated Genes

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

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

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To infer the relative probability of Y→autosomal versus autosomal→Y duplications, we followed the formulas in Betrán et al., (2002) and Vibranovski et al.,(2009), noting that the Y chromosome is present in one copy and autosomes present in two copies in males. The formula is based on the relative size and gene content of the target and recipient regions. For the size of the target and recipient regions, we used the total length of the diploid autosomal genome assembly (2 * 696 Mb) and the minimum conserved region of the haploid Y chromosome (5Mb, as estimated by Almeida et al., 2021). The diploid gene content of the autosomes is estimated at 2 * 20,755 genes, and although an exact gene count of the Y is unclear, the low level of gene degeneration observed suggests that the minimum Y gene content consists of 139 genes (Almeida et al., 2021).

Tajima's D

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

Functional Annotations

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

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Data Accessibility Statement

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DNA-seq data are publicly available in the NCBI SRA (BioProject ID PRJEB39998). Scripts of all the analysis are available on GitHub https://github.com/Lin-Yuying/GENE-DUPLICATION-TO-THE-Y-CHROMOSOME-IN-TRINIDADIAN-GUPPIES.

Author Contributions

J.E.M. and Y.L. designed the study. B.S., F.B. and J.E.M. collected samples. P.A. collected raw 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. 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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Tables and Figures

Table 1. Summary statistics of sexually differentiated genes. Statistically significant values are 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	LG7	**0.0892**	**1.1580**	1.1200	Phosphatidylinositol 3-kinase signalling,

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(si:rp71-17i16.5)					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

1 Ensembl guppy gene number

2 Significance based on Wilcoxon's rank-sum test ($P < 0.01$), compared to autosomal average.

3 Significance based on Fisher's Exact Test ($P < 0.05$), relative to genome-wide M:F SNP density.

4 From Gene Ontology (Ashburner et al., 2000; Carbon et al., 2021) and Gene Cards (Stelzer et al., 2016).

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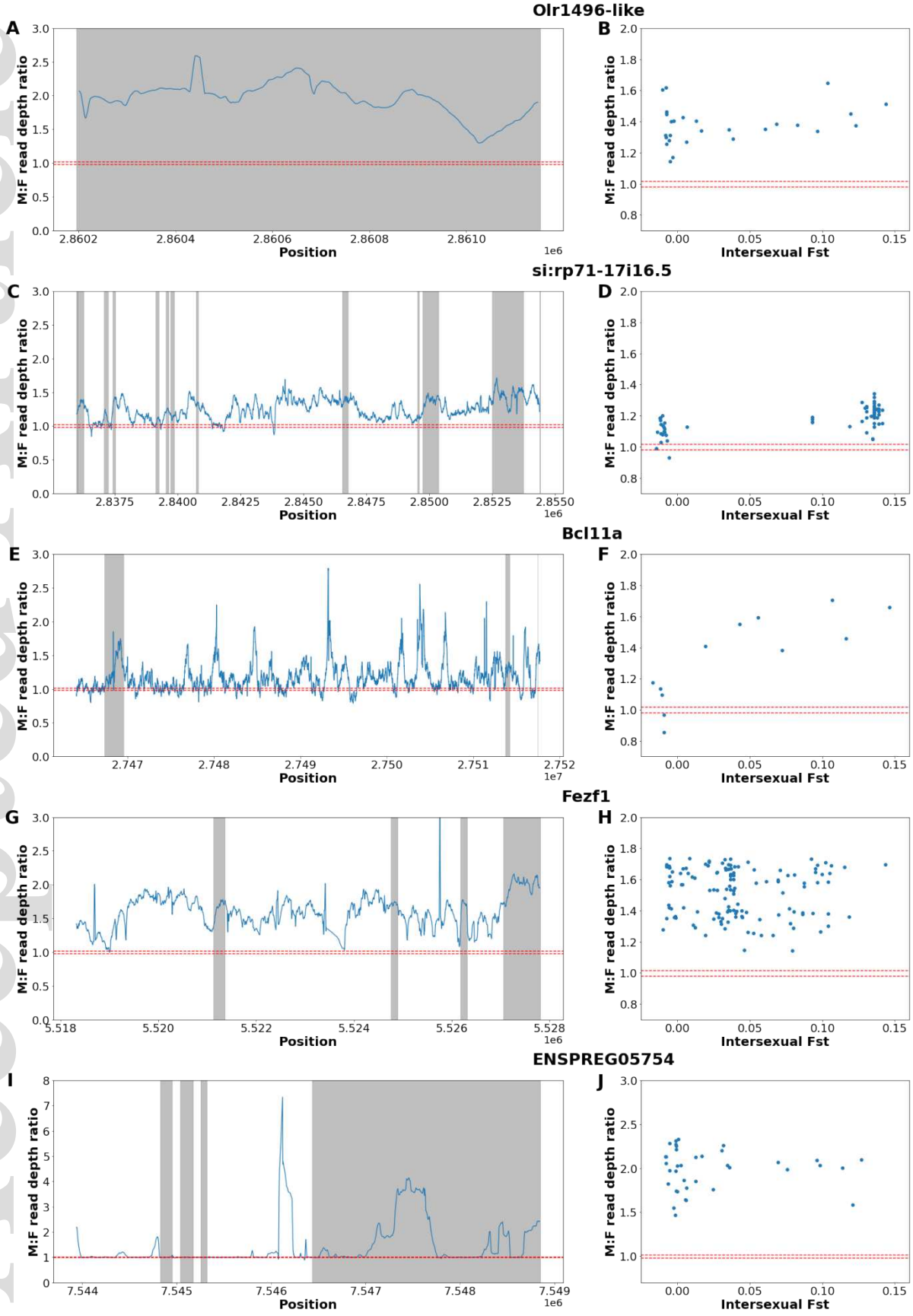

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Fig. 1. Elevated M:F read depth ratio indicates partial gene duplication of Olr1496-like (Panels A and B), si:rp71-17i16.5 (Panels C and D), BCL11A (Panels E and F), Fezf1 (Panels G and H), ENSPREG05754 (Panels I and J). Left panels show M:F read depth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

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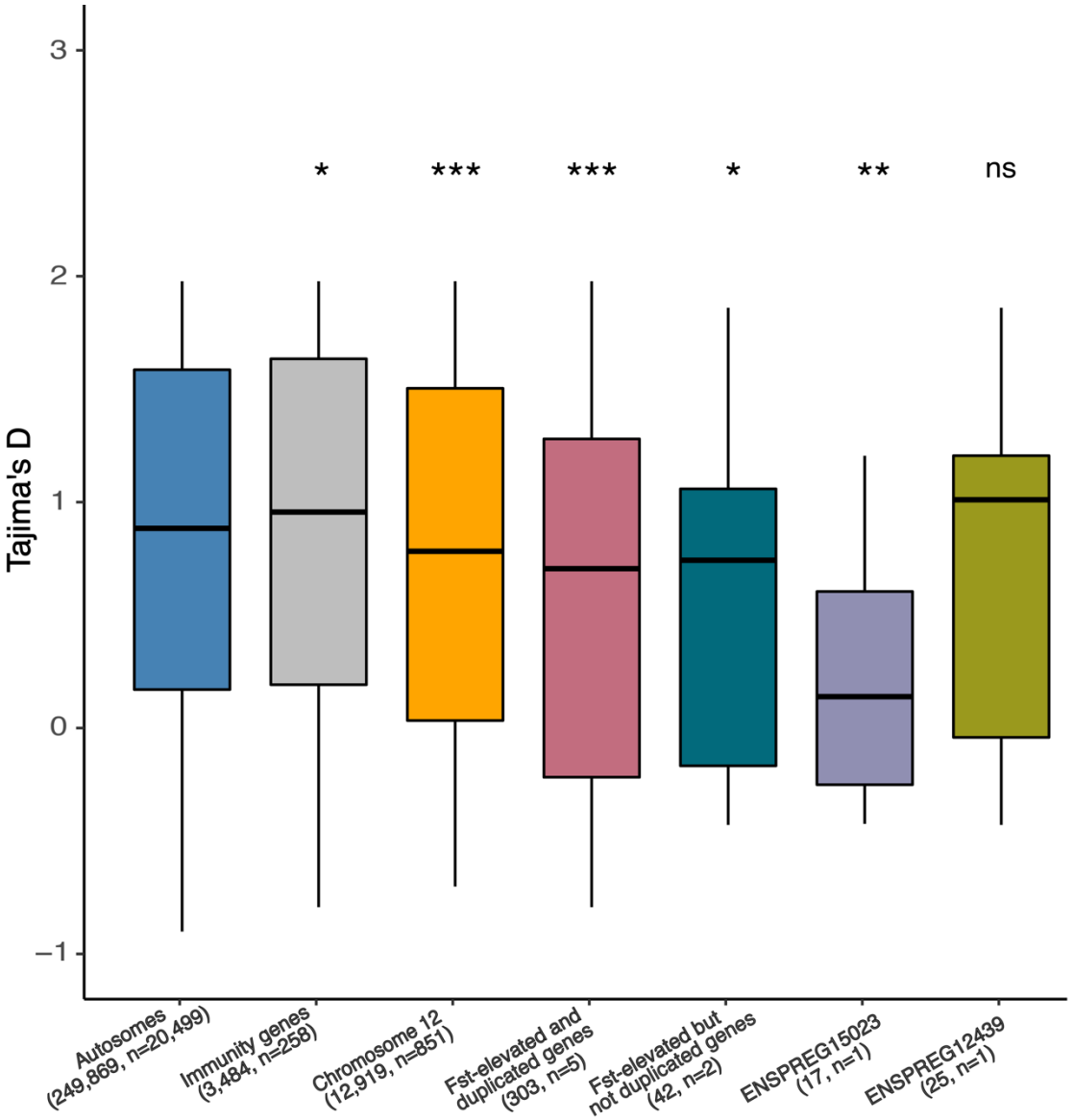

Fig. 2. Distribution of Tajima's D among different gene categories. Number of SNPs and genes in each gene category are indicated in brackets. Significant differences to autosomal distribution are indicated (Wilcoxon rank-sum test, *P < 0.01, **P < 0.001, ***P < 0.0001, ns: non-significant).

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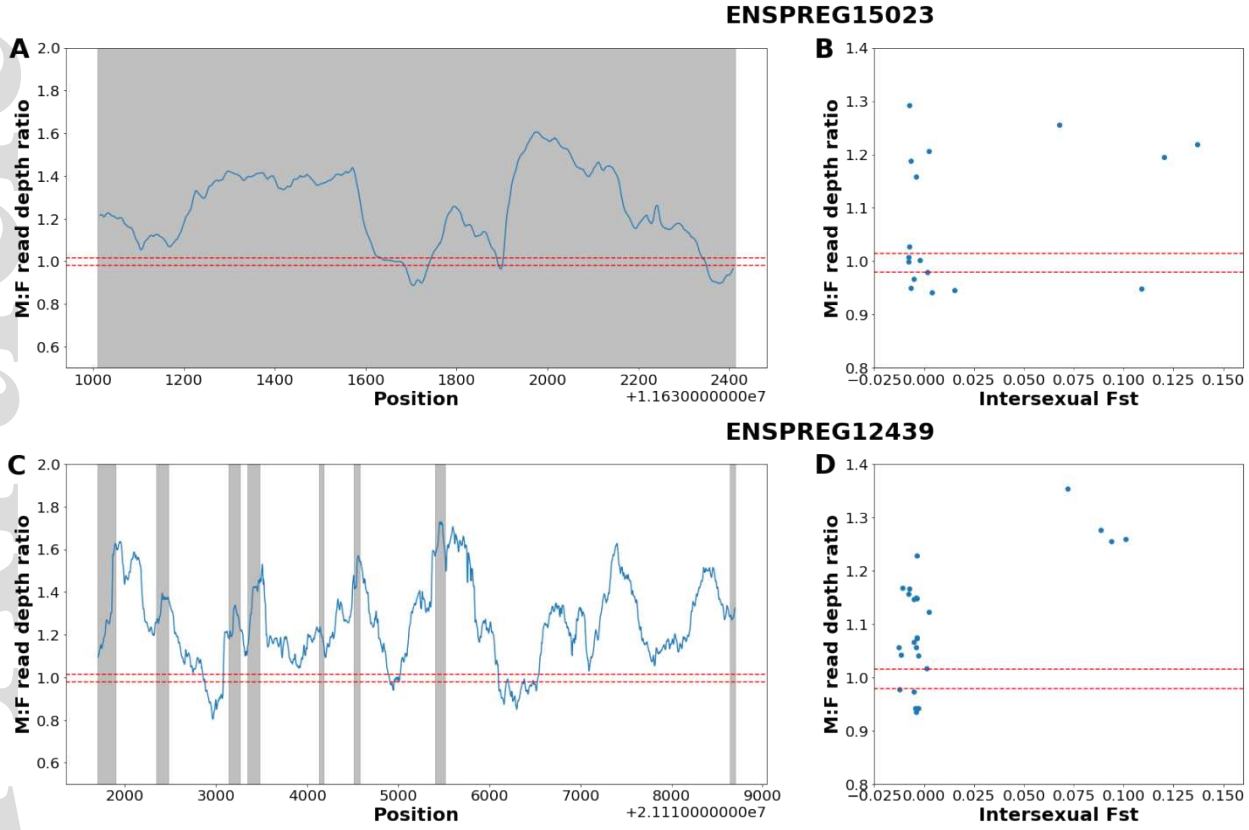

Fig. 3. Normalized M:F read depth ratio for ENSPREG15023 (panels A and B) and ENSPREG12439 (panels C and D). Left panels show M:F read depth as a function of physical position (in Mb), with coding regions shaded in grey. Panels on the right show intersexual F_{ST} and M:F read depth ratio for each SNP.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

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