X chromosome drive in a widespread Palearctic woodland fly, *Drosophila testacea*

Graeme L. Keais, Mark A. Hanson, Brent E. Gowen, and Steve J. Perlman

June 2017

X chromosome drive in a widespread Palearctic woodland fly, *Drosophila testacea*

Graeme L. Keais[1*], Mark A. Hanson[1], Brent E. Gowen[1], and Steve J. Perlman[1*]

Running title: X drive in *Drosophila testacea*

*Corresponding Authors: Email: graeme09@uvic.ca and stevep@uvic.ca; Phone: 250-721-6319; Mailing address: Department of Biology, University of Victoria, 3800 Finnerty Road, Victoria, British Columbia, Canada V8P 5C2

[1]Department of Biology, University of Victoria, 3800 Finnerty Road, Victoria, British Columbia, Canada V8P 5C2

Acknowledgements

We are grateful to Matt Ballinger for providing useful comments on the manuscript. We thank Patrick Nahirney for access to his Jeol TEM. This work was supported by a Discovery grant (Natural Sciences and Engineering Research Council of Canada) to SJP, a Sinergia grant (Swiss National Science Foundation) to SJP, and an NSERC CGS M scholarship to GLK. SJP acknowledges support from the Integrated Microbial Biodiversity program of the Canadian Institute for Advanced Research.

Abstract

Selfish genes that bias their own transmission during meiosis can spread rapidly in populations, even if they contribute negatively to the fitness of their host. Driving X chromosomes provide a clear example of this type of selfish propagation. These chromosomes have important evolutionary and ecological consequences, and can be found in a broad range of taxa including plants, mammals, and insects. Here we report a new case of X chromosome drive (X drive) in a widespread woodland fly, *Drosophila testacea*. We show that males carrying the driving X (SR males) sire 80-100% female offspring, and possess a diagnostic X chromosome haplotype that is perfectly associated with the sex ratio distortion phenotype. We find that the majority of sons produced by SR males are sterile and appear to lack a Y chromosome, suggesting that meiotic defects involving the Y chromosome may underlie X drive in this species. Abnormalities in sperm cysts of SR males reflect that some spermatids are failing to develop properly, confirming that drive is acting during gametogenesis. By screening wild-caught flies using progeny sex ratios and a diagnostic marker, we demonstrate that the driving X is present in wild populations at a frequency of ~10% and that suppressors of drive are segregating in the same population. The testacea species group appears to be a hotspot for X drive, and *D. testacea* is a promising model to compare driving X chromosomes in closely related species, some of which may even be younger than the chromosomes themselves.

Keywords: Meiotic drive, X chromosome drive, selfish genetic elements, *Drosophila*, segregation distortion, genetic conflict

Introduction

Mendelian segregation is effectively 'fair', meaning gene frequencies generally do not change during the process of gene transmission alone. However, some genes cheat, subverting the process of equal segregation **(Crow, 1991)**. Such selfish genetic elements are able to increase their own transmission relative to the rest of the genome, ending up in more than the expected 50% of gametes. The power of non-Mendelian inheritance is striking: in the absence of countervailing selection, even a small transmission advantage above 50% will lead to the rapid spread and eventual fixation of a selfish 'driving' allele **(Burt & Trivers, 2006)**.

X chromosome drive (X drive) provides a clear example of selfish genetic behaviour. This phenomenon was first described in *Drosophila* (where it is called the *sex-ratio* trait) **(Gershenson, 1928)**, but has since been found in a wide range of taxa, including rodents, plants, and numerous flies (Diptera) **(Jaenike, 2001)**. Broadly, X drive is characterized by the unequal transmission of the X chromosome, which can be achieved in a number of ways. In Diptera, X drive is achieved through the action of an X-linked gene product that destroys or incapacitates nearly all of an individual's Y-bearing sperm during gametogenesis. As a result, males carrying a driving X chromosome (X^{SR}) produce predominantly X-bearing gametes, and therefore sire almost exclusively daughters **(Jaenike, 2001)**.

X drive engenders a diverse set of ecological and evolutionary consequences **(Lindholm *et al.*, 2016)**. For example, it has been shown to significantly affect chromosome organization **(Dyer *et al.*, 2007)**, the evolution of mating systems **(Price *et al.*, 2008b; Pinzone & Dyer, 2013)**, and patterns of molecular evolution **(Derome *et al.*, 2004; Phadnis & Orr, 2009; Kingan *et al.*, 2010)**. There is also increasing evidence that X drive plays a role in speciation through the

evolution of hybrid incompatibilities **(Frank, 1991; Hurst & Pomiankowski, 1991; Tao *et al.*, 2001; Phadnis & Orr, 2009; McDermott & Noor, 2010)**. Furthermore, an unhampered rise in the frequency of an X^{SR} chromosome can lead to population sex ratios that are dramatically female biased. In theory, a severe lack of males caused by X drive could drive a species to extinction **(Hamilton, 1967)**.

Despite the threat of extinction, some X drive systems are clearly very old (*e.g.* drive in *Drosophila pseudoobscura* [**Babcock & Anderson, 1996**]), raising the question of how X^{SR} chromosomes are maintained over evolutionary timescales. Several factors contributing to the long-term dynamics of X drive have been explored, both theoretically and experimentally. For example, because any deviation from a 1:1 sex ratio is not evolutionarily stable **(Fisher, 1930)**, X drive instigates an extended genetics arms race over sex ratio, often leading to the evolution of resistant Y chromosomes and autosomal suppressors **(**reviewed in **Jaenike, 2001)**. The presence of suppression is variable: some X drive systems are completely neutralized by suppression in the wild, and are only revealed by interspecific or inter-population crosses **(Dermitzakis *et al.*, 2000; Tao *et al.*, 2001; Tao *et al.*, 2007a,b)**; other systems are polymorphic for suppression, and some lack it entirely (**Jaenike, 2001**). In addition to suppression, selection also plays an important role in maintaining *sex-ratio* (SR) polymorphisms in populations. Inversions that couple driving loci together are frequently found on X^{SR} chromosomes. Due to a lack of recombination in these regions, there can be an accumulation of deleterious mutations linked to drive, which contribute negatively to the fitness of X^{SR} carriers (**Jaenike, 2001**). Therefore, selection against carriers, especially homozygous females ($X^{SR}X^{SR}$), can prevent the spread of an X^{SR} (**Wallace, 1948; Edwards, 1961**). Selection against the X^{SR} may also act in a frequency-

dependent manner. Males carrying the X^{SR} chromosome (hereafter, SR males) do poorly in sperm depletion assays when compared to standard (ST) males **(*e.g.* Beckenbach, 1978; Jaenike, 1996; Atlan *et al.*, 2004; Wilkinson *et al.*, 2006; Unckless *et al.*, 2015)**, reflecting the fact that they are producing roughly half the amount of functional sperm. Therefore, if males become rare due to an increase in X^{SR} frequency, the male mating rate should increase, and ST males will be favoured, assuming a greater number of sperm translates to a greater number of offspring **(Jaenike, 1996)**. Similarly, their reduced sperm production makes SR males poor sperm competitors compared to ST males (***e.g.* Wilkinson & Fry, 2001; Atlan *et al.*, 2004; Wilkinson *et al.*, 2006; Price *et al.*, 2008a**); thus the fitness of SR males is further reduced if females re-mate (polyandry). Overall, the evolutionary maintenance of X drive systems is perplexing, in part because not all drive systems evolve suppression (**Jaenike, 2001**), nor do all species with drive exhibit polyandry (**Verspoor *et al.*, 2016**).

It has long been known that the reduced sperm production of SR males is due to the developmental failure of Y-bearing sperm **(Policansky & Ellison, 1970)**, yet the underlying cause remains poorly understood in most species. The molecular basis of X drive has only been characterized in *Drosophila simulans*, which astonishingly houses at least 3 independent SR systems **(Tao *et al.*, 2007a)**. Two of these systems, called Winters and Paris, have a known genetic basis. In the Paris SR system, X drive results from the missegregation of Y chromatids during meiosis II **(Cazemajor *et al.*, 2000)**. Recently, **Helleu *et al.* (2016)** have shown that mutations in the X-linked heterochromatin protein, *HP1D2*, contribute to this abnormal Y chromosome behaviour, likely by improperly preparing the Y chromosome for meiosis. While this represents the only known molecular mechanism of X drive so far, abnormal behaviour of Y

chromosomes during meiosis II has also been directly observed in *Drosophila pseudoobscura* **(Novitski *et al.*, 1965; Cobbs *et al.*, 1991)** and *Drosophila athabasca* **(Novitski *et al.*, 1965)**, and is inferred in *Drosophila subobscura* **(Hauschteck-Jungen *et al.*, 1972)**. This suggests that causing non-disjunction of the Y chromosome may be a common route for X^{SR} chromosomes to bias their own transmission (**Helleu *et al.*, 2014**). In contrast, the mode of action of the Winters SR trait in *D. simulans* occurs post-meiotically **(Tao *et al.*, 2007a)**. In this case, the causative agent has been mapped to the X-linked gene *Dox*, which codes for a small RNA molecule with limited protein-coding potential **(Tao *et al.*, 2007b)**. Although it is not well understood how the *Dox* gene product causes drive, these various studies of drive in *D. simulans* nevertheless tell us that different SR chromosomes act through different means and at different times during gametogenesis.

Here we report the discovery of X drive in *Drosophila testacea*, a common and widespread Palearctic mushroom-feeding fly belonging to the testacea group of the subgenus *Drosophila*. We characterize several features of X drive in this species, with the aim of contributing to a broader understanding of the shared features of driving X chromosomes. We quantify sex ratio distortion in this species and confirm the inheritance pattern of the SR trait. We also examine the etiology of the X^{SR} by testing the fertility of sons sired by SR males, as well as through transmission electron microscopy of developing sperm in both SR and ST males. Lastly, we examine the frequency of the driving X chromosome in the wild. Interestingly, the testacea group appears to be a hotspot of X drive, with three of its four members now known to harbour examples (**James & Jaenike, 1990; Pieper & Dyer, 2016**), including the well-studied *D. neotestacea*, whose driving X chromosome occurs at very high frequencies in nature, with no

known suppressors **(Dyer, 2012; Pinzone & Dyer, 2013)**. This presents an excellent opportunity to compare driving X chromosomes in closely related species, some of which may even be younger than the chromosomes themselves.

Materials and Methods

Fly stocks

Our lab stock of *D. testacea* was founded with multiple wild caught flies from St. Sulpice, Vaud, Switzerland in September 2012. All flies are reared at 21°C with a 12-h light:dark cycle in vials containing instant *Drosophila* medium (Carolina Biological Supply, Burlington, NC), supplemented with commercial mushroom (*Agaricus bisporus*). We have also established a line lacking the X^{SR}.

Characterization of sex ratio distortion

We first detected a female bias in our stock of *D. testacea* during experiments requiring equal numbers of newly eclosed male and female flies. By quantifying the sex ratio of a single generation emerging from our stock, we found that ~77% were female. A series of initial crosses exploring the nature of this sex ratio distortion suggested that the trait was expressed only in males, a typical characteristic of X drive. Therefore, we took sons of a presumed heterozygous female ($X^{SR}X^{ST}$) and crossed them each individually to 2 virgin $X^{ST}X^{ST}$ females (all ST flies used in this study are from our non-driving line). After 4 days, males were removed and mated females were transferred to fresh vials. Females were subsequently transferred to fresh vials every 4 days for an additional 12 days, and then discarded. The sex ratios of emerging flies

were scored. We tested for deviations from a 1:1 sex ratio using chi-squared tests implemented in R (version 3.3.0).

Inheritance of the *SR* trait

We performed a pedigree analysis to formally demonstrate that the SR trait in our lab population of *D. testacea* is due to an X-linked factor. This pedigree analysis eliminates the possibility that sex ratio distortion is caused by any of the following: a Y-linked gene, a cytoplasmic factor, or an autosomal gene (described in detail in **James & Jaenike, 1990**). We mated females from a highly female biased line (*i.e.* daughters of a presumed $X^{SR}Y$ male, offspring of cross A in Fig. 2) with $X^{ST}Y$ males. We assumed that these daughters were heterozygous for their X chromosome ($X^{SR}X^{ST}$), and we therefore expected them to produce both $X^{SR}Y$ and $X^{ST}Y$ sons. We crossed several of these sons to $X^{ST}X^{ST}$ females (crosses C and D in Fig. 2), and their progeny sex ratios were recorded. As expected, some sons produced offspring with normal (~1:1) sex ratios (cross C), while others produced female biased sex ratios (cross D). The latter were presumed to be $X^{SR}Y$, and their daughters were crossed with $X^{ST}Y$ males (cross E in Fig. 2). Lastly, 20 sons resulting from this final cross were genotyped (see below) as either $X^{SR}Y$ or $X^{ST}Y$.

Fertility of sons sired by SR males

We assessed the fertility of the sons of SR males by mating 38 sons from 7 different SR males to 4 virgin $X^{ST}X^{ST}$ females each. Each male was placed in a vial with 2 virgin $X^{ST}X^{ST}$ females, and allowed to mate for 5 days. Males were then transferred by aspiration to an additional 2

151 virgin $X^{ST}X^{ST}$ females, and again left to mate for 5 days. All pairs of females were turned over to
152 new vials every 5 days, to a total of 3 vials per pair of females.

153 To screen for a Y chromosome in these 38 sons, we extracted their DNA using
154 Prepman™ Ultra (Applied Biosystems), and attempted to amplify the gene *kl-2* using
155 quantitative PCR (qPCR). Previous work has shown this gene to be on the Y chromosome in
156 testacea group flies **(Dyer *et al.*, 2011)**. Gene sequences available from the National Center for
157 Biotechnology Information (NCBI) were used to design a set of qPCR primers (kl2q-F and kl2q-R,
158 Table S2) within the *D. testacea kl-2* gene. We used DNA extracted from a virgin female as a
159 negative DNA template control. The following qPCR thermal cycling conditions were used: 95 °C
160 for 10 min, then 35 cycles of 95 °C for 15s followed by 60 °C for 45s, with the product confirmed
161 using melt curve analysis and Sanger sequencing (Sequetech, USA). We confirmed that all DNA
162 extractions were positive for DNA using a separate PCR reaction amplifying an X-linked gene,
163 *RpL36* (primers in Table S2). The presence of *RpL36* amplicons was confirmed on an agarose gel.
164 An age-matched control for both the fertility assay and qPCR was performed using 24 sons of
165 an ST male following the same procedures.

166 **Genotyping**

167 In an attempt to identify polymorphisms that might be associated with sex ratio
168 distortion, we first extracted DNA from 17 males with known progeny sex ratios. Using PCR, we
169 then amplified two X-linked genes (*RpL36* and *skpA*) from these males using primers previously
170 developed in a study examining molecular evolution in the testacea species group **(Dyer *et al.*,**
171 **2011)**. The following thermal cycling conditions were used: 95 °C for 3 min, then 32 cycles of
172 94 °C for 1 min and 54 °C for 1 min, followed by a final 10 min at 72 °C. PCR amplicons were

then Sanger-sequenced (Macrogen, USA). Sequence handling and analysis was performed using Geneious v5.1.7 **(Kearse *et al.*, 2012)**.

Electron microscopy of sperm cysts

To obtain images of sperm cysts using transmission electron microscopy (TEM), 7 day old ST (n = 3) and SR (n = 4) males were anesthetised with CO_2 and their testes were dissected out. DNA extractions were performed on the head and thorax of each fly and genotyped using the X-linked marker gene s*kpA* (see Genotyping, Methods). The isolated testes were processed using standard TEM methodology (Hayat, 1989): double-fixation and embedding into Epon. TEM sections were stained in uranyl acetate and lead citrate and viewed in a Jeol JEM 1400 TEM equipped with a Gatan SC-1000 digital camera.

Prevalence of X^{SR} in the wild

Wild *D. testacea* were caught near St. Sulpice, Switzerland, in July 2016 by aspirating flies off of mushroom baits. Forty-one wild caught males were mated to 4 laboratory $X^{ST}X^{ST}$ virgin females each and their progeny sex ratios were scored. We also generated *skpA* sequences (See Methods, Genotyping) for all males.

Given our results from these two methods, we suspected a suppressor may be acting in two of the wild caught males. We hypothesized that any males producing a normal offspring sex ratio, but carrying a driving X genotype, may also carry a suppressing element. To explore this possibility, we took daughters of putatively suppressing males (*i.e.* those producing a normal sex ratio but with a driving genotype) and mated them to $X^{ST}Y$ males from our lab stock

193 (and thus containing no suppressors). Sons of these females were then mated to several virgin
194 $X^{ST}X^{ST}$ females each and their progeny sex ratios were scored.
195 All unique DNA sequences for *skpA, RpL36,* and *kl-2* generated in this study were
196 deposited in GenBank (accession numbers KY407222-KY407235 and KY774653-4).
197

198 **Results**

199 **Characterization of sex-ratio distortion**

200 Female-biased sex ratios were common in our driving line. For example, of 23 *D.*
201 *testacea* males mated to $X^{ST}X^{ST}$ females, 13 had a significant excess of female offspring relative
202 to the expected 1:1 ratio ($\chi^2_1 \geq 12.5$, $P \leq 0.000407$)(Fig 1., Table S1). These males produced
203 offspring that were 81%-100% female. The remaining 10 males had normal offspring sex ratios
204 ($\chi^2_1 \geq 0.19679$, $P \geq 0.21$), which ranged between 43% and 54% female (Fig. 1, Table S1).

205 **Inheritance of the *SR* trait**

206 Cross A (Fig. 2) between a presumed SR male ($X^{SR}Y$) and an $X^{ST}X^{ST}$ female yielded 97%
207 female offspring (F_1 females). These F_1 females were presumed heterozygous for their X ($X^{SR}X^{ST}$),
208 and if mated to an $X^{ST}Y$ male (Fig. 2, cross B), they should therefore produce both $X^{SR}Y$ sons and
209 $X^{ST}Y$ sons (F_2 males). Indeed, some F_2 males produced offspring with a ratio of ~1:1 (Fig. 2, cross
210 C), and others produced predominantly female offspring (Fig. 2, cross D). Mating daughters of
211 cross D to $X^{ST}Y$ males generated F_4 males, 15 out of 20 of which were $X^{SR}Y$. This pedigree
212 analysis eliminates the possibility that sex ratio distortion in *D. testacea* is caused by a Y-linked,
213 cytoplasmic, recessive autosomal, or dominant autosomal factor **(see James & Jaenike, 1990)**.

214 **Fertility of sons sired by *SR* males**

215 Out of 38 sons sired by SR fathers, 2 produced viable offspring with normal sex ratios
216 when mated to virgin laboratory females (Table 1, Table S3). In contrast, 23 of 24 sons sired by
217 an ST male produced offspring (Table S3).

218 From these 38 sons of SR males, we were only able to amplify the Y-linked gene *kl-2*
219 from the two sons that produced offspring (Table 1, Table S3). No *kl-2* amplicon was detected
220 from the 36 infertile sons (Table S3). There is therefore a highly significant association between
221 fertility and the presence of the gene *kl-2* in sons of SR males (Fisher's exact test, P = 0.001422).
222 We successfully amplified *kl-2* from all 24 control males (Table S3).

223 **Genotyping**

224 Sequences generated for the *skpA* gene from males with known offspring sex ratios
225 revealed the existence of two haplotypes, one perfectly associated with the SR trait (Fisher's
226 exact test, P = 8.08e-05), which we call the X^{SR} haplotype, and the other perfectly associated
227 with males producing normal offspring sex ratios, which we call the X^{ST} haplotype (Fig. 3, Table
228 S1). The two haplotypes differ by 6 single nucleotide polymorphisms (SNPs) across 442 base
229 pairs (bps), two of which are non-synonymous substitutions in the X^{SR} version (Fig. 3). The X^{ST}
230 haplotype is identical to previously obtained *skpA* sequence from *D. testacea* from Germany
231 (Dyer et al. 2011). Likewise, there were two *RpL36* haplotypes, associated with either the X^{ST} or
232 X^{SR}, and differing at 4 synonymous sites across 323 bp.

233 **Spermatogenesis is abnormal in SR males**

234 Cross sections of sperm cysts indicate that spermatogenesis proceeds normally in ST
235 males: individualized spermatids are tightly arranged, and nearly all spermatids display an

orderly axoneme-Nebenkern pair (Fig. 4A). In contrast, sperm cysts of SR males are highly disorganized, and several spermatids have not developed normally or are absent (Fig. 4B). For example, some spermatids appear to have fused axonemes, with the fused spermatids sharing a common cytoplasm (Fig. 4B, asterisk). Another abnormality is the presence of underdeveloped mitochondrial derivatives (Fig. 4B, arrow). Small vesicles, which may be multivesicular bodies (**see Ramamurthy *et al.*, 1980**), are also present in the sperm cysts of SR males, and not in ST males (Fig. 4B, arrowhead).

Prevalence of X^{SR} in the wild

Most wild caught males produced normal offspring sex ratios when mated to laboratory females (Fig. 5). However, 2 of 37 had significantly female biased offspring (86% and 95%), confirming the presence of the X^{SR} in natural populations of *D. testacea* (Fig. 5, Table S4). Both males with skewed offspring sex ratios had *skpA* sequences that were identical to those generated from our lab X^{SR} individuals (the X^{SR} *skpA* haplotype). Of the 35 males with normal offspring sex ratios, 33 possessed *skpA* genotypes associated with X^{ST}. The remaining 2 males with normal sex ratios (61% and 52% female offspring) carried the X^{SR} *skpA* haplotype; thus, we suspected that these males may carry a suppressing element. Indeed, some F_2 sons from both putative suppressor males produced significantly skewed female offspring sex ratios when mated to females from our non-driving line (Table S5), demonstrating that when put in our non-suppressing laboratory genetic background, both previously suppressed X's were able to drive. Finally, 4 males did not produce offspring; they all carried the X^{ST} *skpA* genotype. Thus the frequency of X^{SR} in our wild sample is ~10% (4/41).

Discussion

This study is the first to identify X drive in the Palearctic woodland fly *Drosophila testacea*, a mycophagous species that ranges from western Europe to Japan. We show that males carrying the X^{SR} sire a significant excess of female offspring. We also show that the majority of male offspring sired by SR males are sterile and appear to lack a Y chromosome. The etiology of X drive in this species is characterized by the irregular development of sperm, as shown by the abnormal appearance of sperm cysts in all SR males. Finally, our results demonstrate that the X^{SR}, as well as suppressors of drive, are segregating in wild populations of *D. testacea* from Switzerland.

X drive in *D. testacea* is strong, with SR males producing 80-100% female offspring. However, drive is much stronger than it appears from progeny sex ratios because nearly all sons produced by SR males are sterile (~95%) – of 38 sons sired by various SR males, only 2 produced offspring when mated to laboratory females. In addition, we were unable to detect the presence of a Y-linked fertility gene from all sterile sons. This strongly suggests that sterile sons of SR males do not possess a Y chromosome. If so, sterile sons are likely derived from nullo-XY sperm, and are therefore XO.

The production of XO males as a result of X drive is a common pattern in *Drosophila*, having been found to occur in *Drosophila simulans* **(Cazemajor *et al.*, 2000)**, *Drosophila pseudoobscura* **(Sturtevant & Dobzhansky, 1936; Henahan & Cobbs, 1983; Cobbs, 1986)**, *Drosophila paramelanica* **(Stalker, 1961)**, and *Drosophila athabasca* **(Voelker & Kojima, 1971)**. X drive in *Drosophila neotestacea* is also presumed to result in the production of XO progeny, as inferred from the sterility of all sons produced by SR males in this species **(James & Jaenike,**

1990). While the production of XO males is clearly a common occurrence in X drive systems, there are subtle differences between them. For instance, in *D. pseudoobscura* all sons of SR males are XO and sterile **(Henahan & Cobbs, 1983)**, whereas SR males in *D. simulans* produce both sterile XO and fertile XY males **(Cazemajor *et al.*, 2000)**. Here we have shown that SR males in *D. testacea*, like *D. simulans*, can produce both sterile XO and fertile XY sons. However, fertile XY sons are far rarer in the progeny of *D. testacea* (~5%) when compared to *D. simulans*, where roughly two-thirds of male progeny of SR males are XY **(Cazemajor *et al.*, 2000)**. The production of nullo-XY sperm by *D. simulans* SR males is explained by the missegregation of the Y chromosomes during meiosis II **(Cazemajor *et al.*, 2000; Helleu *et al.*, 2016)**. As we observe XO males as a result of X drive in *D. testacea*, a similar abnormality could be occurring during meiosis in *D. testacea* SR males.

We found a haplotype that is unique to the X^{SR}, spanning several hundred base pairs within the genes *skpA* and *RpL36*, demonstrating that there is reduced recombination between driving and non-driving X chromosomes. Since both X-linked genes we sequenced showed so many sequence differences, it is likely that recombination is suppressed across a large portion of the *D. testacea* driving X chromosome. Suppressed recombination, often in the form of chromosomal inversions, is a common feature of driving X chromosomes, as it prevents the decoupling of interacting loci that contribute to drive **(Jaenike, 2001)**. A lack of recombination benefits the X^{SR} in the short term but will eventually lead to the accumulation of deleterious mutations **(Dyer *et al.*, 2007)**. In accordance with this expectation, we find that even though *skpA* is a highly conserved gene, two of the six nucleotide changes found in X^{SR} *skpA* are nonsynonymous amino acid changes that are not found in any sequenced Diptera.

The sequence differences existing between the X^{SR} and X^{ST} allow us to use *skpA* as a marker for SR, making it a powerful tool for studying X drive in *D. testacea*. In the present study, we took advantage of this marker to help screen for males carrying the X^{SR} in the wild, in the Swiss population where we first identified drive. We found that the X^{SR} exists at relatively low frequencies in males (~10%). When mated to laboratory females, 2 of 37 wild caught males expressed drive, both of which carried the X^{SR} chromosome. However, two males with normal sex ratios also carried the X^{SR}. By placing these X chromosomes in our laboratory genetic background, we show that both of these chromosomes exhibit drive, demonstrating that the initial wild caught males carried suppressing elements. Attempting to characterize the genetic nature of suppression in *D. testacea* is beyond the scope of this paper. However, resistant Y chromosomes are predicted to evolve more readily than autosomal suppressors, as susceptible Y chromosomes have little to no fitness when paired with an X^{SR} **(Helleu *et al.*, 2014)**. Future work will attempt to confirm whether suppression of drive is Y-linked.

The testacea group is an especially promising system for studying the evolution and ecology of driving X chromosomes. Testacea group species are mycophagous, spending most of their life cycle on mushroom. Their ecology is therefore comparatively well understood, making them amenable to research under ecologically relevant contexts. Furthermore, three (*D. testacea*, *D. neotestacea,* and *D. orientacea*) of the four known members of the testacea species group now have been reported to harbour driving X chromosomes (James & Jaenike, 1990; Pieper & Dyer, 2016), indicating that this lineage may be a 'hotspot' for the evolution of X drive. These three species are very closely related, with incomplete reproductive isolation, and were only recently recognized to be different **(Grimaldi *et al.*, 1992)**. Significantly, X drive in

these closely related species differs in a number of important ways. The presence of suppression, its low frequency in the wild, and its divergence from the X^{ST} point to an ancient origin of the X^{SR} in *D. testacea*, perhaps even pre-dating the origin of the species. In contrast, X drive in *D. neotestacea* appears to be comparatively young: it persists at high frequencies (>30%) in the wild and no suppressors of drive have been found in this species **(Dyer, 2012)**. Also, recent dating work suggests that it evolved after *D. neotestacea* split from its relatives **(Pieper & Dyer, 2016)**. X drive in *D. testacea* provides the unique opportunity to compare driving X chromosomes of different ages in closely related taxa, which should provide insight into the evolution of these selfish genetic elements, as well as their role in speciation.

References

Atlan, A., Joly, D., Capillon, C. & Montchamp-Moreau, C. 2004. *Sex-ratio* distorter of *Drosophila simulans* reduces male productivity and sperm competition ability. *J. Evol. Biol.* **17**: 744-751.

Babcock, C.S. & Anderson, W.W. 1996. Molecular evolution of the Sex-Ratio inversion complex in *Drosophila pseudoobscura*: analysis of the *Esterase-5* gene region. *Mol. Biol. Evol.* **13**: 297-308.

352 Beckenbach, A.T. 1978. The “sex-ratio” trait in *Drosophila pseudoobscura*: fertility relations of
353 males and meiotic drive. *Am. Nat.* **112**: 97-117.
354 Beckenbach, A.T. 1996. Selection and the “sex-ratio” polymorphism in natural populations of
355 *Drosophila pseudoobscura*. *Evolution* **50**: 787-794.
356 Burt, A. & Trivers R. 2006. *Genes in conflict: the biology of selfish genetic elements.* Belknap
357 Press of Harvard University Press, Cambridge, MA.
358 Cazemajor, M., Joly, D. & Montchamp-Moreau C. 2000. *Sex-ratio* meiotic drive in *Drosophila*
359 *simulans* is related to equational nondisjunction of the Y chromosome. *Genetics* **154**:
360 229-236.
361 Cobbs, G. 1986. An investigation of the genetics of “male sex-ratio” phenotype in *Drosophila*
362 *pseudoobscura*. *Genetics* **113**: 355-365.
363 Cobbs, G., Jewell, L. & Gordon, L. 1991. Male-sex-ratio trait in *Drosophila pseudoobscura*:
364 frequency of autosomal aneuploid sperm. **127**: 381-390.
365 Crow, J.F. 1991. Why is mendelian segregation so exact? *BioEssays* **13**: 305-312.
366 Dermitzakis, E.T., Masly, J.P., Waldrip, H.M. & Clark, A.G. 2000. Non-mendelian segregation of
367 sex chromosomes in heterospecific *Drosophila* males. *Genetics* **154**: 687-694.
368 Derome, N., Métayer, K., Montchamp-Moreau, C. & Veuille, M. 2004. Signature of selective
369 sweep associated with the evolution of sex-ratio drive in *Drosophila simulans*. *Genetics*
370 **166**: 1357-1366.
371 Dyer, K.A., Charlesworth, B. & Jaenike, J. 2007. Chromosome-wide linkage disequilibrium as a
372 consequence of meiotic drive. *Proc. Natl. Acad. Sci. U. S. A.* **104**: 1587-1592.

373 Dyer, K.A., White, B.E., Bray, M.J., Piqué, D.G. & Betancourt, A.J. 2011. Molecular evolution of a
374 Y chromosome to autosome gene duplication in *Drosophila*. *Mol. Biol. Evol.* **28**: 1293-
375 1306.
376 Dyer, K.A. 2012. Local selection underlies the geographic distribution of sex-ratio drive in
377 *Drosophila neotestacea*. *Evolution* **66**: 973-984.
378 Edwards, A.W.F. The population genetics of "sex-ratio" in *Drosophila pseudoobscura. Heredity*
379 **16**: 291-304.
380 Fisher, R.A. 1930. *The genetical theory of natural selection*. The Claredon Press, Oxford.
381 Frank, S.A. 1991. Divergence of meiotic drive-suppression systems as an explanation for sex-
382 biased hybrid sterility and inviability. *Evolution* **45**: 262-267.
383 Gershenson, S. 1928. A new sex-ratio abnormality in *Drosophila obscura*. *Genetics* **13**: 488-507.
384 Grimaldi, D., James, A.C. & Jaenike, J. 1992. Systematics and modes of reproductive isolation in
385 the Holarctic *Drosophila testacea* species group (Diptera: Drosophilidae). *Ann. Entomol.*
386 *Soc. Am.* **85**: 671-685.
387 Hamilton, W.D. 1967. Extraordinary sex ratios. *Science* **156**: 477-488.
388 Hauschteck-Jungen, E., Jungen, H. & Muller, M. 1972. Karyotyp und meiose bei wild- und sex
389 ratio-mannchen von *Drosophila subobscura*. Revue Suisse Zool. **79**:297-305.
390 Hayat, M.A. 1989. *Principles and techniques of electron microscopy: biological applications*, 3rd
391 edn. CRC Press, Boca Raton, FL.
392 Helleu, Q., Gérard, P.R., Dubruille, R., Ogereau, D., Prud'homme, B., Loppin, B. *et al.* 2016. Rapid
393 evolution of a Y-chromosome heterochromatin protein underlies sex chromosome
394 meiotic drive. *Proc. Natl. Acad. Sci. U. S. A.* **113**: 4110-4115.

Helleu, Q., Gérard, P.R. & Montchamp-Moreau, C. 2014. Sex chromosome drive. *Cold Spring Harb. Perspect. Biol.* **7**: a017616.

Henahan, J. & Cobbs G. 1983. Origin of X/O progeny from crosses of sex-ratio trait males of *Drosophila pseudoobscura*. *J. Hered.* **74**: 145-148.

Hurst, L.D. & Pomiankowski, A. 1991. Causes of sex ratio bias may account for unisexual sterility in hybrids: a new explanation of Haldane's rule and related phenomena. *Genetics* **128**: 841-858.

James, A.C. & Jaenike, J. 1990. "Sex ratio" meiotic drive in *Drosophila testacea*. *Genetics* **126**: 651-656.

Jaenike, J. 1996. Sex-ratio meiotic drive in the *Drosophila quinaria* group. *Am. Nat.* **148**: 237-254.

Jaenike, J. 2001. Sex chromosome meiotic drive. *Annu. Rev. Ecol. and Syst.* **32**: 25-49.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S. *et al.* 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics **28:*** 1647-1649.

Kingan, S.B., Garrigan, D. & Hartl, D.L. 2010. Recurrent selection on the Winters *sex-ratio* genes in *Drosophila simulans*. *Genetics* **184**: 253-265.

Lindholm, A.K., Dyer, K.A., Firman, R.C., Fishman, L., Forstmeier, W., Holman, L. *et al.* 2016. The ecology and evolutionary dynamics of meiotic drive. *Trends Ecol. Evol.* **31**: 315-326.

McDermott, S.R. & Noor, M.A.F. 2010. The role of meiotic drive in hybrid male sterility. *Phil. Trans. R. Soc. B* **365**: 1265-1272.

Novitski, E., Peacock, W.J. & Engel, J. 1965. Cytological basis of "sex ratio" in *Drosophila pseudoobscura*. *Science* **148**: 516-517.

Phadnis, N. & Orr, H.A. 2009. A single gene causes both male sterility and segregation distortion in *Drosophila* hybrids. *Science* **323**: 376-379.

Pieper, K.E. & Dyer, K.A. 2016. Occasional recombination of a selfish X-chromosome may permit its persistence at high frequencies in the wild. *J. Evol. Biol.* **29**: 2229-2241.

Pinzone, C.A. & Dyer, K.A. 2013. Association of polyandry and sex-ratio drive prevalence in natural populations of *Drosophila neotestacea*. *Proc. R. Soc. London, B.* **280**: 20131397.

Policansky, D. & Ellison, J. 1970. "Sex ratio" in *Drosophila pseudoobscura*: spermiogenic failure. *Science* **168**: 888-889.

Price, T.A.R., Bretman, A.J., Avent, T.D., Snook, R.R., Hurst, G.D.D. & Wedell, N. 2008a. Sex ratio distorter reduces sperm competitive ability in an insect. *Evolution* **62**: 1644-1652.

Price, T.A.R, Hodgson, D.J., Lewis, Z., Hurst, G.D.D. & Wedell, N. 2008b. Selfish genetic elements promote polyandry in a fly. *Science* **322**: 1241-1243.

Ramamurthy, G., Alfert, M. & Stern, C. 1980. Ultrastructural studies on spermatogenesis in a sex-ratio-mutant strain of *Drosophila simulans*. *Am. J. Anat.* **157**: 205-219.

R Core Team 2016. *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria.

Stalker, H.D. 1961. The genetic systems modifying meiotic drive in *Drosophila paramelanica*. *Genetics* **46**: 117-202.

Sturtevant, A.H. & Dobzhanksy, T. 1936. Geographical distribution and cytology of "sex ratio" in *Drosophila pseudoobscura* and related species. *Genetics* **21**: 473-490.

Tao, Y., Hartl, D.L. & Laurie, C.C. 2001. Sex-ratio segregation distortion associated with reproductive isolation in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* **108**: 13183-13188.

Tao, Y., Masly, J.P., Araripe, L., Ke, Y. & Hartl, D.L. 2007. A *sex-ratio* meiotic drive system in *Drosophila simulans.* I: an autsosomal suppressor. *PLoS Biol.* **5**: e292.

Tao, Y., Araripe, L., Kingan, S.B., Ke, Y., Xiao, H. & Hartl, D.L. 2007. A *sex-ratio* meiotic drive system in *Drosophila simulans.* II: an X-linked distorter. *PLoS Biol.* **5**: e292.

Unckless, R.L., Larracuente, A.M. & Clark, A.G. 2015. Sex-ratio meiotic drive and Y-linked resistance in *Drosophila affinis*. *Genetics* **199**: 831-840.

Verspoor, R.L., Hurst, G.D.D. & Price, T.A.R. 2016. The ability to gain matings, not sperm competition, reduces the success of males carrying a selfish genetic element in a fly. *Anim. Behav.* **115**: 207-215.

Voelker, R.A. & Kojima, K. 1971. Fertility and fitness of XO males in *Drosophila* I. qualitative study. *Evolution* **25**: 119-128.

Wallace, B. 1948. Studies on “sex-ratio” in *Drosophila pseudoobscura*. I. selection and “sex-ratio”. *Evolution* **2**: 189-217.

Wilkinson, G.S. & Fry, C.L. 2001. Meiotic drive alters sperm competitive ability in stalk-eyed flies. *Proc. R. Soc. London, B.* **22**: 2559-2564.

Wilkinson, G.S., Johns, P.M., Kelleher, E.S., Muscedere, M.L. & Lorsong, A. 2006. Fitness effects of X chromosome drive in the stalk-eyed fly, *Cyrtodiopsis dalmanni*. *J. Evol. Biol.* **19**: 1851-1860.

Table 1. Fertility of 38 sons sired by 7 different SR males. Sons were mated to 4 virgin laboratory females each to assess their fertility. DNA extractions were subsequently performed on these sons in order to screen for the Y-linked gene *kl-2* using qPCR.

SR male	Offspring sex ratio (proportion female)	Sons tested	Fertile	*kl-2* amplified
1	0.98	3	0	0
2	0.83	15	1	1
3	0.91	5	0	0
4	0.94	5	0	0
5	0.97	3	0	0
6	0.86	5	1	1
7	0.98	2	0	0

Figure 1. Progeny sex ratios of male *Drosophila testacea* (SR or ST). Each male was mated to 2 virgin females. The number of offspring sired by each male is shown in brackets. Only males that produced >15 offspring are included. Progeny sex ratios that significantly deviate from the expected 1:1 are shown as light bars (χ^2_1: $P < 0.05$). The dashed horizontal line denotes the expected proportion of female offspring (0.5).

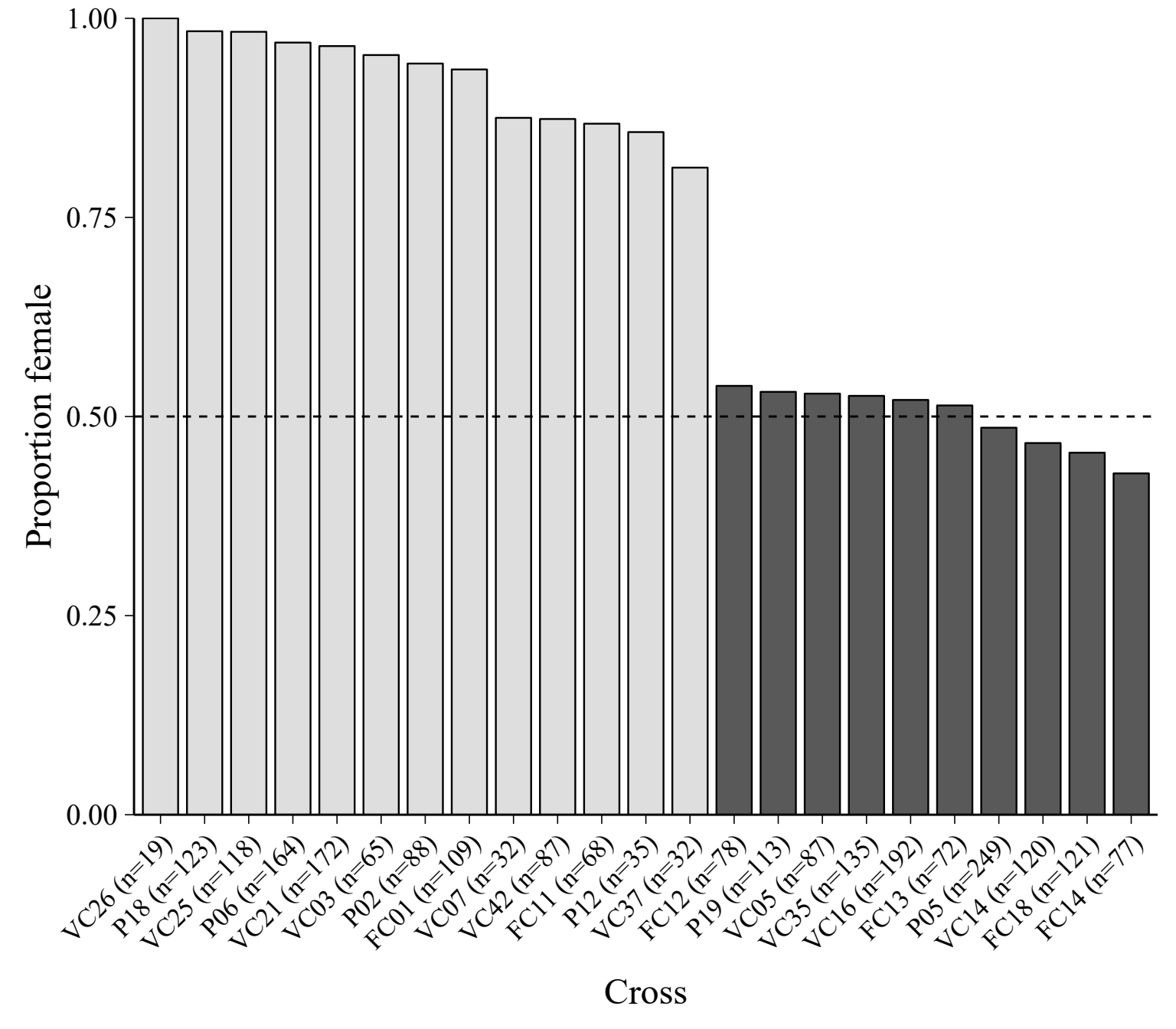
Figure 2. Crossing scheme used to determine the inheritance pattern of the *SR* trait in *Drosophila testacea.* Squares and circles denote males and females, respectively.

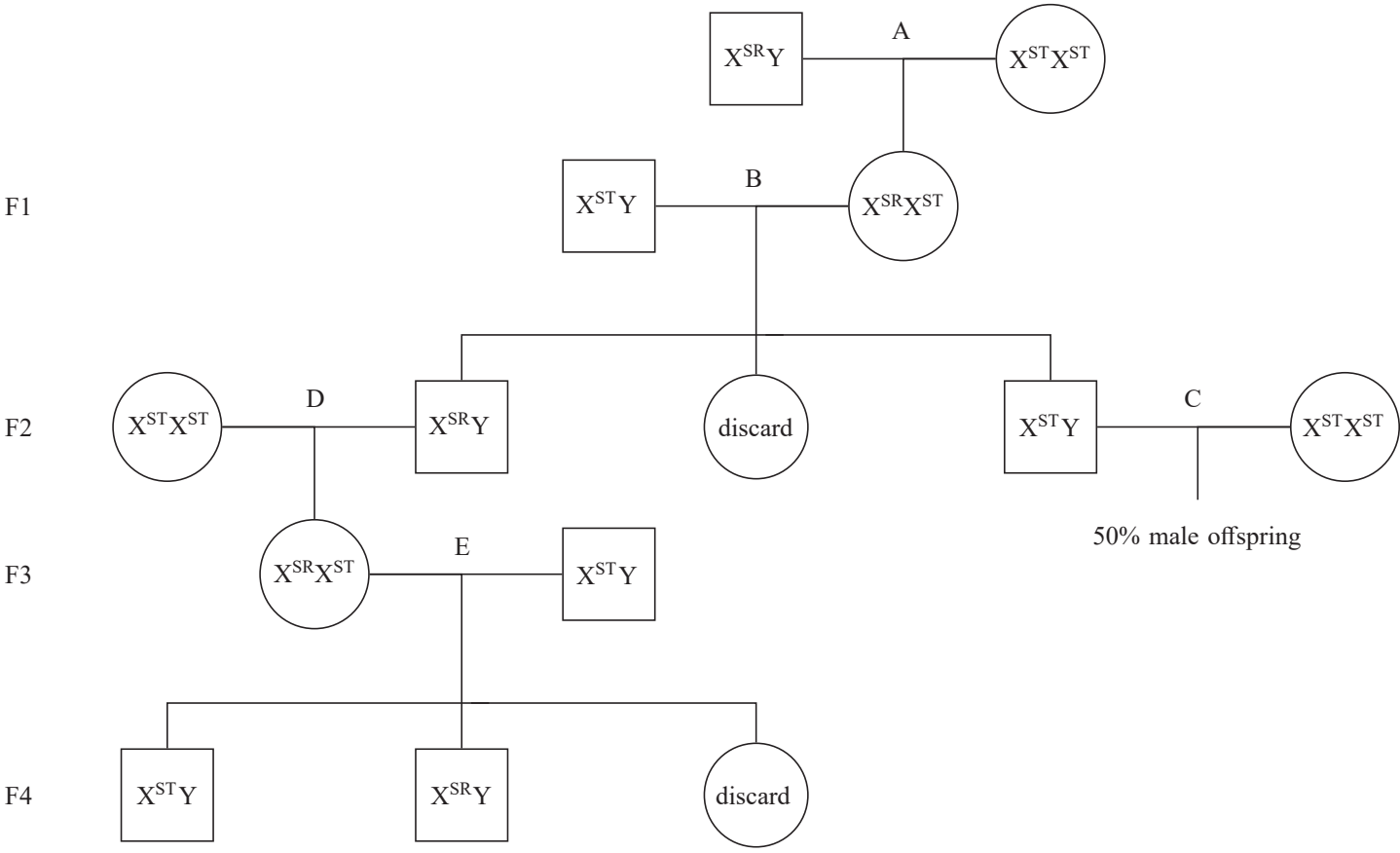
Figure 3. Alignment of the s*kpA* gene region used to differentiate between the X^{SR} and X^{ST} chromosomes. The X^{SR} and X^{ST} differ by 6 SNPs, 2 of which are non-synonymous in the X^{SR} version.

Figure 4. Electron micrographs of sperm cysts from 7-day old ST and SR males in cross section. (A) Cysts from ST males that have undergone individualization. Spermatids are tightly arranged, each with an orderly axoneme-Nebenkern pair. (B) Cysts from SR males are disorganized, and show several abnormalities. Some spermatids have fused and share a common cytoplasm (*).

Others have underdeveloped mitochondrial derivatives (arrow). Small vesicles, potentially multivesicular bodies, are present in the cytoplasm surrounding spermatids in SR males (arrowhead). Bars, 1µm.

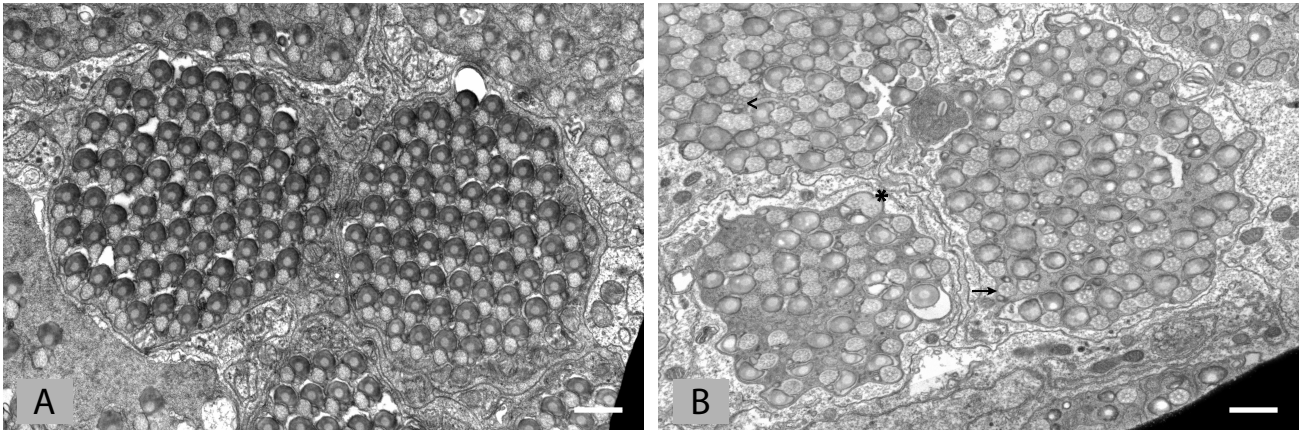
Figure 5. Offspring sex ratios of male *Drosophila testacea* from St. Sulpice, Switzerland, caught in August 2016. Each male was mated to 4 lab virgin females ($X^{ST}X^{ST}$). Asterisks indicate the 4 males with a driving X chromosome, two that produced heavily female-biased sex ratios, and two that were suppressed in the wild.

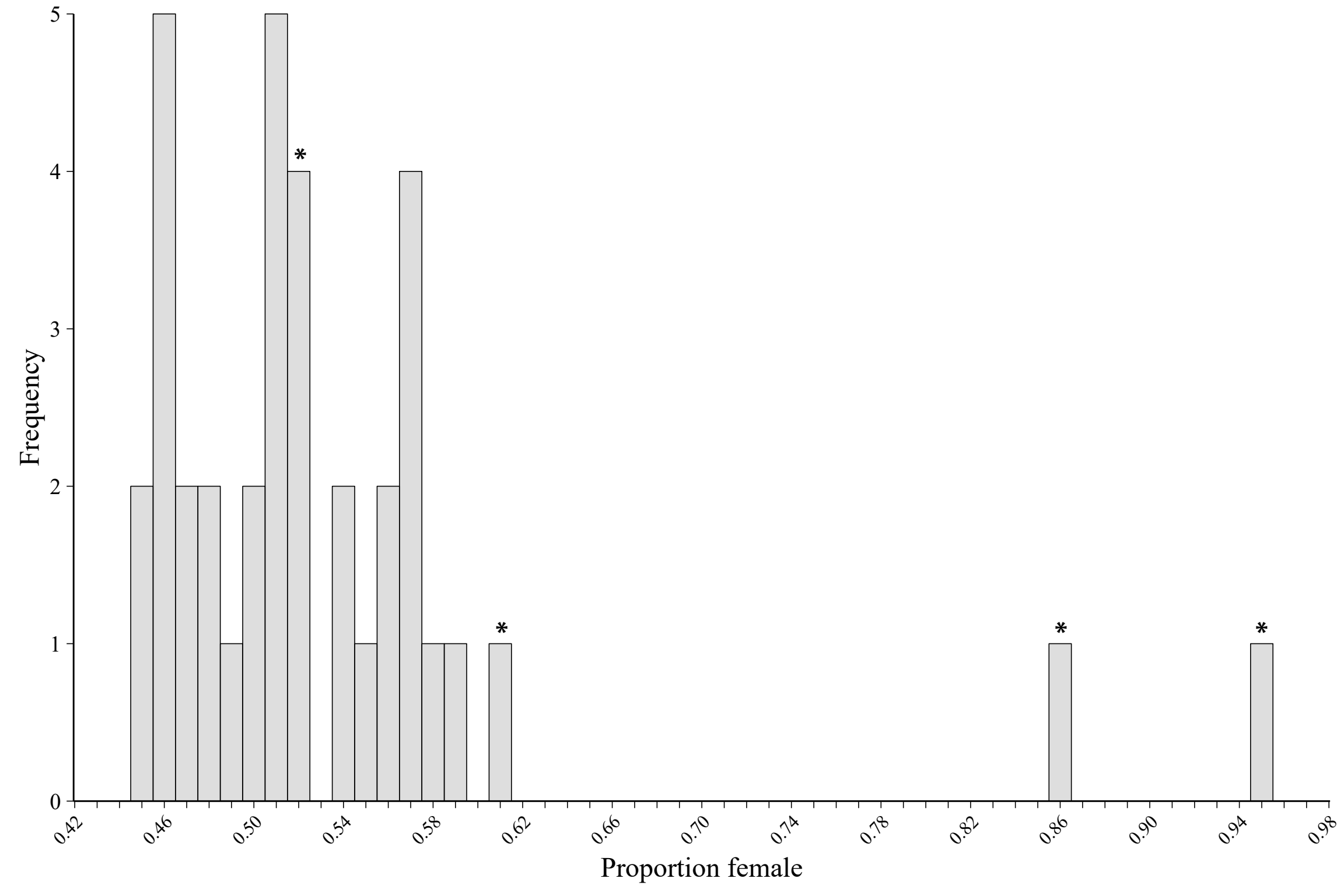

1.00
0.75
0.50
0.25
0.00
Proportion female
VC26 (n=19)
P18 (n=123)
VC25 (n=118)
P06 (n=164)
VC21 (n=172)
VC03 (n=65)
P02 (n=88)
FC01 (n=109)
VC07 (n=32)
VC42 (n=87)
FC11 (n=68)
P12 (n=35)
VC37 (n=32)
FC12 (n=78)
P19 (n=113)
VC05 (n=87)
VC35 (n=135)
VC16 (n=192)
FC13 (n=72)
P05 (n=249)
VC14 (n=120)
FC18 (n=121)
FC14 (n=77)
Cross



Determine genotypes of 20 sons from cross E


X^SR CTCGGATGAGGAGATTTTTGATACCGATATAGAAATCGCCAAGTGCTCTGGCACAATTCGCACCATGTTGGAGGATTGTGGCATGGAGGAGGATGAGAATGC
S D E E I F D T D I E I A K C S G T I R T M L E D C G M E E D E N A
X^ST CTCGGATGAGGAGATTTTTGATACCGATATACAAATCGCCAAGTGCTCTGGCACAATTCGCACCATGTTGGAGGATTGTGGCATGGAGGAGGATGAGAACGC
S D E E I F D T D I Q I A K C S G T I R T M L E D C G M E E D E N A
X^SR AATTGTTCCATTGCCAAATGTGAATTCAACAATATTGCGCAAAGTATTGACTTGGGCCAATTATCATAAGGATGATCCTAAGCCAACTGAGGATGATGAGAG
I V P L P N V N S T I L R K V L T W A N Y H K D D P K P T E D D E S
X^ST AATTGTTCCATTGCCAAATGTGAATTCAACAATATTGCGCAAAGTATTAACTTGGGCCAATTATCACAAGGATGATCCTCAGCCAACTGAGGATGATGAGAG
I V P L P N V N S T I L R K V L T W A N Y H K D D P Q P T E D D E S
X^SR CAAGGAGAAACGCACCGATGACATCACATCATGGGATGCTGATTTTCTGAAAGTTGATCAGGGAACACTCTTTGAATTAATTTTGGCTGCCAACTATTTGGA
K E K R T D D I T S W D A D F L K V D Q G T L F E L I L A A N Y L D
X^ST CAAGGAGAAACGCACCGATGACATCACATCATGGGATGCTGATTTTCTGAAAGTTGATCAGGGAACACTCTTTGAATTAATTTTGGCTGCCAACTATTTGGA
K E K R T D D I T S W D A D F L K V D Q G T L F E L I L A A N Y L D
X^SR TATCAAAGGATTGCTAGAATTAACTTGCAAGACTGTCGCCAATATGATTAAGGGCAAGACTCCAGAGGACATCCGTAAGACATTCAACATCAAAAAGGACTTT
I K G L L E L T C K T V A N M I K G K T P E D I R K T F N I K K D F
X^ST TATCAAAGGATTGCTAGAATTAACTTGCAAGACTGTCGCCAATATGATTAAGGGCAAGACTCCAGAGGACATCCGTAAGACATTCAACATCAAAAAGGACTTT
I K G L L E L T C K T V A N M I K G K T P E D I R K T F N I K K D F
X^SR CACTCCCGCCGAGGAGGAGCAGGTTCGCAAAGAG
T P A E E E Q V R K E
X^ST CACTCCCGCCGAGGAGGAGCAGGTTCGCAAGGAG
T P A E E E Q V R K E


A
B


Frequency
0
1
2
3
4
5
*
*
*
*
0.42
0.46
0.50
0.54
0.58
0.62
0.66
0.70
0.74
0.78
0.82
0.86
0.90
0.94
0.98
Proportion female

Supporting information

Supplemental Table S1. Offspring sex ratio and *skpA* haplotype data from 23 *Drosophila testacea* males (mated to 2 laboratory females each).

Cross	n	Female/male	Proportion female	p-value (chi-squared, null = 1:1)	*skpA* haplotype
VC26	19	19/0	1	1.307e-0.5	X^{SR}
P18	123	121/2	0.98374	2.2e-16	X^{SR}
VC25	118	116/2	0.983051	2.2e-16	X^{SR}
P06	164	159/5	0.969512	2.2e-16	X^{SR}
VC21	172	164/6	0.965116	2.2e-16	X^{SR}
VC03	65	62/3	0.953846	2.516e-13	X^{SR}
P02	88	83/5	0.943182	2.2e-16	X^{SR}
FC01	109	102/7	0.93578	2.2e-16	Not determined
VC07	32	28/4	0.875	2.209e-05	X^{SR}
VC42	87	76/11	0.873563	3.198e-12	X^{SR}
FC11	68	59/9	0.867647	1.333e-09	Not determined
P12	35	30/5	0.857143	2.381e-05	X^{SR}
VC37	32	26/6	0.8125	0.000407	X^{SR}
FC12	78	42/36	0.538462	0.4969	Not determined
P19	113	60/53	0.530973	0.5102	X^{ST}
VC05	87	46/41	0.528736	0.5919	X^{ST}
VC35	135	71/64	0.525926	0.5469	X^{ST}
VC16	192	100/92	0.520833	0.5637	X^{ST}
FC13	72	37/35	0.513889	0.8137	Not determined
P05	249	121/128	0.485944	0.6573	X^{ST}
VC14	120	56/64	0.466667	0.4652	X^{ST}
FC18	121	55/66	0.454545	0.3173	Not determined
FC14	77	33/44	0.428571	0.21	Not determined

Supplemental Table S2. Primers used in this study.

Primer name	Sequence (5' → 3')	Source
SkpA-F	AAVATGCCBARYATYAARYTGCARTC	Dyer *et al.* (2011)
SkpA-R	CTTCTCCTCRCACCAYTCRTT	Dyer *et al.* (2011)
Kl2q-F	AAGAACGCCTACGAAAGCAA	This study
Kl2q-R	TGAGATGCCTCCACTTGTTG	This study
Rpl-F	CMRVGSCCACAAGACCWCSAARRTC	Dyer *et al.* (2011)
Rpl-R	CRTGRGTCTGRGCCTTCC	Dyer *et al.* (2011)

Supplemental Table S3. Fertility data for 38 sons sired by 7 different SR fathers, and 24 sons sired by an ST father.

Father	Son	Produced offspring?	Offspring sex ratio (female/male)	*kl-2* amplified?
1 (SR male)	2-1	n	n/a	n
	2-2	n	n/a	n
2 (SR male)	4-1	n	n/a	n
	4-2	n	n/a	n
	4-3	n	n/a	n
	4-4	**y**	**20/17**	**y**
	4-5	n	n/a	n
	4-6	n	n/a	n
	4-7	n	n/a	n
	4-8	n	n/a	n
	4-9	n	n/a	n
	4-10	n	n/a	n
	4-11	n	n/a	n
	4-12	n	n/a	n
	4-13	n	n/a	n
	4-14	n	n/a	n
	4-16	n	n/a	n
3 (SR male)	8-1	n	n/a	n
	8-2	n	n/a	n
	8-3	n	n/a	n
	8-4	n	n/a	n
	8-5	n	n/a	n
4 (SR male)	P2-1	n	n/a	n
	P2-2	n	n/a	n
	P2-3	n	n/a	n
	P2-4	n	n/a	n
	P2-5	n	n/a	n
5 (SR male)	P6-1	n	n/a	n
	P6-2	n	n/a	n
	P6-3	n	n/a	n
6 (SR male)	P12-1	n	n/a	n
	P12-2	**y**	**41/36**	**y**
	P12-3	n	n/a	n
	P12-4	n	n/a	n
	P12-5	n	n/a	n
	P12-3	n	n/a	n
	P12-4	n	n/a	n
	P12-4	n	n/a	n
	P12-5	n	n/a	n
	P12-5	n	n/a	n
	P12-1	n	n/a	n
7 (SR male)	P18-1	n	n/a	n
	P18-2	n	n/a	n
1 (ST male)	6-1	y	Not counted	y

Supplemental Table S3. *Continued.*

Father	Son	Produced offspring?	Offspring sex ratio (female/male)	*kl-2* amplified?
	6-2	y	Not counted	y
	6-3	y	Not counted	y
	6-4	y	Not counted	y
	6-5	y	Not counted	y
	6-6	y	Not counted	y
	6-7	y	Not counted	y
	6-8	y	Not counted	y
	6-9	y	Not counted	y
	6-10	y	Not counted	y
	6-11	n	n/a	y
	6-12	y	Not counted	y
	6-13	y	Not counted	y
	6-14	y	Not counted	y
	6-15	y	Not counted	y
	6-16	y	Not counted	y
	6-17	y	Not counted	y
	6-18	y	Not counted	y
	6-19	y	Not counted	y
	6-20	y	Not counted	y
	6-21	y	Not counted	y
	6-22	y	Not counted	y
	6-23	y	29/22	y
	6-24	y	34/33	y

Supplemental Table S4. Offspring sex ratio and *skpA* haplotype data from wild caught male *Drosophila testacea* from Saint-Sulpice, Switzerland (mated to 4 laboratory females each).

Wild male	n	Female/male	Proportion female	*skpA* haplotype
S2*	300	184/116	0.6133333	X^{SR}
S3	279	140/139	0.5017921	X^{ST}
S4	227	118/109	0.5198238	X^{ST}
S5	0	n/a	n/a	X^{ST}
S36	220	122/98	0.5545455	X^{ST}
S37	160	82/78	0.5125	X^{ST}
S38	151	76/75	0.5033113	X^{ST}
S39	238	204/34	0.8571429	X^{SR}
S40	207	118/89	0.5700483	X^{ST}
S41	128	57/71	0.4453125	X^{ST}
S42	306	174/132	0.5686275	X^{ST}
S43	109	61/48	0.559633	X^{ST}
S44	193	99/94	0.5129534	X^{ST}
S45	315	155/160	0.4920635	X^{ST}
S47	0	n/a	n/a	X^{ST}
S48	189	102/87	0.5396825	X^{ST}
S49	122	56/66	0.4590164	X^{ST}
S50	159	91/68	0.572327	X^{ST}
S51	0	n/a	n/a	X^{ST}
S52	140	82/58	0.5857143	X^{ST}
S53	130	60/70	0.4615385	X^{ST}
S54	136	69/67	0.5073529	X^{ST}
S55	101	46/55	0.4554455	X^{ST}
S56	216	100/116	0.462963	X^{ST}
S57	226	108/118	0.4778761	X^{ST}
S58	110	51/59	0.4636364	X^{ST}
S59	254	115/139	0.4527559	X^{ST}
S60	129	62/67	0.4806202	X^{ST}
S61	219	102/117	0.4657534	X^{ST}
S62	0	n/a	n/a	X^{ST}
S63*	190	98/92	0.5157895	X^{SR}
S64	108	55/53	0.5092593	X^{ST}
S65	179	103/76	0.575419	X^{ST}
S66	88	50/38	0.5681818	X^{ST}
S67	242	131/111	0.5413223	X^{ST}
S68	155	80/75	0.516129	X^{ST}
S69	157	82/75	0.522293	X^{ST}
S70	113	63/50	0.5575221	X^{ST}
S71	133	127/6	0.9548872	X^{SR}
S72	124	58/66	0.4677419	X^{ST}
S73	259	133/126	0.5135135	X^{ST}

*putatively suppressed males, see table S5

Supplemental Table S5. Offspring sex ratio and *skpA* haplotype data from F_2 sons of wild-caught males carrying a suppressing element. F_2 males are siblings produced by crossing $X^{SR}X^{ST}$ females (F_1 daughters of the wild-caught males S2 and S63) to laboratory $X^{ST}Y$ males.

Wild-caught male (see table S4)	F_2 son	n	Female/male	Proportion female	*skpA* haplotype
S2	2-2	64	34/30	0.53125	X^{ST}
	2-38	25	24/1	0.96	X^{SR}
S63	63-2	75	38/37	0.50667	X^{ST}
	63-6	41	41/0	1.0	X^{SR}

References

Dyer, K.A., White, B.E., Bray, M.J., Piqué, D.G. & Betancourt, A.J. 2011. Molecular evolution of a Y chromosome to autosome gene duplication in *Drosophila*. *Mol. Biol. Evol.* **28**: 1293-1306.