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Sex Chromosome Evolution in Muscid Flies

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ABSTRACT Sex chromosomes and sex determining genes can evolve fast, with the sex-linked chromosomes often differing between closely related species. Population genetics theory has been developed and tested to explain the rapid evolution of sex chromosomes and sex determination. However, we do not know why the sex chromosomes are divergent in some taxa and conserved in others. Addressing this question requires comparing closely related taxa with conserved and divergent sex chromosomes to identify biological features that could explain these differences. Cytological karyotypes suggest that muscid flies (*e.g.*, house fly) and blow flies are such a taxonomic pair. The sex chromosomes appear to differ across muscid species, whereas they are conserved across blow flies. Despite the cytological evidence, we do not know the extent to which muscid sex chromosomes are independently derived along different evolutionary lineages. To address that question, we used genomic and transcriptomic sequence data to identify young sex chromosomes in two closely related muscid species, horn fly (*Haematobia irritans*) and stable fly (*Stomoxys calcitrans*). We provide evidence that the nascent sex chromosomes of horn fly and stable fly were derived independently from each other and from the young sex chromosomes of the closely related house fly (*Musca domestica*). We present three different scenarios that could have given rise to the sex chromosomes of horn fly and stable fly, and we describe how the scenarios could be distinguished. Distinguishing between these scenarios in future work could identify features of muscid genomes that promote sex chromosome divergence.

KEYWORDS
Diptera
Calyptratae
Muscidae
Horn fly
Stable fly
Genetics of Sex

In species where sex is determined by genetic differences between males and females, sex determining loci can reside on sex chromosomes, such as the male-limited Y chromosome in mammals that carries a male-determining gene (Swain and Lovell-Badge 1999). When the X and Y (or Z and W) chromosomes are highly differentiated, the Y (or W) chromosome contains only a handful of genes with male- (female-) specific functions (Charlesworth *et al.* 2005; Bachtrog 2013). X (or Z) chromosomes, on the other hand, typically resemble autosomes in gene density, with some differences in the types of genes found on the X and autosomes (Vicoso and Charlesworth 2006; Meisel *et al.* 2012). Other sex chromosome pairs are homomorphic, with little sequence differentiation between the X and Y (or Z and W) chromosomes (Wright *et al.* 2016).

Sex determining genes and sex chromosomes often differ across species (Bachtrog *et al.* 2014; Beukeboom and Perrin 2014), predominantly as a result of two general processes. First, when a new sex determining locus arises on an autosome, it can convert the autosome into a "proto-sex-chromosome", and the ancestral sex chromosome can revert to an autosome (Carvalho and Clark 2005; Larracuente *et al.* 2010; van Doorn 2014). Second, autosomes can fuse with X, Y, Z, or W chromosomes to create "neo-sex-chromosomes" (Pennell *et al.* 2015; Vicoso and Bachtrog 2015). A special case of chromosomal fusions are reciprocal translocations, in which an autosomal region is translocated to a sex chromosome and *vice versa* (*e.g.*, Toups *et al.* 2019). Population genetics theory suggests that sex-specific selection

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pressures (including sexual antagonism) are important contributors to the evolution of sex determination pathways, evolutionary turnover in sex chromosomes, and the fixation of neo-sex chromosomes (Charlesworth and Charlesworth 1980; Rice 1986; van Doorn and Kirkpatrick 2007, 2010). This theory has been tested in many plants and animals, and those tests have generally supported the hypothesis that sex-specific selection is important for the evolution of sex chromosomes and sex determination (*e.g.*, Roberts *et al.* 2009; Wang *et al.* 2012; Zhou and Bachtrog 2012; Qiu *et al.* 2013).

In some taxa, the sex-linked chromosomes or sex determining genes differ across many species, whereas in other taxa the X and Y (or Z and W) chromosomes are conserved across (nearly) all species (Beukeboom and Perrin 2014; Blackmon and Demuth 2014). Despite the well constructed theory explaining how sex chromosomes and sex determination evolve, and the empirical work supporting that theory, we know very little about why the rates of evolution differ across taxa (Bachtrog *et al.* 2014; Abbott *et al.* 2017). Contrasting closely related taxa with conserved and divergent sex chromosomes could allow for the identification of biological factors that affect sex chromosome divergence.

Brachyceran flies (*i.e.*, higher Diptera) are well-suited models for identifying factors that promote or inhibit sex chromosome evolution because they have variable rates of sex chromosome divergence across lineages (Vicoso and Bachtrog 2015). The karyotype of the most recent common ancestor (MRCA) of Brachycera consists of five gene-rich autosomes (Muller elements A–E), a gene-poor heterochromatic X chromosome (Muller element F), and a Y chromosome that carries a male-determining locus (Figure 1) (Boyes and Van Brink 1965; Foster *et al.* 1981; Weller and Foster 1993; Vicoso and Bachtrog 2013). Elements A–E correspond to the five gene-rich chromosome arms of *Drosophila melanogaster* (X, 2L, 2R, 3L, and 3R), and element F is homologous to the gene-poor *D. melanogaster* dot chromosome, *i.e.*, chromosome 4 (Muller 1940). Element F has been conserved as the X chromosome for ~ 175 million years (My) in some phylogenetic lineages within flies, while new sex chromosomes have arisen along other lineages (Baker and Wilkinson 2010; Wiegmann *et al.* 2011; Vicoso and Bachtrog 2013, 2015).

Within Brachycera, the sister families of Muscidae (house flies and their allies), Calliphoridae (blow flies), and Sarcophagidae (flesh flies) could be especially informative for comparative studies because they appear to have family-specific rates of sex chromosome evolution (Figure 1). These three families diverged from their common ancestor ~ 50 My ago (Wiegmann *et al.* 2011). Nearly all blow flies and flesh flies have the ancestral fly karyotype, with five autosomes, a heterochromatic X, and a male-determining locus on a Y chromosome (Boyes 1961; Boyes and Van Brink 1965; Scott *et al.* 2014b; Vicoso and Bachtrog 2015). The only exceptions are sex determination by maternal genotype in the blow fly *Chrysomya rufifacies* (Ullerich 1963), and an expanded karyotype of 19–20 chromosomes in the flesh fly *Agria* (*Pseudosarcophaga*) *affinis* (Boyes 1953). The X chromosomes of the Australian sheep blow fly (*Lucilia cuprina*) and gray flesh fly (*Sarcophaga bullata*) both correspond to element F (Vicoso and Bachtrog 2013, 2015), suggesting that element F is the ancestral X of these families. In addition, the Y chromosomes of *L. cuprina* and *S. bullata* are extremely differentiated from their homologous X chromosomes, suggesting that they have existed as an X-Y pair for many millions of years (Vicoso and Bachtrog 2013, 2015). Furthermore, the haploid X chromosome in *L. cuprina* males is up-regulated (*i.e.*, dosage compensated) by an RNA-binding protein that is homologous to a *Drosophila* protein that localizes nearly exclusively to element F (Linger *et al.* 2015; Davis *et al.* 2018). As expected because of the genetic differentiation between the

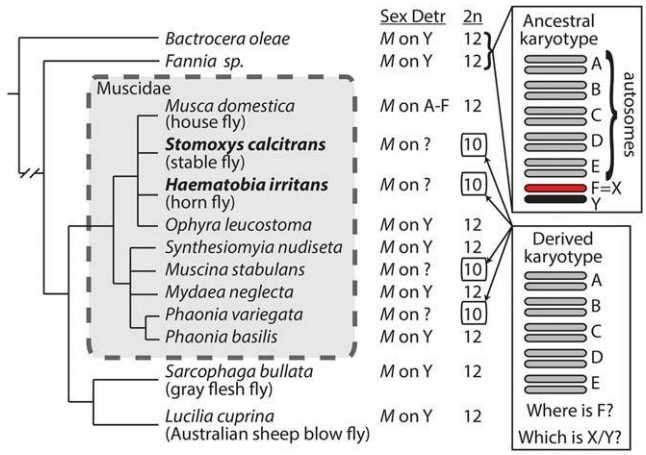

Figure 1 Cryptic sex chromosomes in Muscidae. Phylogenetic relationships and karyotypes of muscid flies and their relatives (Boyes *et al.* 1964; Boyes and Van Brink 1965; Schnell e Schuehli *et al.* 2007; Vicoso and Bachtrog 2015). The inferred mechanism of sex determination (Sex Detr) and the diploid chromosome number (2n) are listed for each species. "*M*" refers to a generic male-determining locus.

L. cuprina X and Y, loss of function mutations in the *L. cuprina* gene encoding the dosage compensation protein are lethal specifically in males (Davis *et al.* 2018).

In contrast to flesh flies and blow flies, multiple lineages in the family Muscidae seem to have evolved new sex chromosomes in the < 40 My since the common ancestor of the family (Ding *et al.* 2015). The iconic example of sex chromosome evolution in Muscidae is the house fly (*Musca domestica*), which has a well-characterized polygenic sex determination system (Hamm *et al.* 2015). House fly appears to have the ancestral brachyceran karyotype (*i.e.*, 5 pairs of euchromatic chromosomes and a heterochromatic sex chromosome pair), but the X and Y chromosomes are not differentiated (Meisel *et al.* 2017). This is because the house fly male-determining locus (*Mdmd*) is a recently derived duplicated gene that can be found on at least 5 of the 6 chromosomes (Sharma *et al.* 2017). The invasion and spread of this new male-determiner in the house fly genome since the divergence with closely related species is such that every house fly chromosome can be an undifferentiated proto-sex-chromosome pair.

In addition to house fly, other muscid fly species have derived karyotypes that were evidenced by cytological examination (Figure 1). For example, the heterochromatic element F is missing from the karyotypes of stable fly (*Stomoxys calcitrans*), horn fly (*Haematobia irritans*), and some other muscids (Boyes *et al.* 1964; LaChance 1964; Joslyn *et al.* 1979; Avancini and Weinzierl 1994; Parise-Maltempi and Avancini 2007), possibly because it fused to another element. Stable fly and horn fly both have genetic sex determination with a dominant male-determining locus (Willis *et al.* 1981; McDonald and Schmidt 1987), but the specific identities of the X and Y chromosomes remains unresolved. We hypothesize that species with these derived karyotypes have cryptic sex chromosomes that arose when an ancestral sex chromosome (element F and/or the Y chromosome) recently fused to one of the other five chromosomes. Here, we describe the identification of the cryptic sex chromosomes in stable fly and horn fly using genomic and transcriptomic sequence data. We also provide evidence that the stable fly and horn fly sex chromosomes are young and of independent origins. These results demonstrate that muscid flies are a good model system for studying the factors that permit rapid evolution of sex chromosomes.

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MATERIALS AND METHODS

Assigning scaffolds to Muller elements

We used a homology-based approach that we had previously developed in house fly to map stable fly and horn fly scaffolds to Muller elements (Meisel *et al.* 2015; Meisel and Scott 2018). This approach works because Muller element gene content (synteny) is conserved across Brachycera (Foster *et al.* 1981; Weller and Foster 1993; Vicoso and Bachtrog 2013; Sved *et al.* 2016). For stable fly, we selected OrthoGroups from the OrthoDB annotation that contain a single *D. melanogaster* gene and a single *S. calcitrans* gene (Kriventseva *et al.* 2018; Olafson *et al.* 2019 *preprint*). For horn fly, we obtained annotated genes from the initial analysis of the genome, and we extracted the inferred *D. melanogaster* homologs for each gene (Konganti *et al.* 2018). We assigned the stable fly and horn fly genes to the same Muller element as their *D. melanogaster* homologs. Each of these genes is part of a chromosomal scaffold. We used a majority-rules approach to assign those scaffolds to Muller elements if $>50\%$ of the genes on a scaffold are assigned to the same Muller element (Supplementary tables S1 and S2). This allowed us to assign 94.6% (1,482/1,566) of stable fly scaffolds and 97.5% (4,778/4,889) of horn fly scaffolds containing annotated genes to Muller elements. All genes on a scaffold are then assigned to the Muller element of that scaffold regardless of the Muller element designation of their annotated ortholog. Assigning genes to Muller elements based on their scaffold should control for individual genes that are positionally relocated between elements across flies (Bhutkar *et al.* 2007; Baker and Wilkinson 2010).

Variant calling

Our approach to identifying sex chromosomes involves testing for Muller elements with increased heterozygosity in males, which is the expectation for young, undifferentiated X-Y chromosome pairs (Vicoso and Bachtrog 2015; Meisel *et al.* 2017). To those ends, we used the Genome Analysis Toolkit (GATK) version 3.4-0 to identify heterozygous single nucleotide polymorphisms (SNPs) in stable fly and horn fly genomes and transcriptomes, following the GATK best practices (McKenna *et al.* 2010; DePristo *et al.* 2011; Van der Auwera *et al.* 2013). The Illumina sequencing reads used to assemble the stable fly genome were generated from DNA extracted from males of a strain that had been inbred for 7 generations (Olafson *et al.* 2019 *preprint*). These data allow us to identify nascent sex chromosomes based on elevated male heterozygosity. The same cannot be done for horn fly because the sequences used to assemble the genome came from DNA isolated from mixed pools of males and females (Konganti *et al.* 2018).

To quantify heterozygosity in stable fly males, we first mapped the sequencing reads (SRR1975009, SRR1975010, SRR1975042, SRR1996621, SRR1996622, SRR1996626, SRR1996627, SRR1996630) to the assembled genomic scaffolds (GCF_001015335.1) using the MEM algorithm implemented in BWA with the default parameters (Li and Durbin 2009). Next, we used Picard Tools version 1.133 to identify and remove duplicate reads, and we realigned indels with GATK. Then, we performed naïve variant calling using the GATK HaplotypeCaller with a phred-scaled confidence threshold of 20, and we selected the highest confidence SNPs from that first-pass (QD <2.0, MQ <40, FS >60, SOR >4, MQRankSum <-12.5, ReadPosRankSum <-8). We used those high quality variants to perform base recalibration, we re-input those recalibrated bases into another round of variant calling, and we extracted the highest quality variants. We repeated the process so that we had performed three rounds of recalibration, which was sufficient for convergence of variant calls. We applied GenotypeGVCFs to the variant calls from all of the Illumina libraries for joint genotyping. We then used the GATK HaplotypeCaller to genotype all of the variable sites (phred-scaled confidence >20), and we selected only the high quality variants (FS >30 and QD <2).

Comparing male and female heterozygosity, rather than only analyzing male heterozygosity, may be a better way to identify elevated heterozygosity in males. There are no genomic sequences available from female stable fly or from single-sex horn fly to use in such an analysis. However, Illumina RNA-seq data were collected from female and male tissues separately for both species. From stable fly, RNA-seq libraries were sequenced from female and male whole adults (SRX229930 and SRX229931), the reproductive tract (SRX995859 and SRX995857), and heads (SRX995858 and SRX995860). The reproductive tract from males includes testis, vas deferens, and ejaculatory duct. The female reproductive tract includes ovary, oviduct, and accessory glands, but not the external ovipositor. RNA from whole adult stable flies was extracted from the same inbred strain that supplied the DNA for the genome assembly, and RNA from reproductive tissues and head was extracted from the stable flies in the lab colony from which the inbred line was derived. From horn fly, RNA-seq libraries were sequenced from ovary (SRX3340090) and testis (SRX3340086). Horn flies were sampled from the USDA-ARS Knipling-Bushland U.S. Livestock Insects Research Laboratory strain, which has been maintained since 1961 (Konganti *et al.* 2018). One RNA-seq library was sequenced for each tissue from each species (Konganti *et al.* 2018; Olafson *et al.* 2019 *preprint*).

We used a modified GATK pipeline to identify SNPs in stable fly and horn fly RNA-seq data (Meisel *et al.* 2017). First, RNA-seq reads were aligned to the reference genomes of the appropriate species (GCF_001015335.1 for stable fly and GCA_003123925.1 for horn fly) using STAR version 2.4.0.1 (Dobin *et al.* 2013). We used the aligned reads to create a new reference genome index (*i.e.*, transcript annotation) from the inferred spliced junctions in the first alignment, and then we performed a second alignment with the new reference. Defining a new genome index allows us to align RNA-seq reads to transcripts or splice variants that are not annotated in the reference genome. We next marked duplicate reads and used SplitNCigarReads to reassign mapping qualities to 60 with the ReassignOneMappingQuality read filter for alignments with a mapping quality of 255. Indels were realigned, and three rounds of variant calling and base recalibration were performed as described above for the stable fly genomic sequencing reads. We applied GenotypeGVCFs to the variant calls from all tissues for joint genotyping of males and females from each species. Finally, we used the same filtering parameters that we applied to the stable fly genomic sequencing reads to extract high-quality SNPs from our variant calls.

Once we had identified sites in the sequence data that differ from the reference genome, we extracted heterozygous sites across the genome within annotated genes. We used those data to calculate the number of heterozygous SNPs per Mb within each annotated gene separately for each sex (Supplementary tables S3–S5). Genes with no heterozygous sites were omitted from the results we present, but we obtain the same general patterns when these genes are included. For each gene, we calculated relative male heterozygosity, or the fraction of all heterozygous sites in either sex that are heterozygous in males. This was calculated for each gene using the equation $H_m/(H_m+H_f)$, where H_m and H_f are the number of heterozygous SNPs in males and females, respectively, within a given gene. This value ranges from 0 (if heterozygous sites are only observed in females) to 1 (if heterozygous sites are only observed in males), with 0.5 indicating equal heterozygosity in males and females.

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Sequencing coverage

We measured the sequencing coverage per stable fly gene using the alignments of genome sequencing reads described above. We kept only pairs of sequencing reads where both reads mapped to the same scaffold. A mapped pair of reads was assigned to a gene if at least one read overlapped with the genomic region within the start and end coordinates of the gene. The reads mapped to each gene were counted separately for each sequencing library, that value was divided by the length of gene (difference between start and end coordinates in kilobases, kb), and the mapped reads per kb was divided by the total number of reads mapped to genes in that library (in millions). We then calculated the median reads per kb per million mapped reads across all libraries for each gene. These values were compared for genes across Muller elements.

Gene expression analysis

We aligned the same RNA-seq data described above to the annotated transcripts in the stable fly (GCF_001015335.1) and horn fly (Konganti *et al.* 2018) genomes and calculated transcripts per million reads (TPM) for each transcript using kallisto version 0.44.0 (Bray *et al.* 2016). We also used kallisto to align 454 GS FLX reads from whole adult female (SRR003191) and male (SRR003191) horn flies to the horn fly reference transcripts (Konganti *et al.* 2018). In addition, we used the same approach to align Illumina RNA-seq reads from whole adult male (SRX208993 and SRX208994) and female (SRX208996 and SRX208997) house flies to the annotated house fly transcripts (GCA_000371365.1; Scott *et al.* 2014a). We then summed TPM for all transcripts from each gene for each sample type (*e.g.*, stable fly female head) to obtain a gene-level estimate of expression in each sample (Supplementary tables S6–S11). In house fly, where there are two RNA-seq libraries for each sex, we calculated the mean TPM for each gene across both libraries. Using these data, we calculated the $\log_2$-fold male:female expression level ($\log_2 \frac{M}{F}$) of each gene for each tissue type. In our analysis, we only considered genes with TPM >0 in both males and females in a particular tissue and TPM >1 in at least one sex.

Data availability

All data analyzed are available from the appropriate NCBI archives. The assembled and annotated genomes are available from accession GCF_001015335.1 for stable fly, GCA_003123925.1 for horn fly, and GCA_000371365.1 for house fly. Stable fly genome sequencing reads are available from accessions SRR1975009, SRR1975010, SRR1975042, SRR1996621, SRR1996622, SRR1996626, SRR1996627, and SRR1996630. Stable fly RNA-seq reads are available from accessions SRX229930, SRX229931, SRX995859, SRX995857, SRX995858, and SRX995860. Horn fly RNA-seq reads are available from accessions SRX3340090, SRX3340086, SRR003191, and SRR003191. House fly RNA-seq reads are available from accessions SRX208993, SRX208994, SRX208996, and SRX208997. Supplementary tables S1–S13 with data analysis are available online at figshare: https://doi.org/10.25387/g3.11782317.

RESULTS

The stable fly sex chromosomes consist of elements D and F

We hypothesize that stable fly has young, cryptic sex chromosomes (Fig 1). Young X/Y chromosome pairs will have started to differentiate in the sequences of their genes, but they should still have similar gene content (Charlesworth *et al.* 2005). Therefore, reads from the Y chromosome will align to X chromosome genes, and *vice versa*. Nascent sex chromosomes can thus be identified based on elevated heterozygosity in the heterogametic sex, *i.e.*, XY males (Vicoso and Bachtrog 2015; Meisel *et al.* 2017). The stable fly genome was sequenced from male DNA (Olafson *et al.* 2019 *preprint*), allowing us to identify the sex-linked element(s) by testing for elevated heterozygosity in the genome sequencing reads. To those ends, we first assigned most of the genes in the stable fly genome to Muller elements using homology relationships with *D. melanogaster* (Supplementary table S1). Next, we identified heterozygous SNPs in the sequencing reads generated from stable fly males (Supplementary table S3). Because multiple males were sampled for genome sequencing, our approach will capture two different types of variable sites: fixed differences between the X and Y chromosomes, as well as polymorphisms on the X, Y, and autosomes that segregate in the lab strain that was sampled. We expect the sex chromosomes to have elevated heterozygosity because they will contain both types of variable sites, whereas the autosomes will only have the latter. We performed all of our analyses on heterozygous variants that we identified within annotated genes. Here, we report results for gene-level heterozygosity.

We found that stable fly genes assigned to Muller element D have more heterozygous SNPs in males than genes on scaffolds mapped to the other four major elements (Figure 2A). We performed a similar analysis comparing each of the other four major elements against all others, and we did not detect any other elements with elevated heterozygosity. The elevated heterozygosity of element D genes is not a result of increased power to detect heterozygous variants—element D genes have lower sequencing coverage than genes on elements A, B, C, and E (Figure 3A), which should result in reduced power to detect heterozygous variants on element D. This suggests that element D is part of the stable fly X and Y chromosomes.

Notably, element F has reduced heterozygosity (Figure 2A), although not significantly because only five stable fly genes with heterozygous sites were assigned to element F, which means we had low power to detect a significant reduction of heterozygosity on element F. Element F also has reduced variation along most of its length in *Drosophila*, likely because a lack of recombination enhances the diversity-reducing effects of selective sweeps and background selection (Berry *et al.* 1991; Hilton *et al.* 1994; Jensen *et al.* 2002; Wang *et al.* 2002, 2004; Arguello *et al.* 2010). Therefore, the low heterozygosity of element F genes in stable fly could be explained if they also experience reduced recombination. Comparing with female heterozygosity is necessary to determine if the low male heterozygosity on element F (and elevated male heterozygosity on element D) is consistent with X-linkage.

To compare male and female heterozygosity in stable fly, we used available RNA-seq data to identify heterozygous SNPs separately in each sex (Olafson *et al.* 2019 *preprint*). We then calculated relative male heterozygosity as the fraction of all SNPs in each gene that are heterozygous in males (Supplementary table S4). Relative male heterozygosity ranges from 0, if all heterozygous SNPs in a gene are in females, to 1, if all heterozygous SNPs in a gene are in males (Meisel *et al.* 2017). As in our analysis of absolute male heterozygosity, we expect the sex chromosomes to have elevated relative male heterozygosity because sex-linked genes will harbor fixed differences between the X and Y chromosomes, in addition to segregating polymorphisms. Consistent with our analysis of absolute heterozygosity, there is elevated relative male heterozygosity in genes on element D (Figure 2B). None of the other major elements (A, B, C, or E) have elevated relative male heterozygosity. It is also curious that male heterozygosity is, on average, higher than female heterozygosity across the entire genome. More individual stable flies were used to generate the male RNA-seq libraries (36 total flies) than the female libraries (26 total flies) as part of the stable fly genome project (Olafson *et al.* 2019 *preprint*). Sampling more male flies could have caused us to identify more variable sites in the males than females. We cannot examine heterozygosity along the full length of

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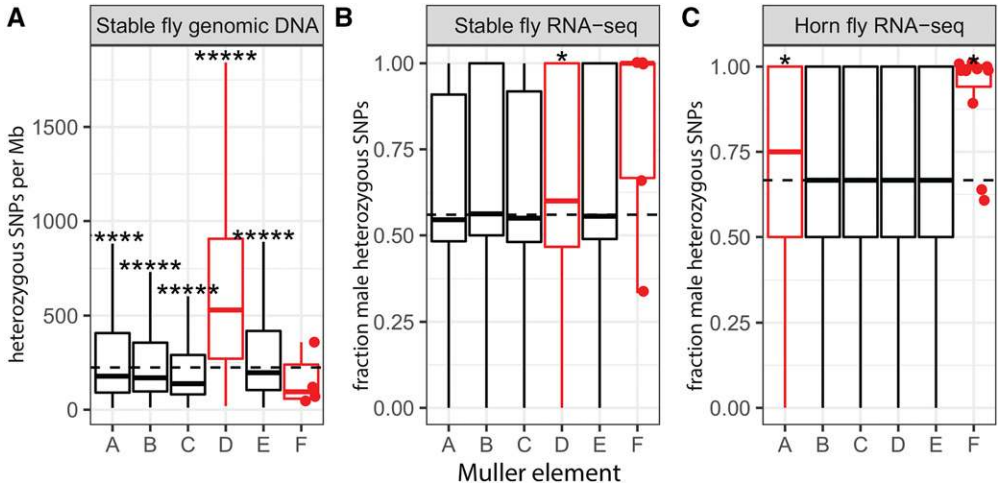

Figure 2 Identifying stable fly and horn fly sex chromosomes. A. Boxplots show the number of heterozygous SNPs per megabase (Mb) identified using genomic sequencing reads from males within annotated stable fly genes mapped to each of the Muller elements. B–C. Boxplots show the fraction of heterozygous SNPs in males relative to females within annotated stable fly and horn fly genes mapped to each of the Muller elements, using RNA-seq data. Each data point used to generate the boxplots corresponds to an individual gene. Dots indicate the values for element F genes. Dashed lines indicate the genome-wide average for all genes. Outliers were omitted from all plots. Asterisks represent p-values from Mann-Whitney tests comparing each Muller element with the other five elements ($^{*}P < 0.05$, $^{****}P < 0.00005$, $^{*****}P < 0.000005$). Inferred sex-linked elements are drawn in red.

any individual Muller elements because there is not a chromosome-scale assembly of the stable fly genome, and the order of the assembled scaffolds along each chromosome has not yet been determined.

Other comparisons of male and female heterozygosity support the sex-linkage of element D in stable fly. For example, there is a higher fraction of element D genes that only have heterozygous SNPs in males and not in females (518/1850 = 28.0%) when compared to genes on the other major elements (2168/8625 = 25.1%; $z = 2.56$, $P = 0.005$). Moreover, when we limit our analysis to only those heterozygous SNPs identified in both the genomic DNA and RNA-seq data, we find that an excess of element D genes have at least one heterozygous SNP (507/2437) when compared to genes on the other four major elements (503/11226; $P < 10^{-15}$ in Fisher's exact test).

We also detect evidence for elevated relative male heterozygosity on stable fly element F (the ancestral X chromosome). Only 10 annotated stable fly genes are assigned to element F, of which five have heterozygous SNPs. Four of those five genes have more heterozygous SNPs in males than females. It is unlikely that $\geq 4/5$ of element F genes would have $>50\%$ male heterozygous SNPs if heterozygosity were equal in males and female ($z = 1.34$, $P = 0.09$). In addition, three of the five element F genes only have heterozygous SNPs in males, which is more than the frequency of autosomal genes (25.1%) that only have male heterozygous SNPs ($z = 1.80$, $P = 0.04$). These results support the hypothesis that the stable fly X and Y chromosomes are young and minimally differentiated. They also suggest that the X chromosome consists of elements D and F, as does the Y chromosome.

It is curious that only 10 stable fly genes were assigned to element F because *D. melanogaster* element F has $\sim$ 80 genes (Riddle and Elgin 2018). The small number of stable fly element F genes in our dataset is a result of the way we assigned genes to Muller elements based on the scaffold upon which they are located (see Materials and Methods.). Only 7 scaffolds were assigned to element F, and those scaffolds contain the 10 element F genes described above. However, there are 32 additional stable fly genes with orthologs on *D. melanogaster* element F, but those 32 genes are on scaffolds either assigned to other elements or not assigned to any element (Supplementary table S12). The scaffolds containing element F genes are not enriched for assignment to any single element. These results suggest that most element F genes have translocated to other other elements in the stable fly genome, but additional work is necessary to test this hypothesis.

The genome sequence data and assembly of stable fly provides a third line of support that element D has higher male heterozygosity and is therefore part of the X and Y chromosomes. First, the reduced read mapping coverage of element D genes (Figure 3A) could be the result of variable sites in element D genes interfering with their assignment to genomic locations. In addition, we expect heterozygosity to interfere with genome assembly, leading to smaller contigs and scaffolds (Vinson *et al.* 2005; Kajitani *et al.* 2014; Pryszcz and Gabaldón 2016). In the case of XY males with nascent sex chromosomes, this assembly fragmentation should be greater on sex-chromosome-derived scaffolds than autosomal scaffolds. Consistent with this prediction, scaffolds assigned to stable fly element D are shorter and have fewer genes than scaffolds assigned to the other four major chromosomes (Figure 3B–C). Furthermore, more scaffolds in the stable fly assembly are assigned to element D than any of the other elements, even though element D does not have more genes or a larger inferred length than the other elements (Figure 3D–F).

Therefore, there are three lines of evidence that are all consistent with elevated heterozygosity on element D in stable fly males, providing a congruent picture that element D is part of a young X-Y chromosome pair. Stable fly element D is also enriched for a unique set of transposable elements (*e.g.*, *Vingi* and *Dada*) that are not enriched on any other element (Olafson *et al.* 2019 *preprint*), which is also expected for an evolving sex chromosome (Steinemann and Steinemann 2005; Ellison and Bachtrog 2013). The stable fly X and Y chromosomes also likely both contain a remnant of element F, which probably fused to element D, because genes on element F scaffolds have elevated male heterozygosity (Figure 2B). However, most element F genes have likely translocated to other elements, as described above.

The stable fly male-determining locus was previously mapped to chromosome 1 (Willis *et al.* 1981). We therefore conclude that stable fly chromosome 1 corresponds to Muller elements D and F. The stable fly Y chromosome carries a male-determining locus, but we do not know the nature of this gene. The house fly male-determining gene (*Mdmd*) was not found in the stable fly genome or any other fly relatives (Sharma *et al.* 2017). We also searched for the male-determining gene from tephritid flies (*MoY*; Meccariello *et al.* 2019) in the stable fly genome using BLAST (Altschul *et al.* 1990), and we failed to find anything resembling the *MoY* protein sequence. Therefore, either stable fly has an independently derived new male-determiner or it has

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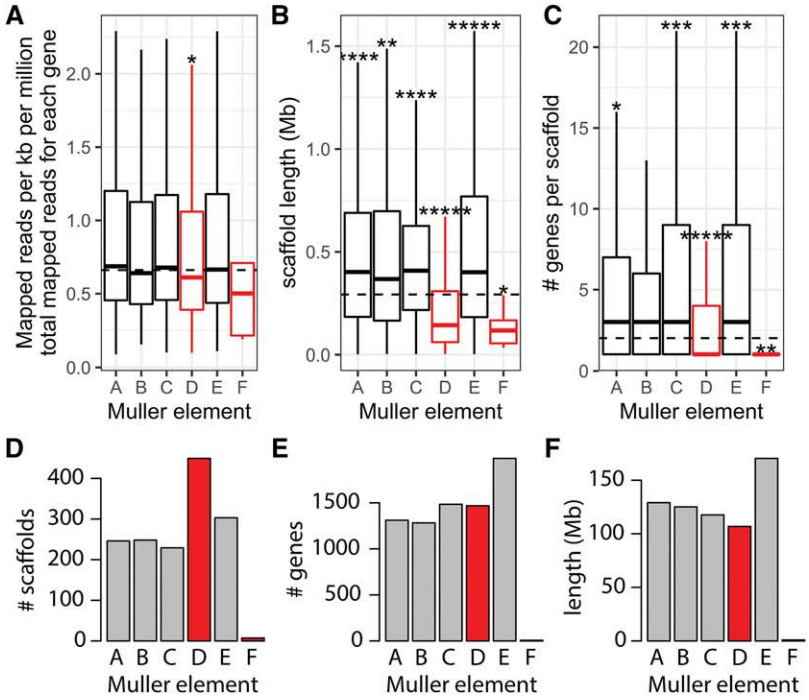

Figure 3 Fragmented assembly of the stable fly sex chromosome. A. The distributions of the number of reads mapped to genes assigned to each Muller element are shown with boxplots. B. The distributions of scaffold lengths and C. the distributions of genes per scaffold for scaffolds assigned to each stable fly Muller element are shown with boxplots. Outliers are omitted from each boxplot to more clearly show the majority of data. Dashed lines indicate the median value across all genes assigned to a Muller element. Asterisks represent p-values from Mann-Whitney tests comparing each Muller element with the other five elements ($^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$, $^{****}P < 0.00005$, $^{*****}P < 0.000005$). D. The number of genomic scaffolds and E. the number of genes assigned to each stable fly Muller element. F. The composite length of all scaffolds assigned to each stable fly Muller element. Inferred sex-linked elements are highlighted in red.

retained an ancestral male-determining locus that was replaced in house fly.

The horn fly sex chromosomes consist of elements A and F

We hypothesize that horn fly also has a young X-Y chromosome pair. The horn fly genome was sequenced using DNA extracted from a mixed sample of males and females (Konganti *et al.* 2018), which prevents us from using heterozygosity in the genome sequencing reads to identify the horn fly sex chromosomes. However, there is available RNA-seq data from horn fly testis and ovary, which we used to identify the horn fly sex chromosomes using the same approach as we did in stable fly (Supplementary tables S2 and S5). In horn fly, there is elevated relative male heterozygosity in genes assigned to element A and element F, but none of the other major elements (Figure 2C). This suggest that elements A and F are both sex-linked in horn fly. While it may appear that elements B–E have the same distributions from the boxplots shown in Figure 2C, they do in fact differ when viewed with greater resolution (Supplementary figure S1).

Other analyses of heterozygosity support the sex-linkage of element A and F in horn fly. First, there is a higher fraction of element A genes that only have heterozygous SNPs in males and not in females ($385/889 = 43.3\%$) when compared to genes on the other major elements ($1571/4163 = 37.7\%$; $z = 3.09$, $P = 0.001$). In addition, of the 44 horn fly genes assigned to element F, 11 have heterozygous SNPs. Of those 11 genes, 8 are only heterozygous in males, and the remaining 3 have more heterozygous SNPs in males than females. It is highly unlikely that $>50\%$ of heterogyzous SNPs would only be observed in males for all 11 of the horn fly element F genes if heterozygosity were equal in males and females ($z = 3.32$, $P = 0.0005$). There is also a higher fraction of element F genes that only have heterozygous SNPs in males ($8/11 = 72.7\%$) when compared to genes on the autosomes ($z = 2.39$, $P = 0.008$).

We therefore conclude that the horn fly X and Y chromosomes are likely both composed of elements A and F, and the sex chromosomes arose through a fusion of those two elements. However, unlike in stable fly, the majority (20/28) of horn fly genes with *D. melanogaster* element F homologs are assigned to horn fly element F, and an additional 24 horn fly genes with *D. melanogaster* orthologs are also assigned to element F (Supplementary table S13). This is comparable to the conservation of element F between *D. melanogaster* the blow fly *L. cuprina* (Davis *et al.* 2018). Therefore, it appears that horn fly element F has remained largely intact following the fusion with an autosome.

As with stable fly, we cannot examine heterozygosity along the full length of any individual Muller elements in horn fly because we lack a chromosome-scale assembly, and scaffold order along each chromosome has not been determined. Also, as in stable fly, male heterozygosity is, on average, higher than female heterozygosity across the entire horn fly genome (Figure 2C). In horn fly, we believe that this is caused by more RNA-seq reads generated from the male sample (SRX3340086 has 8.9 billion reads) than the female sample (SRX3340090 has 3.9 billion reads). This results in more power to detect heterozygous SNPs in males than females. Unlike stable fly, we cannot test for a more fragmented assembly of the horn fly sex chromosomes because the entire horn fly genome assembly is fragmented. The scaffold N50 of the horn fly assembly is 23 kb (Konganti *et al.* 2018), and the vast majority ($4112/4778 = 86\%$) of horn fly scaffolds assigned to Muller elements have only 1 annotated gene. We also searched for both the house fly and tephritid male-determining genes in the horn fly genome using BLAST (Altschul *et al.* 1990; Sharma *et al.* 2017; Meccariello *et al.* 2019), and we failed to find either.

Sex-biased gene expression on the sex chromosomes

Genes that are expressed at different levels between females and males are said to have "sex-biased" expression (Parsch and Ellegren 2013). Genes with male-biased (female-biased) expression are often under-(over-) represented on fly X chromosomes as a result of the haploid dose of the X chromosome in males or sex-specific selection pressures that prevent (favor) the evolution of male-biased (female-biased) expression on the X (Rice 1984; Parisi *et al.* 2003; Sturgill *et al.* 2007;

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Meiklejohn and Presgraves 2012; Meisel *et al.* 2012). We cannot perform statistical tests for differentially expressed genes between males and females using available data from either stable fly or horn fly because only one replicate RNA-seq sample was collected for each tissue type from each sex for each species. However, we can calculate the relative expression of genes in males and females ($\log_2\frac{M}{F}$) for each tissue type (Supplementary tables S6–S11), which differs between the X and autosomes in many flies (Vicoso and Bachtrog 2015).

We compared the distributions of $\log_2\frac{M}{F}$ for genes assigned to each Muller element in stable fly, horn fly, and the closely related house fly (Figure 4). Genes on stable fly element D (part of the X and Y chromosomes) have significantly lower $\log_2\frac{M}{F}$ in reproductive tissues than genes on the other major elements ($P = 0.0032$ in a Mann-Whitney test). The slight but significantly reduced $\log_2\frac{M}{F}$ on element D is consistent with the "demasculinization" or "feminization" of the X chromosome observed in reproductive tissues of other flies (Parisi *et al.* 2003; Sturgill *et al.* 2007; Meiklejohn and Presgraves 2012; Meisel *et al.* 2012; Vicoso and Bachtrog 2013, 2015). In contrast, genes on horn fly element F have higher $\log_2\frac{M}{F}$ in gonad than genes on the autosomal elements ($P = 10^{-4}$ in a Mann-Whitney test), suggesting a "masculinization" of the ancestral X chromosome. The small number of annotated element F genes in stable fly likely limits our ability to detect significant masculinization of stable fly element F. There is no significant difference in $\log_2\frac{M}{F}$ between genes on horn fly element A (the new portion of the sex chromosomes) and the autosomes in either gonad or whole adult (Fig 4). Element F is also part of a young sex chromosome pair in house fly (Meisel *et al.* 2017). There is no evidence for a difference in $\log_2\frac{M}{F}$ between genes on element F and the autosomes in house fly. The minimal evidence for demasculinization or feminization of the muscid X chromosomes is consistent with the sex chromosomes being diploid in both males and females in all three species. Similar expression in males and females is also consistent with young sex chromosomes that have not yet had time to accumulate sexually antagonistic alleles that could lead to sex-biased expression.

DISCUSSION

Stable fly and horn fly have derived karyotypes in which the X chromosome found in most other flies (Muller element F) and Y chromosome are not visible (Boyes *et al.* 1964; LaChance 1964; Joslyn *et al.* 1979; Avancini and Weinzierl 1994; Parise-Maltempi and Avancini 2007). We show, based on elevated male heterozygosity, that the X and Y chromosomes of stable fly both contain elements D and F (Figure 2A-B). The reduced assembly quality of element D is further evidence that it is sex-linked in stable fly (Figure 3). We also present evidence that the X and Y chromosomes of horn fly contain elements A and F (Figure 2C). Elevated male heterozygosity is a hallmark of a young and undifferentiated sex chromosome pair (Vicoso and Bachtrog 2015), suggesting that stable fly and horn fly have independently and recently derived nascent sex chromosomes. In addition, house fly also has multiple young proto-Y chromosomes (Meisel *et al.* 2017; Sharma *et al.* 2017). The minimal feminization/masculinization of gene expression on the sex chromosomes across these three species is consistent with their recent origins (Figure 4). House fly, stable fly, and horn fly diverged $\sim$ 27 My ago, and the order of their divergence is difficult to resolve (Ding *et al.* 2015). This is the upper-bound on the age of the sex chromosomes in each species, but the sex chromosomes may have arisen more recently given the minimal X-Y sequence divergence in all three species.

Our evidence for the sex-linkage of element D in stable fly is strong because there is a consistent signal from absolute male heterozygosity (Figure 2A), relative male heterozygosity (Figure 2B), and assembly quality (Figure 3). In contrast, the only evidence for sex-linkage of element A in horn fly is elevated relative male heterozygosity (Figure 2C). However, there is support from other work that suggests elevated relative male heterozygosity is a reliable indicator of an undifferentiated X-Y pair (Yoshida *et al.* 2014; Vicoso and Bachtrog 2015; Meisel *et al.* 2017; Wright *et al.* 2017; Toups *et al.* 2019; Veltsos *et al.* 2019). We are therefore confident that element A is indeed sex-linked in horn fly. Future work could be done to further evaluate the sex-linkage of horn fly elements A and F, as well as stable fly elements D and F.

Curiously, we observe a pattern consistent with masculinization of the ancestral X chromosome (element F) in stable fly and horn fly (Figure 4), although only significant in horn fly due to a small sample size of stable fly element F genes. This is surprising because element F genes trend toward female-biased expression both in flies with the ancestral karyotype (X-linked element F) and in *Drosophila* where element F has reverted to an autosome (Vicoso and Bachtrog 2013). The masculinization of element F in stable fly and horn fly suggests that a Y-linked copy of element F may have accumulated alleles that increase male expression (Zhou and Bachtrog 2012). Alternatively, element F could be hyper-expressed in stable fly and horn fly males because it is both diploid and transcription is up-regulated by an ancestral dosage compensation system. Dosage compensation in a closely related blow fly, which has the ancestral fly karyotype (*i.e.*, only element F is X-linked), is regulated by an RNA-binding protein that increases the transcriptional output of element F genes in hemizygous males (Linger *et al.* 2015; Davis *et al.* 2018). Stable fly and horn fly could have elevated element F expression in males because those genes are both up-regulated and diploid. Additional work is necessary to test these hypotheses, including collecting more genome sequence and RNA-seq data in order to examine allele-specific expression.

We propose three different scenarios that could have given rise to the stable fly and horn fly cryptic sex chromosomes (Figure 5). The order of most events in each scenario is arbitrary, and it is not necessary for the sex chromosomes of stable fly and horn fly to have arisen by the same scenario. All three scenarios assume that the MRCA of muscid flies had a karyotype with five euchromatic autosomes (elements A–E) and a heterochromatic sex chromosome pair (where element F is the X chromosome) because this is the ancestral karyotype of Brachycera (Vicoso and Bachtrog 2013, 2015) and is still conserved in some Muscidae (Figure 1). We additionally assume that the Y chromosome of the MRCA of Muscidae carried a male-determining locus because that is the most common mechanism of sex determination in closely and distantly related families of flies (Willhoeft and Franz 1996; Bopp *et al.* 2014; Scott *et al.* 2014b; Meccariello *et al.* 2019).

In the first scenario, we hypothesize that both the X and Y chromosomes of the MRCA of Muscidae fused to the same ancestral autosome (Figure 5). These X-autosome and Y-autosome fusions would convert one copy of the ancestral autosome into a neo-X chromosome and the other copy into a neo-Y chromosome. Concurrent fusions between the X and Y to the same autosomal element may seem unlikely, but it has been observed in *Drosophila* and birds (Flores *et al.* 2008; Pala *et al.* 2011). Element F genes have elevated heterozygosity in both stable fly and horn fly males (Figure 2), suggesting that males carry two copies of element F that have the same gene content and are only slightly differentiated at the sequence level. Therefore, this scenario requires that the ancestral X and Y of Muscidae was undifferentiated, with the Y chromosome essentially a copy of element F that carries a male-determining locus. The X and Y chromosomes of house fly are very similar in gene content (Meisel *et al.* 2017), but we do not

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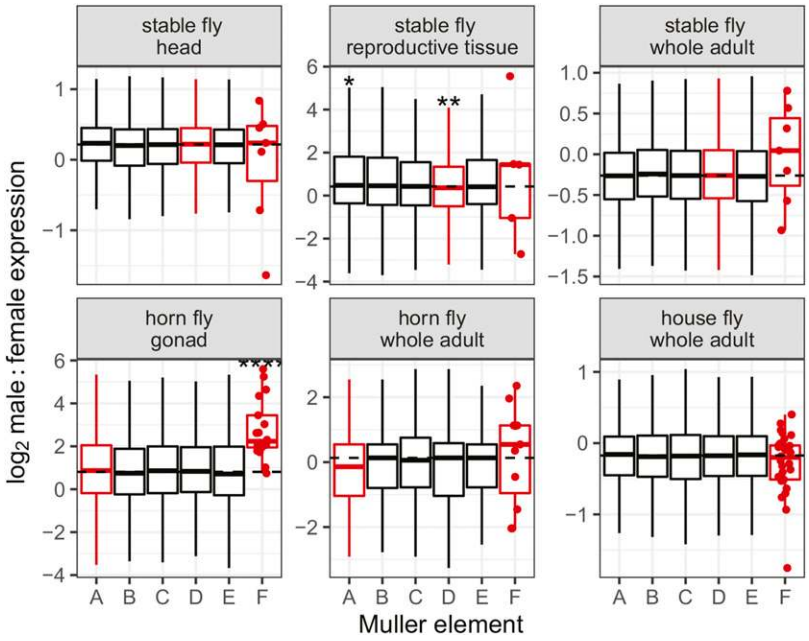

Figure 4 Sex-biased expression across Muller elements. The distributions of $\log_2$ male:female expression ($\log_2\frac{M}{F}$) for genes on each Muller element in stable fly, horn fly, and house fly are shown with boxplots. Expression data are from either whole adult (all species), gonad (horn fly only), reproductive tissues (stable fly only), or head (stable fly only). Each data point used to generate the boxplots corresponds to an individual gene. Dots show the expression levels of individual genes on element F. Dashed lines indicate the genome-wide average for all genes. Outliers were omitted from the boxplots. Asterisks represent p-values from Mann-Whitney tests comparing each Muller element with the other five elements ($^{*}P < 0.05$, $^{**}P < 0.005$, $^{****}P < 0.00005$). Inferred sex-linked elements are drawn in red.

know if this is the ancestral state of Muscidae or a derived condition in house fly.

In the second scenario, we hypothesize that the ancestral male-determining gene transposed from the ancestral Y chromosome to an autosome (Figure 5). This transposition event would convert the autosome into a proto-Y chromosome, and its homolog would be a proto-X chromosome. A transposing male-determining gene (*Mdmd*) was identified in house fly (Sharma *et al.* 2017), demonstrating the feasibility of this scenario. We also hypothesize that the ancestral X chromosome (element F) fused to the same autosome containing the male-determining locus in this scenario. We acknowledge that element F fusing to the same chromosome that carries the male-determining gene would be a remarkable coincidence. Moreover, element F must have fused to both a copy of the autosome with the male-determiner (the proto-Y) and a copy of the autosome without a male-determiner (the proto-X). This is because males must have two differentiated copies of element F (one X-linked and the other Y-linked) in order to explain the elevated male heterozygosity of element F genes in both stable fly and horn fly (Figure 2). The fusion of element F to both copies of the proto-sex chromosome may be favored if those particular fusions are more likely to resolve an inter-sexual conflict (Charlesworth and Charlesworth 1980; Matsumoto and Kitano 2016). The order of the F-autosome fusion and the transposition of the male-determiner is arbitrary in this model.

In the last step of the second scenario, the ancestral Y chromosome either fused to one of the autosomes or it was lost from the genome. This Y-autosome fusion or loss of a Y could have happened before or after the steps described above. A Y-autosome fusion is possible in this scenario if the Y chromosome lost its male-determining activity, possibly via a pseudogenizing mutation. Such a transposition of Y chromosome genes to an autosome happened following the creation of a neo-X chromosome in *Drosophila pseudoobscura* (Carvalho and Clark 2005; Larracuente *et al.* 2010). Alternatively, the ancestral Y chromosome could have fused to the element carrying the transposed male-determiner (the proto-Y), which would allow the ancestral Y to retain the male-determiner without creating an independently segregating second Y chromosome. If the ancestral Y chromosome was lost, we hypothesize that the ancestral Y did not contain any essential genes other than the male-determiner. Some *Drosophila* species have Y chromosomes that lack essential genes (Voelker and Kojima 1971), demonstrating that it is feasible for a fly Y chromosome to not be essential for male viability or fertility. Moreover, the genetic differentiation of X and Y chromosomes in both blow fly and flesh fly could be explained by a lack of essential genes on their Y chromosomes other than the male-determiner (Vicoso and Bachtrog 2013; Linger *et al.* 2015; Vicoso and Bachtrog 2015).

The third scenario differs from the second in that instead of the ancestral male-determiner transposing to an autosome, a new male-determiner arises on one of the autosomes (Figure 5). The new male-determiner would convert the autosome into a proto-Y chromosome, and its homolog would be a proto-X. The male-determining *Mdmd* gene in house fly arose from a highly conserved splicing factor that was duplicated after the divergence between house fly and stable fly (Sharma *et al.* 2017), demonstrating that new male-determining genes can arise within Muscidae. As in the second scenario, element F would have fused to the same chromosome carrying the new male-determiner (*i.e.*, the proto-Y), and it must have also fused to the homologous proto-X to produce the elevated male heterozygosity we observe in element F genes (Figure 2). The order of the F-autosome fusion and the origin of the new male-determiner is arbitrary in this model. Once again, like the second scenario, fusion of element F to the same chromosome that carries the male-determining gene would be a remarkable coincidence. In addition, the ancestral Y was either lost or fused to an autosome, as in the second scenario.

In all three scenarios, invasion (and fixation) of the fusion between element F and an autosome may be favored if one copy (more likely a Y-autosome fusion, but also possibly an X-autosome fusion) confers a sex-specific fitness benefit (Charlesworth and Charlesworth 1980; Matsumoto and Kitano 2016). The effect of sex-specific selection on the invasion of Y-autosome fusion will be greater if there is no recombination between the neo-X and neo-Y (Charlesworth and Charlesworth 1980). One way for recombination to be suppressed is if there is no recombination in males, as is the case in *Drosophila* and many other flies (Gethmann 1988). There have been no tests for male recombination in either stable fly or horn fly, but there is some evidence

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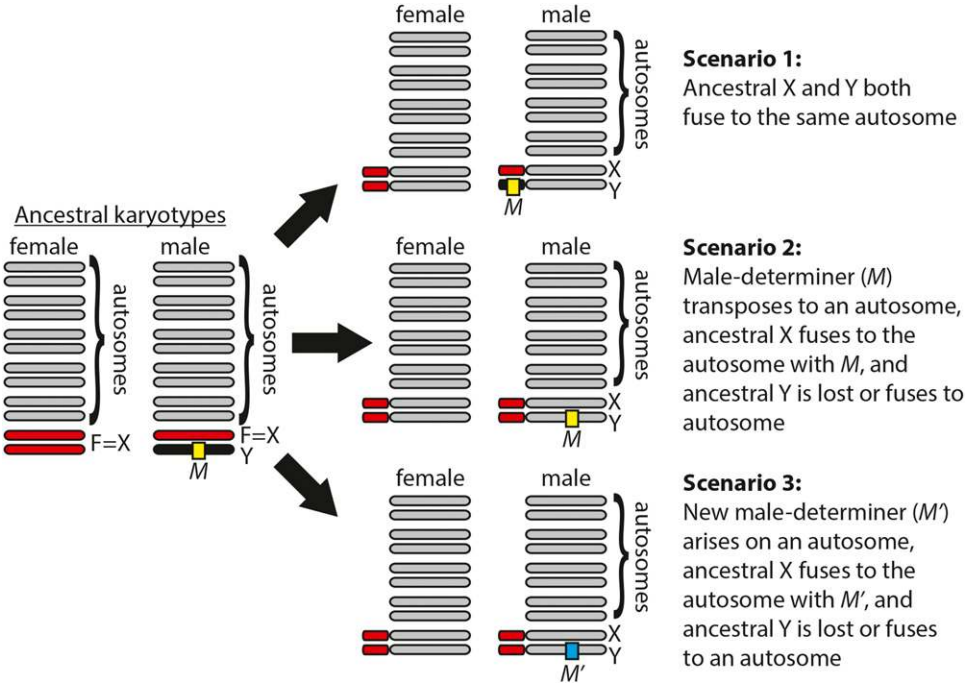

Figure 5 Potential scenarios that could give rise to muscid cryptic sex chromosomes. Ancestral autosomes (gray), X chromosome (red), and Y chromosome (black) are shown in both male and female karyotypes. Possible derived male and female genotypes are shown for three potential scenarios that could give rise to the cryptic sex chromosomes in stable fly and horn fly.

for male recombination in the closely related house fly (Feldmeyer *et al.* 2010). If there is no male recombination, then the entire neo-Y chromosome should become differentiated from the neo-X, although the rate at which genes differentiate may be heterogeneous (Kaiser *et al.* 2011; Nozawa *et al.* 2016). In contrast, if there is male recombination, then we would expect X-Y differentiation only in chromosomal regions where X-Y recombination has been suppressed via, for example, chromosomal inversions (Lahn and Page 1999). Testing for male recombination in other muscid flies will be important for evaluating which selective forces could be responsible for the new sex-linked elements in Muscidae. In addition, more contiguous assemblies of muscid genomes would allow for evaluation of the extent of differentiation across the young sex chromosomes in this family.

Each of the three scenarios makes predictions about the sex chromosomes of the MRCA of Muscidae, and testing those predictions is necessary in order to evaluate the hypotheses. For example, if all muscid species with the ancestral karyotype have undifferentiated X and Y chromosomes (as in house fly), then we would infer that the MRCA had undifferentiated sex chromosomes, supporting scenario 1. Alternatively, if extant muscids with the ancestral karyotype have differentiated X and Y chromosomes, then we could infer that the MRCA had differentiated X and Y chromosomes. This would support scenarios 2 and 3. In addition, identifying the male-determining genes across Muscidae would allow us to test if the same gene is used for male-determination across species or if new male-determiners have arisen in species other than the house fly (Sharma *et al.* 2017). If the same male-determiner is used across most species, then scenarios 1 and 2 would be supported. If new male-determiners arose in species with derived karyotypes, then scenario 3 would be supported.

Therefore, by characterizing the sex chromosomes and male-determining genes across muscid flies, we can distinguish between all three scenarios. Distinguishing between the scenarios would provide valuable insights into the factors that promote sex chromosome evolution in muscid flies, which would serve as an informative model for understanding why rates of sex chromosome evolution differ across taxa. For example, is a high rate of sex chromosome evolution in Muscidae promoted by ancestrally undifferentiated X and Y chromosomes, a gene-poor ancestral Y, transposing male-determiners, a high rate of new male-determiners, or some combination of multiple factors? Testing these hypotheses should motivate future work in this system.

In summary, we have identified independently derived young sex chromosomes in stable fly and horn fly (Figure 2), which are different from the proto-sex chromosomes of house fly (Meisel *et al.* 2017; Sharma *et al.* 2017). Therefore, there are at least three independently derived young sex chromosome systems in Muscidae, and probably more based on the derived karyotypes distributed across the family (Figure 1). In addition, we present three possible scenarios for the origins of the stable fly and horn fly sex chromosomes (Figure 5). Each scenario makes specific predictions about the male-determining genes and sex chromosomes in the MRCA of Muscidae and in other muscid species. Notably, the scenarios include two important factors that could allow for faster rates of sex chromosome evolution in some taxa (*e.g.*, Muscidae) than in closely related taxa (*e.g.*, blow flies and flesh flies). First, the ancestral X and Y chromosomes of Muscidae could have been undifferentiated, in contrast to the differentiated blow fly and flesh fly sex chromosomes (Vicoso and Bachtrog 2013; Linger *et al.* 2015; Vicoso and Bachtrog 2015; Davis *et al.* 2018). Undifferentiated sex chromosomes could allow for the formation of new sex chromosomes (Stöck *et al.* 2011, 2013; Dufresnes *et al.* 2015). Second, there could be a high rate of new or transposing male-determining genes across Muscidae, as is the case in house fly (Sharma *et al.* 2017). These new or transposable male-determining genes could allow allow for a faster rate of sex chromosome turnover. Testing for undifferentiated sex chromosomes, new male-determining genes, and transposing male-determiners in Muscidae is therefore a promising approach to assess the relative importance of these factors in permitting or promoting frequent and rapid sex chromosome turnover or neo-sex chromosomes.

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LITERATURE CITED

Abbott, J. K., A. K. Nordén, and B. Hansson, 2017 Sex chromosome evolution: historical insights and future perspectives. Proc. R. Soc. Lond. B Biol. Sci. 284: 20162806. https://doi.org/10.1098/rspb.2016.2806

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, 1990 Basic local alignment search tool. J. Mol. Biol. 215: 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Arguello, J. R., Y. Zhang, T. Kado, C. Fan, R. Zhao *et al.*, 2010 Recombination yet inefficient selection along the *Drosophila melanogaster* subgroup's fourth chromosome. Mol. Biol. Evol. 27: 848–861. https://doi.org/10.1093/molbev/msp291

Avancini, R. M. P., and R. A. Weinzierl, 1994 Karyotype of the horn fly, *Haematobia irritans* (L.) (Diptera, Muscidae). Cytologia (Tokyo) 59: 269–272. https://doi.org/10.1508/cytologia.59.269

Bachtrog, D., 2013 Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. Nat. Rev. Genet. 14: 113–124. https://doi.org/10.1038/nrg3366

Bachtrog, D., J. E. Mank, C. L. Peichel, M. Kirkpatrick, S. P. Otto *et al.*, 2014 Sex determination: why so many ways of doing it? PLoS Biol. 12: e1001899. https://doi.org/10.1371/journal.pbio.1001899

Baker, R. H., and G. S. Wilkinson, 2010 Comparative genomic hybridization (CGH) reveals a neo-X chromosome and biased gene movement in stalk-eyed flies (genus *Teleopsis*). PLoS Genet. 6: e1001121. https://doi.org/10.1371/journal.pgen.1001121

Berry, A. J., J. W. Ajioka, and M. Kreitman, 1991 Lack of polymorphism on the *Drosophila* fourth chromosome resulting from selection. Genetics 129: 1111–1117.

Beukeboom, L., and N. Perrin, 2014 *The Evolution of Sex Determination*, Oxford University Press, New York, NY. https://doi.org/10.1093/acprof:oso/9780199657148.001.0001

Bhutkar, A., S. M. Russo, T. F. Smith, and W. M. Gelbart, 2007 Genome-scale analysis of positionally relocated genes. Genome Res. 17: 1880–1887. https://doi.org/10.1101/gr.7062307

Blackmon, H., and J. P. Demuth, 2014 Estimating tempo and mode of Y chromosome turnover: Explaining Y chromosome loss with the fragile Y hypothesis. Genetics 197: 561–572. https://doi.org/10.1534/genetics.114.164269

Bopp, D., G. Saccone, and M. Beye, 2014 Sex determination in insects: variations on a common theme. Sex Dev. 8: 20–28. https://doi.org/10.1159/000356458

Boyes, J. W., 1953 Somatic chromosomes of higher diptera: II. Differentiation of sarcophagid species. Can. J. Zool. 31: 561–576. https://doi.org/10.1139/z53-040

Boyes, J. W., 1961 Somatic chromosomes of higher diptera: V. Interspecific and intraspecific variation in the Calliphoridae. Can. J. Zool. 39: 549–570. https://doi.org/10.1139/z61-059

Boyes, J. W., M. J. Corey, and H. E. Paterson, 1964 Somatic chromosomes of higher diptera IX. Karyotypes of some muscid species. Can J Cytol 42: 1025–1036.

Boyes, J. W., and J. M. Van Brink, 1965 Chromosomes of calyptrate diptera. Can. J. Genet. Cytol. 7: 537–550. https://doi.org/10.1139/g65-073

Bray, N. L., H. Pimentel, P. Melsted, and L. Pachter, 2016 Near-optimal probabilistic RNA-seq quantification. Nat. Biotechnol. 34: 525–527. https://doi.org/10.1038/nbt.3519

Carvalho, A. B., and A. G. Clark, 2005 Y chromosome of *D. pseudoobscura* is not homologous to the ancestral *Drosophila* Y. Science 307: 108–110. https://doi.org/10.1126/science.1101675

Charlesworth, D., and B. Charlesworth, 1980 Sex differences in fitness and selection for centric fusions between sex-chromosomes and autosomes. Genet. Res. 35: 205–214. https://doi.org/10.1017/S0016672300014051

Charlesworth, D., B. Charlesworth, and G. Marais, 2005 Steps in the evolution of heteromorphic sex chromosomes. Heredity 95: 118–128. https://doi.org/10.1038/sj.hdy.6800697

Davis, R. J., E. J. Belikoff, E. H. Scholl, F. Li, and M. J. Scott, 2018 *no blokes* is essential for male viability and X chromosome gene expression in the Australian sheep blowfly. Curr. Biol. 28: 1987–1992.e3. https://doi.org/10.1016/j.cub.2018.05.005

DePristo, M. A., E. Banks, R. Poplin, K. V. Garimella, J. R. Maguire *et al.*, 2011 A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat. Genet. 43: 491–498. https://doi.org/10.1038/ng.806

Ding, S., X. Li, N. Wang, S. L. Cameron, M. Mao *et al.*, 2015 The phylogeny and evolutionary timescale of Muscoidea (Diptera: Brachycera: Calyptratae) inferred from mitochondrial genomes. PLoS One 10: e0134170. https://doi.org/10.1371/journal.pone.0134170

Dobin, A., C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski *et al.*, 2013 STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29: 15–21. https://doi.org/10.1093/bioinformatics/bts635

Dufresnes, C., A. Borzée, A. Horn, M. Stöck, M. Ostini *et al.*, 2015 Sex-chromosome homomorphy in Palearctic tree frogs results from both turnovers and X-Y recombination. Mol. Biol. Evol. 32: 2328–2337. https://doi.org/10.1093/molbev/msv113

Ellison, C. E., and D. Bachtrog, 2013 Dosage compensation via transposable element mediated rewiring of a regulatory network. Science 342: 846–850. https://doi.org/10.1126/science.1239552

Feldmeyer, B., I. Pen, and L. W. Beukeboom, 2010 A microsatellite marker linkage map of the housefly, *Musca domestica*: evidence for male recombination. Insect Mol. Biol. 19: 575–581. https://doi.org/10.1111/j.1365-2583.2010.01016.x

Flores, S., A. Evans, and B. McAllister, 2008 Independent origins of new sex-linked chromosomes in the *melanica* and *robusta* species groups of *Drosophila*. BMC Evol. Biol. 8: 33. https://doi.org/10.1186/1471-2148-8-33

Foster, G. G., M. J. Whitten, C. Konovalov, J. T. A. Arnold, and G. Maffi, 1981 Autosomal genetic maps of the Australian sheep blowfly, *Lucilia cuprina dorsalis* R.-D. (Diptera: Calliphoridae), and possible correlations with the linkage maps of *Musca domestica* L. and *Drosophila melanogaster* (Mg.). Genet. Res. 37: 55–69. https://doi.org/10.1017/S0016672300020012

Gethmann, R. C., 1988 Crossing over in males of higher Diptera (Brachycera). J. Hered. 79: 344–350. https://doi.org/10.1093/oxfordjournals.jhered.a110526

Hamm, R. L., R. P. Meisel, and J. G. Scott, 2015 The evolving puzzle of autosomal *vs.* Y-linked male determination in *Musca domestica*. G3 (Bethesda) 5: 371–384. https://doi.org/10.1534/g3.114.014795

Hilton, H., R. M. Kliman, and J. Hey, 1994 Using hitchhiking genes to study adaptation and divergence during speciation within the *Drosophila melanogaster* species compelx. Evolution 48: 1900–1913. https://doi.org/10.1111/j.1558-5646.1994.tb02222.x

Jensen, M. A., B. Charlesworth, and M. Kreitman, 2002 Patterns of genetic variation at a chromosome 4 locus of *Drosophila melanogaster* and *D. simulans*. Genetics 160: 493–507.

Joslyn, D. J., J. A. Seawright, and N. L. Willis, 1979 The karyotype of the stable fly, *Stomoxys Calcitrans* (L.) (Diptera: Muscidae). Caryologia 32: 349–354. https://doi.org/10.1080/00087114.1979.10796798

Kaiser, V. B., Q. Zhou, and D. Bachtrog, 2011 Nonrandom gene loss from the *Drosophila miranda* neo-Y chromosome. Genome Biol. Evol. 3: 1329–1337. https://doi.org/10.1093/gbe/evr103

Kajitani, R., K. Toshimoto, H. Noguchi, A. Toyoda, Y. Ogura *et al.*, 2014 Efficient de novo assembly of highly heterozygous genomes from whole-genome shotgun short reads. Genome Res. 24: 1384–1395. https://doi.org/10.1101/gr.170720.113

Konganti, K., F. D. Guerrero, F. Schilkey, P. Ngam, J. L. Jacobi *et al.*, 2018 A whole genome assembly of the horn fly, *Haematobia irritans*, and prediction of genes with roles in metabolism and sex determination. G3 (Bethesda) 8: 1675–1686. https://doi.org/10.1534/g3.118.200154

Kriventseva, E. V., D. Kuznetsov, F. Tegenfeldt, M. Manni, R. Dias *et al.*, 2018 OrthoDB v10: sampling the diversity of animal, plant, fungal, protist, bacterial and viral genomes for evolutionary and functional annotations of orthologs. Nucleic Acids Res. 47: D807–D811. https://doi.org/10.1093/nar/gky1053

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LaChance, L. E., 1964 Chromosome studies in three species of Diptera (Muscidae and Hypodermatidae). Ann. Entomol. Soc. Am. 57: 69–73. https://doi.org/10.1093/aesa/57.1.69

Lahn, B. T., and D. C. Page, 1999 Four evolutionary strata on the human X chromosome. Science 286: 964–967. https://doi.org/10.1126/science.286.5441.964

Larracuente, A. M., M. A. F. Noor, and A. G. Clark, 2010 Translocation of Y-linked genes to the dot chromosome in *Drosophila pseudoobscura*. Mol. Biol. Evol. 27: 1612–1620. https://doi.org/10.1093/molbev/msq045

Li, H., and R. Durbin, 2009 Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Linger, R. J., E. J. Belikoff, and M. J. Scott, 2015 Dosage compensation of X-linked Muller element F genes but not X-linked transgenes in the Australian sheep blowfly. PLoS One 10: e0141544. https://doi.org/10.1371/journal.pone.0141544

Matsumoto, T., and J. Kitano, 2016 The intricate relationship between sexually antagonistic selection and the evolution of sex chromosome fusions. J. Theor. Biol. 404: 97–108. https://doi.org/10.1016/j.jtbi.2016.05.036

McDonald, P. T., and C. D. Schmidt, 1987 Genetics of permethrin resistance in the horn fly (Diptera: Muscidae). J. Econ. Entomol. 80: 433–437. https://doi.org/10.1093/jee/80.2.433

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis *et al.*, 2010 The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20: 1297–1303. https://doi.org/10.1101/gr.107524.110

Meccariello, A., M. Salvemini, P. Primo, B. Hall, P. Koskinioti *et al.*, 2019 Maleness-on-the-Y (MoY) orchestrates male sex determination in major agricultural fruit fly pests. Science 365: 1457–1460.

Meiklejohn, C. D., and D. C. Presgraves, 2012 Little evidence for demasculinization of the *Drosophila* X chromosome among genes expressed in the male germline. Genome Biol. Evol. 4: 1007–1016. https://doi.org/10.1093/gbe/evs077

Meisel, R. P., C. A. Gonzales, and H. Luu, 2017 The house fly Y Chromosome is young and minimally differentiated from its ancient X Chromosome partner. Genome Res. 27: 1417–1426. https://doi.org/10.1101/gr.215509.116

Meisel, R. P., J. H. Malone, and A. G. Clark, 2012 Disentangling the relationship between sex-biased gene expression and X-linkage. Genome Res. 22: 1255–1265. https://doi.org/10.1101/gr.132100.111

Meisel, R. P., and J. G. Scott, 2018 Using genomic data to study insecticide resistance in the house fly, *Musca domestica*. Pestic. Biochem. Physiol. 151: 76–81. https://doi.org/10.1016/j.pestbp.2018.01.001

Meisel, R. P., J. G. Scott, and A. G. Clark, 2015 Transcriptome differences between alternative sex determining genotypes in the house fly, *Musca domestica*. Genome Biol. Evol. 7: 2051–2061. https://doi.org/10.1093/gbe/evv128

Muller, H. J., 1940 Bearings of the '*Drosophila*' work on systematics, pp. 185–268 in *The New Systematics*, edited by Huxley, J. Clarendon Press, Oxford, Oxford.

Nozawa, M., K. Onizuka, M. Fujimi, K. Ikeo, and T. Gojobori, 2016 Accelerated pseudogenization on the neo-X chromosome in *Drosophila miranda*. Nat. Commun. 7: 13659. https://doi.org/10.1038/ncomms13659

Olafson, P. U., S. Aksoy, G. M. Attardo, G. Buckmeier, X. Chen *et al.*, 2019 Functional genomics of the stable fly, *Stomoxys calcitrans*, reveals mechanisms underlying reproduction, host interactions, and novel targets for pest control. bioRxiv. doi: 10.1101/623009 (Preprint posted May 2, 2019).

Pala, I., S. Naurin, M. Stervander, D. Hasselquist, S. Bensch *et al.*, 2011 Evidence of a neo-sex chromosome in birds. Heredity 108: 264–272. https://doi.org/10.1038/hdy.2011.70

Parise-Maltempi, P., and R. Avancini, 2007 Comparative cytogenetic study in Muscidae flies. Braz. J. Biol. 67: 945–950. https://doi.org/10.1590/S1519-69842007000500020

Parisi, M., R. Nuttall, D. Naiman, G. Bouffard, J. Malley *et al.*, 2003 Paucity of genes on the *Drosophila* X chromosome showing male-biased expression. Science 299: 697–700. https://doi.org/10.1126/science.1079190

Parsch, J., and H. Ellegren, 2013 The evolutionary causes and consequences of sex-biased gene expression. Nat. Rev. Genet. 14: 83–87. https://doi.org/10.1038/nrg3376

Pennell, M. W., M. Kirkpatrick, S. P. Otto, J. C. Vamosi, C. L. Peichel *et al.*, 2015 Y fuse? sex chromosome fusions in fishes and reptiles. PLoS Genet. 11: e1005237. https://doi.org/10.1371/journal.pgen.1005237

Pryszcz, L. P., and T. Gabaldón, 2016 Redundans: an assembly pipeline for highly heterozygous genomes. Nucleic Acids Res. 44: e113. https://doi.org/10.1093/nar/gkw294

Qiu, S., R. Bergero, and D. Charlesworth, 2013 Testing for the footprint of sexually antagonistic polymorphisms in the pseudoautosomal region of a plant sex chromosome pair. Genetics 194: 663–672. https://doi.org/10.1534/genetics.113.152397

Rice, W. R., 1984 Sex chromosomes and the evolution of sexual dimorphism. Evolution 38: 735–742. https://doi.org/10.1111/j.1558-5646.1984.tb00346.x

Rice, W. R., 1986 On the instability of polygenic sex determination: the effect of sex-specific selection. Evolution 40: 633–639.

Riddle, N. C., and S. C. R. Elgin, 2018 The *Drosophila* dot chromosome: Where genes flourish amidst repeats. Genetics 210: 757–772. https://doi.org/10.1534/genetics.118.301146

Roberts, R. B., J. R. Ser, and T. D. Kocher, 2009 Sexual conflict resolved by invasion of a novel sex determiner in Lake Malawi cichlid fishes. Science 326: 998–1001. https://doi.org/10.1126/science.1174705

Schnell e Schuehli, G., C. J. B. de Carvalho, and B. M. Wiegmann, 2007 Molecular phylogenetics of the Muscidae (Diptera: Calyptratae): new ideas in a congruence context. Invertebr. Syst. 21: 263–278. https://doi.org/10.1071/IS06026

Scott, J. G., W. C. Warren, L. W. Beukeboom, D. Bopp, A. G. Clark *et al.*, 2014a Genome of the house fly, *Musca domestica* L., a global vector of diseases with adaptations to a septic environment. Genome Biol. 15: 466. https://doi.org/10.1186/s13059-014-0466-3

Scott, M. J., M. L. Pimsler, and A. M. Tarone, 2014b Sex determination mechanisms in the Calliphoridae (blow flies). Sex Dev. 8: 29–37. https://doi.org/10.1159/000357132

Sharma, A., S. D. Heinze, Y. Wu, T. Kohlbrenner, I. Morilla *et al.*, 2017 Male sex in houseflies is determined by *Mdmd*, a paralog of the generic splice factor gene *CWC22*. Science 356: 642–645. https://doi.org/10.1126/science.aam5498

Steinemann, S., and M. Steinemann, 2005 Y chromosomes: born to be destroyed. BioEssays 27: 1076–1083. https://doi.org/10.1002/bies.20288

Stöck, M., A. Horn, C. Grossen, D. Lindtke, R. Sermier *et al.*, 2011 Ever-young sex chromosomes in european tree frogs. PLoS Biol. 9: e1001062. https://doi.org/10.1371/journal.pbio.1001062

Stöck, M., R. Savary, C. Betto-Colliard, S. Biollay, H. Jourdan-Pineau *et al.*, 2013 Low rates of X-Y recombination, not turnovers, account for homomorphic sex chromosomes in several diploid species of Palearctic green toads (*Bufo viridis* subgroup). J. Evol. Biol. 26: 674–682. https://doi.org/10.1111/jeb.12086

Sturgill, D., Y. Zhang, M. Parisi, and B. Oliver, 2007 Demasculinization of X chromosomes in the *Drosophila* genus. Nature 450: 238–241. https://doi.org/10.1038/nature06330

Sved, J. A., Y. Chen, D. Shearman, M. Frommer, A. S. Gilchrist *et al.*, 2016 Extraordinary conservation of entire chromosomes in insects over long evolutionary periods. Evolution 70: 229–234. https://doi.org/10.1111/evo.12831

Swain, A., and R. Lovell-Badge, 1999 Mammalian sex determination: a molecular drama. Genes Dev. 13: 755–767. https://doi.org/10.1101/gad.13.7.755

Toups, M. A., N. Rodrigues, N. Perrin, and M. Kirkpatrick, 2019 A reciprocal translocation radically reshapes sex-linked inheritance in the common frog. Mol. Ecol. 28: 1877–1889. https://doi.org/10.1111/mec.14990

Ullerich, F.-H., 1963 Geschlechtschromosomen und Geschlechtsbestimmung bei einigen Calliphorinen (Calliphoridae, Diptera). Chromosoma 14: 45–110. https://doi.org/10.1007/BF00332610

Van der Auwera, G. A., M. O. Carneiro, C. Hartl, R. Poplin, G. del Angel *et al.*, 2013 From fastq data to high-confidence variant calls:

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The genome analysis toolkit best practices pipeline. Curr. Protoc. Bioinformatics 43: 1–33.
van Doorn, G. S., 2014 Patterns and mechanisms of evolutionary transitions between genetic sex-determining systems. Cold Spring Harb. Perspect. Biol. 6: a017681. https://doi.org/10.1101/cshperspect.a017681
van Doorn, G. S., and M. Kirkpatrick, 2007 Turnover of sex chromosomes induced by sexual conflict. Nature 449: 909–912. https://doi.org/10.1038/nature06178
van Doorn, G. S., and M. Kirkpatrick, 2010 Transitions between male and female heterogamety caused by sex-antagonistic selection. Genetics 186: 629–645. https://doi.org/10.1534/genetics.110.118596
Veltsos, P., K. E. Ridout, M. A. Toups, S. C. González-Martínez, A. Muyle *et al.*, 2019 Early sex-chromosome evolution in the diploid dioecious plant *Mercurialis annua*. Genetics 212: 815–835. https://doi.org/10.1534/genetics.119.302045
Vicoso, B., and D. Bachtrog, 2013 Reversal of an ancient sex chromosome to an autosome in *Drosophila*. Nature 499: 332–335. https://doi.org/10.1038/nature12235
Vicoso, B., and D. Bachtrog, 2015 Numerous transitions of sex chromosomes in Diptera. PLoS Biol. 13: e1002078. https://doi.org/10.1371/journal.pbio.1002078
Vicoso, B., and B. Charlesworth, 2006 Evolution on the X chromosome: unusual patterns and processes. Nat. Rev. Genet. 7: 645–653. https://doi.org/10.1038/nrg1914
Vinson, J. P., D. B. Jaffe, K. O'Neill, E. K. Karlsson, N. Stange-Thomann *et al.*, 2005 Assembly of polymorphic genomes: Algorithms and application to *Ciona savignyi*. Genome Res. 15: 1127–1135. https://doi.org/10.1101/gr.3722605
Voelker, R. A., and K.-i. Kojima, 1971 Fertility and fitness of XO males in *Drosophila* i. qualitative study. Evolution 25: 119–128. https://doi.org/10.1111/j.1558-5646.1971.tb01865.x
Wang, J., J.-K. Na, Q. Yu, A. R. Gschwend, J. Han *et al.*, 2012 Sequencing papaya X and Yh chromosomes reveals molecular basis of incipient sex chromosome evolution. Proc. Natl. Acad. Sci. USA 109: 13710–13715. https://doi.org/10.1073/pnas.1207833109
Wang, W., K. Thornton, A. Berry, and M. Long, 2002 Nucleotide variation along the *Drosophila melanogaster* fourth chromosome. Science 295: 134–137. https://doi.org/10.1126/science.1064521
Wang, W., K. Thornton, J. J. Emerson, and M. Long, 2004 Nucleotide variation and recombination along the fourth chromosome in *Drosophila simulans*. Genetics 166: 1783–1794. https://doi.org/10.1534/genetics.166.4.1783
Weller, G. L., and G. G. Foster, 1993 Genetic maps of the sheep blowfly *Lucilia cuprina*: linkage-group correlations with other dipteran genera. Genome 36: 495–506. https://doi.org/10.1139/g93-068
Wiegmann, B. M., M. D. Trautwein, I. S. Winkler, N. B. Barr, J.-W. Kim *et al.*, 2011 Episodic radiations in the fly tree of life. Proc. Natl. Acad. Sci. USA 108: 5690–5695. https://doi.org/10.1073/pnas.1012675108
Willhoeft, U., and G. Franz, 1996 Identification of the sex-determining region of the *Ceratitis capitata* Y chromosome by deletion mapping. Genetics 144: 737–745.
Willis, N. L., J. A. Seawright, C. Nickel, and D. J. Joslyn, 1981 Reciprocal translocations and partial correlation of chromosomes in the stable fly. J. Hered. 72: 104–106. https://doi.org/10.1093/oxfordjournals.jhered.a109435
Wright, A. E., I. Darolti, N. I. Bloch, V. Oostra, B. Sandkam *et al.*, 2017 Convergent recombination suppression suggests role of sexual selection in guppy sex chromosome formation. Nat. Commun. 8: 14251. https://doi.org/10.1038/ncomms14251
Wright, A. E., R. Dean, F. Zimmer, and J. E. Mank, 2016 How to make a sex chromosome. Nat. Commun. 7: 12087. https://doi.org/10.1038/ncomms12087
Yoshida, K., T. Makino, K. Yamaguchi, S. Shigenobu, M. Hasebe *et al.*, 2014 Sex chromosome turnover contributes to genomic divergence between incipient stickleback species. PLoS Genet. 10: e1004223. https://doi.org/10.1371/journal.pgen.1004223
Zhou, Q., and D. Bachtrog, 2012 Sex-specific adaptation drives early sex chromosome evolution in *Drosophila*. Science 337: 341–345. https://doi.org/10.1126/science.1225385

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