Fitness and transmission of a selfish X chromosome in female *Drosophila testacea*

by

Candice Powell
B.Sc., MacEwan University, 2018

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Biology

Supervisory Committee

Fitness and transmission of a selfish X chromosome in female *Drosophila testacea*

by

Candice Powell
B.Sc., MacEwan University, 2018

Supervisory Committee

Dr. Steve J. Perlman, Department of Biology
Supervisor

Dr. Gerry Allen, Department of Biology
Departmental Member

Dr. Bob Chow Department of Biology
Departmental Member

Abstract

Supervisory Committee
Dr. Steve J. Perlman, Department of Biology
Supervisor
Dr. Gerry Allen, Department of Biology
Departmental Member
Dr. Bob Chow, Department of Biology
Departmental Member

Selfish genetic elements break the rules of Mendelian inheritance to bias their transmission to following generations, often with negative fitness consequences. A striking example involves selfish X chromosomes that operate in males and interfere with the production of sperm that carry a Y chromosome. Only X chromosome-bearing sperm are produced, and this can result in extraordinary female-biased sex-ratio distortions. Most studies have focused on how selfish X chromosomes operate in and affect males, and there has been relatively little work on their consequences in females. In this thesis, I characterize fitness effects and transmission in females, in a recently discovered selfish X chromosome system in *Drosophila testacea*, a common woodland fly. I show that females with two copies of the selfish X chromosome have reduced fitness compared to females carrying zero, or one copy. Specifically, these females have a lower hatch rate and lifetime fecundity. Additionally, I show that heterozygous females are more likely to transmit the selfish X chromosome than the wildtype copy to their offspring. I observe this transmission bias in eggs, larvae, and adults, which suggests that the selfish X chromosome is preferentially segregating into the egg, rather than the polar bodies, during oogenesis. We believe this is the first documented case of a selfish X chromosome acting through both sexes. The negative fitness effects and the biased transmission in males and females will have important consequences on the evolutionary dynamics of the selfish X chromosome. In addition, the phenomenon of biased transmission in both sexes has the potential to yield interesting insights in the mechanism of meiotic drive.

Table of Contents

List of Tables

List of Figures

Acknowledgments

Finishing my degree and writing my thesis would not have been possible without the guidance and support from my mentor, Steve Perlman. He managed to keep a positive and productive atmosphere even through a global pandemic; and is the type of supervisor that will work late into the night to help make his students' lives easier. I always felt like I could approach him with any questions and concerns, and he would have my back.

I would like to thank my committee members, Gerry and Bob for their support and kind words about my research. Their enthusiasm about my project was encouraging and motivating.

Many lab members (past and present) supported me in my degree by answering my many questions and promoting a positive, inclusive atmosphere that made me feel at home, even though I was a province away from it. I would especially like to thank Caitlin, Jan, Audrey, Ryan, Natasha, and Graeme. You all made this possible.

To my previous supervisor, Agata Becalska, and many other influential mentors from the Biology department at MacEwan University, thank you for encouraging me and giving me so many amazing opportunities to learn and grow as a person.

I would also like to thank my family, friends, and Matt for always supporting my decisions and letting me practice so many presentations in front of them. I appreciated how you all always tried so hard to be interested in what I was doing, even if you did not "get it".

To my dogs and bird (Zoltron, Pepper and Sappie), thanks for the cuddles and company while I worked on my project.

And finally, to the flies, without them this project would have never existed.

Fitness and transmission of a selfish X chromosome in female *Drosophila testacea*

Introduction

Selfish Genetic Elements

Inheritance of alleles in diploid organisms is often thought to be "fair", as there is an equal chance of getting either allele from each parent. However, there are cases where genes can cheat, or manipulate, the rest of the genome to result in a transmission bias (more than expected 1:1 allele ratios) (Burt and Trivers, 2006). Selfish genetic elements (SGEs) are sequences of DNA that cheat normal patterns of Mendelian genetics and bias their transmission to following generations at the expense of the rest of the genome. Due to their ability to manipulate transmission (and resulting intragenomic conflict), SGEs can have profound cellular and evolutionary consequences. SGEs have been suggested to be involved in the evolution of meiotic processes and mating systems (Yoshida and Kitano, 2012), extinction and speciation events (Brown and O'Neill, 2010; Crespi and Nosil, 2013; Lindholm *et al.*, 2016), and genome evolution (De Villena and Sapienza, 2001). SGEs provide an excellent opportunity to further our overall understanding of how intragenomic conflict can drive evolutionary processes.

In general, SGEs are thought to be common and diverse, yet they often go unnoticed, especially if they do not produce an easily detected phenotype (John *et al.*, 2016; Kruger and Mueller, 2021). Unfortunately, this means that we still do not understand how many of these systems work on a molecular level. To get a general understanding of how SGEs work mechanistically in a cell, they can be broadly classified into two main types: elements that overreplicate, or produce multiple copies of themselves (e.g., transposable elements); and elements that bias transmission by interfering with their homolog, often referred to as segregation distorters (Burt and Trivers, 2006). There is a large and rich literature on transposable elements and overreplicators (e.g., Alu sequence in humans, P-elements in *Drosophila*, and Ac-Ds in maize); however, my thesis will focus on selfish genetic elements that use the strategy of interference.

Segregation distorters

In contrast to overreplicators, segregation distorters will typically work through interference, where the alternate copy is prevented from being passed on to offspring (Burt and Trivers, 2006). In this system, the SGE can interfere with the alternate allele directly (target-killer), by expressing a toxin that requires inheritance of the selfish element to provide the antidote (poison/antidote), or by manipulating players involved in chromosomal segregation to ensure its presence in gametes. Target-killers function intercellularly where a trans-acting "killer" encoded on the distorting chromosome will disable, or interfere, with a target sequence on the non-distorting chromosome. Often, the killer will be in tight genetic linkage with the alternate homologous copy of the target sequence. This is thought to prevent the target-killer from acting within its own cell, thereby, killing itself.

In a poison-antidote system, the selfish element encodes a toxin that is expressed in all developing gametes, or embryos, in contrast to target-killers, which specifically target the alternate allele. Cells inheriting the selfish genetic element will also have an antidote to the poison, which neutralizes its effect. In contrast, wildtype gametes or embryos, die because they do not have the antidote. Similar to a target-killer and its target's homolog, the sequences of poison and antidote are often physically linked. This ensures that the embryos inheriting the SGE also inherit the antidote, resulting in their overrepresentation in the progeny of SGE carriers.

Some SGEs achieve transmission bias by manipulating how chromosomes are segregated during cell division. This often occurs during chromosomal segregation and movement to either pole during cellular division. Overall, this ensures that the SGE is directed to the side that will result in the functional gamete instead of a supporting cell that will eventually by degraded. Within each of these categories, there is an immense amount of diversity in how these SGEs manipulate cellular processes to ensure a transmission bias and our understanding on how these systems work is still very limited.

Another useful way to classify different types of interference elements is to consider when they act in development – a) during meiosis, b) after meiosis but at the gamete stage, or c) after fertilization. Also, the timing of SGE interference is often constrained by whether it functions through males, or females. This can occur for a number of reasons. For example, SGEs can take advantage of pathways through which females provision their eggs. In addition, male and females gametogenesis are fundamentally different (Pardo-Manuel De

Villena and Sapienza, 2001; Kruger and Mueller, 2021), and these differences play a major role in shaping the evolution of SGEs.

Gamete production in males is symmetrical, in the sense that one diploid pre-spermatocyte will become four haploid gametes (sperm cells) (Zedek and Bureš, 2016). In contrast to males, female meiosis is asymmetrical as it results in the production of one haploid gamete (ovum/megaspore), and three polar bodies. The polar bodies assist the developing egg and are degraded once oogenesis is complete. Any genetic material going into the polar bodies would then be an evolutionary dead-end. It has even been suggested that these meiotic differences could place a biological and mechanistic constraints on how SGEs arise and evolve in populations (Kruger and Mueller, 2021). This may explain why we see different types of SGEs that act through either male, or female meiosis, but not both.

Post-zygotic embryo killers

Post-zygotic segregation distorters typically operate during early stages of embryogenesis (Burt and Trivers, 2006; Lindholm *et al.*, 2016). Typically, we see this style of interference (maternal-effect killers) through females. In general, maternal-effect killers are maternally provided proteins, or factors, that are required for embryogenesis and are usually expressed in the cytoplasm of the egg (Burt and Trivers, 2006). These factors can act as target-killers, or poison-antidote systems, either by targeting the alternate allele directly, or as a globally expressed toxin that requires the SGE-linked antidote to rescue the cell. The resulting disruption of the alternate allele often causes early death in progeny that do not carry a copy of the driver (usually embryonic arrest). The first example of this type of selfish element was discovered in *Tribolium castaneum* flour beetles, and is named Medea (M) (**M**aternal **e**ffect **d**ominant **e**mbryonic **a**rrest) (Beeman *et al.*, 1992). Heterozygous females (M/+) have a transmission bias of the Medea factor; offspring that do not inherit it die early in development.

Meiotic and post-meiotic gamete killers in males

Often, SGEs act through male meiosis by disrupting the production of the sperm, either as a target-killer, or a poison-antidote system (Burt and Trivers, 2006). In contrast to post-zygotic female systems, this style of interference can occur at both meiotic and post-meiotic stages during gamete production, prior to fertilization. However, the same overall concept of the target-killer and poison-antidote systems remains. In this scenario, the target-killer would disrupt the sperm carrying the alternate allele. Or alternatively, sperm cells that

did not inherit the SGE would not have the antidote to an intercellular expressed toxin. Both mechanisms result in the overrepresentation of the SGE in the male's gametes.

Male gamete killers have been frequently documented and are especially common in flies (Jaenike, 2001; Courret *et al.*, 2019). One example is the trans-acting target-killer, "Segregation Distorter" (*SD*), system in *Drosophila melanogaster* (Sandler *et al.*, 1958; Larracuente and Presgraves, 2012). In this system, the driver evolved from a gene duplication, with the duplication resulting in a truncated version of a RanGTPase activating gene ((Powers and Ganetzky, 1991; Merrill *et al.*, 1999). The truncated RanGAP protein mislocates to the nucleus, where it targets the intracellular expressed *Responder* (*Rsp*) (Kusano *et al.*, 2001). Wild-type sperm cells carry a *Rsp* haplotype that has a high copy number, while the *SD* carriers have a low copy number (Temin *et al.*, 1991). The SGE targets the high copy number *Rsp*, and the overall result is the sperm fail to undergo chromatin compaction. This results in a ~95 % transmission bias of the *SD* chromosome in progeny produced by *SD/SD+* males (Sandler *et al.*, 1958). However, the exact mechanism of how this occurs is still unknown.

Another well-documented example of a male gamete killer is the *t*-haplotype in mice (*Mus musculus*) (Burt and Trivers, 2006; Herrmann and Bauer, 2012). The t-haplotype is a variant of autosomal chromosome 17 that results in a transmission bias of *t* allele to ~90-95% of offspring from heterozygous males (*t*/+) but is inherited normally through females (~50%) (Chesley and Dunn, 1936). Genetic analysis has shown that this transmission bias results from interactions between the *t*-complex responder (*Tcr*) and the *t*-complex distorters (*Tcd1*, *Tcd2*, *Tcd3*, and *Tcd4*) (Herrmann and Bauer, 2012). Currently, studies have shown that two distorters are regulators of RHO small G proteins (Bauer *et al.*, 2005, 2007) and one distorter encodes a hypomorph of a nucleoside diphosphate kinase gene (Bauer *et al.*, 2012). It is believed that the distorters work together to impair sperm motility of all (*t*/+) mice, however, the *t* bearing sperm are rescued by the responder which encodes a novel sperm-motility-kinase ($Smok^{Tcr}$) that restores normal sperm motility (Herrmann *et al.*, 1999).

The *wtf* drivers in the yeast species *Schizosaccharomyces pombe* provide an example of how a poison-antidote system can work (Angélica *et al.*, 2018). In this case, we can consider spore production as the equivalent of sperm in yeast. The first *wtf* driver (*wtf4*) was found by crossing two yeast species *S. pombe* and *Schizosaccharomyces kambucha* (Zanders *et al.*, 2014). The *wtf4* family encodes for two proteins, a poison, and an antidote (Zanders *et al.*, 2014; Nuckolls *et al.*, 2017). During spore production, the *wtf4* gene produces a poison that is expressed in all the spores. Only the spores that inherit the *wtf4* gene also have the

antidote; the rest of the spores will die. Further research has shown that this family of genes is diverse and highly prevalent in *S. pombe* (Hu *et al.*, 2017; Eickbush *et al.*, 2019; Nuñez *et al.*, 2020).

Female meiotic drive

In females, meiotic drive acts by exploiting the asymmetry in female meiosis to bias transmission into the developing oocyte, thus avoiding the polar bodies and allowing the element to transmit to the next generation (Burt and Trivers, 2006; Lindholm *et al.*, 2016; Kruger and Mueller, 2021). This transmission advantage is linked to centromere segregation during meiosis and is often referred to as centromere drive, due to the intragenomic conflict between competing homologous centromeres for inclusion into the functional gamete (Henikoff *et al.*, 2001). However, there are also some cases of "non-centromeric drive", such as the knob neo-centromeres in maize (Rhoades, 1942), where the selfish element resides outside the centromere or pericentromere regions.

In general, centromere drive works by spatially orientating the SGE in a way that will ensure it goes into the egg (Akera *et al.*, 2017; Kursel and Malik, 2018). This can occur before the spindles attach to the centromere, or after spindle formation during cortical signalling. There are several ways this may occur, but a common theme is via large amounts of repetitive DNA sequence in centromere and pericentromere regions. These repetitive sequences can influence how spindles attach to the centromeres during meiosis, with the more stable attachment being retained in the egg (Malik and Henikoff, 2009; Iwata-Otsubo *et al.*, 2017; Kursel and Malik, 2018). Additionally, these SGEs are often able to recruit proteins that can reorient the chromosome into a more favourable spindle attachment (Chmátal *et al.*, 2015, 2017). This allows the SGE to ensure its attachment to the egg pole if it was not initially successful.

Evolution and molecular basis of meiotic drivers

Although incredibly diverse, there appear to be some general patterns with respect to how meiotic drivers arise and their molecular mechanisms. In general, it appears that SGEs often arise from gene duplications events (Courret *et al.*, 2019). This is seen in *Drosophila melanogaster* (Larracuente and Presgraves, 2012), *Drosophila simulans* (Tao *et al.*, 2007), and *Drosophila neotestacea* (Pieper *et al.*, 2018). Specifically, *D. simulans* has two independent drive systems that are a result of gene duplications (Tao, *et al.*, 2007; Fouvry *et al.*, 2011). Some ways that we see these duplications working to facilitate drive, include: the

disruption of heterochromatin-binding proteins, nuclear transport, and small RNAs. However, it is still unclear how exactly these mechanisms facilitate a transmission bias in drive systems.

A drive system in *Drosophila simulans* (*Paris*) has been shown to result from interactions between duplications of two separate loci (Montchamp-Moreau *et al.*, 2006; Fouvry *et al.*, 2011). We do not have evidence of the exact identity of one of the duplications (37kb tandem repeat); however, the second component is a duplicated heterochromatin gene called *HP1D2* that is involved in Y chromosome binding and heterochromatin organization (Helleu *et al.*, 2016). Both duplications are required for the driver to act, and although it is not yet well understood, it is believed that dysfunctional *HP1D2* alleles fail to prepare the Y chromosome for meiosis after binding, resulting in biased transmission of the X chromosome.

A few cases of male meiotic drive have been linked to disruptions in nuclear transport, including the Segregation Distorter system of *D. melanogaster* that is caused by a truncated RanGAP that mislocalizes to the nucleus (Temin *et al.*, 1991; Merrill *et al.*, 1999; Kusano *et al.*, 2001). In *D. neotestacea*, a candidate gene for meiotic drive resulted from a duplication of an X-linked nuclear transport gene, called importin-alpha2 (Pieper *et al.*, 2018).

Although we are starting to get some hints at how these systems manipulate gametogenesis, it should be emphasized that very little is known about the genetic basis and mechanisms of meiotic drivers. The *D. neotestacea* example is tantalizing but has not been functionally confirmed and we do not fully understand how Segregation Distorter works in *D. melanogaster* yet. It also is very likely that multiple genetic loci could disrupt several different cellular processes that together cause drive, making it difficult to not only elucidate these mechanisms, but also to directly compare different systems.

Sex chromosome meiotic drive

Meiotic drivers can arise in any sequence of DNA, including noncoding DNA, pieces of genes, whole genes, entire chromosomes, or sets of chromosomes (Burt and Trivers, 2006). While they can be found on autosomes (such as Segregation Distorter in *D. melanogaster*), drivers that reside on a sex chromosome are particular noticeable because of their striking phenotype (Jaenike, 2001; Burt and Trivers, 2006; Helleu *et al.*, 2015). In this case, the transmission bias results in an over-representation of one sex over the other (sex ratio distortion). Often, the effects of these drivers are severe, and extreme sex biases may be seen (up to 100% of offspring inheriting the meiotic driver).

The overall structure of sex chromosomes may make them particularly susceptible to SGEs. SGEs tend to arise in areas where there is reduced recombination. Sex chromosomes tend to be very divergent from each other and lack recombination. This puts them in a vulnerable position compared to other areas of the genome for SGE infiltration. Selfish (also commonly referred to as driving, for meiotic drive) X (X^d) chromosomes appear to be quite common in rodents and flies (the insect order Diptera), and have also been found in plants.

Selfish X chromosomes in some rodent species (denoted X*) operate by disabling 'male factors' on the Y chromosome resulting in the feminization of males, leading to X*Y, or X*O, females (Fredga and Bulmer, 1988; Burt and Trivers, 2006). This results in a female-biased sex ratio, not only because X*Y individuals are female, but also because the feminized males often do not produce functional Y bearing eggs due to defects in meiosis or embryogenesis (Winking *et al.*, 1981; Akhverdyan and Fredga, 2001). Additionally, any Y bearing eggs that are produced and fertilized by Y bearing sperm are inviable. Feminizing X chromosomes are seen in Arctic lemmings [previously: varying lemmings] (*Dicrostonyx torqualus*) (Gileva, 1975, 1987; Gileva *et al.*, 1982) and wood lemmings (*Myopus schisticolor*) (Fredga *et al.*, 1976).

In flies, X^d chromosomes have been reported in *Drosophila* (Drosophilidae) (the focus of my thesis), stalk-eyed flies (Diopsidae), and tsetse flies (Glossinidae) (Jaenike, 2001). The genus *Drosophila* in particular, appears to be quite susceptible, with the first discovery in *Drosophila obscura* in 1928 (Gershenson, 1928). Thus far, 18 different X^d chromosomes, in 16 *Drosophila* species, have been documented (Courret *et al.*, 2019).

One possibility for why X^d chromosomes seem to be so common in *Drosophila* is that male meiosis is achiasmatic (meaning that males do not produce chiasmata between chromosomes) (Burt and Trivers, 2006). This means that cross-over events (or, recombination events) are not possible, and recombination can only occur through females. This reduces the possibility for the removal of SGEs through recombination events since recombination already does not happen between the X and Y chromosome. This furthers to limit the genome's ability to break up regions that may be harbouring or assisting an SGE.

X chromosome drive is more commonly seen than Y chromosome drive. There are a few possibilities as to why this might occur. One reason why Y chromosome drive may be relatively rare is that its effects are expected to be much more rapid and severe than X chromosome drive (Hamilton, 1967). This is because Y chromosome drive would be expressed every generation and, in every individual, whereas X drive would alternate generations (i.e., driving Y males will sire driving Y sons, whereas driving X males produce

daughters). This occurs because the SGE does not operate through female meiosis to disable sperm production, so it must follow Mendelian rules while segregating in females. Additionally, the consequences of a male bias are more severe because one male can mate with numerous females to produce offspring. This means that in the early stages of SGE evolution, a female bias may be somewhat beneficial if males are still present and able to mate with many females (Mackintosh *et al.*, 2021). In contrast, a male bias cannot increase offspring production as this is limited by the number of females that are reproductively mature. This, added with the faster spread of the driving Y chromosome, could lead to population extinction.

Y chromosome drive may also be relatively rare because Y chromosomes often have very few genes (Charlesworth and Charlesworth, 2000; Helleu *et al.*, 2015). This means that there is less genetic material that a SGE could evolve from on a Y chromosome. In support of this hypothesis, the only documented cases of Y chromosome drive occur in *Aedes aegypti* and *Culex pipiens* mosquitoes (Newton *et al.*, 1976; Sweeny and Barr, 1978; Wood, 1991), which have young, gene-rich Y chromosomes that are cytologically indistinguishable from the X chromosome (Wood, 1991; Burt and Trivers, 2006; Helleu *et al.*, 2015). In both cases, male spermatogenesis is disrupted by the preferential breakage of the X chromosome during meiosis, leading to the overrepresentation of the Y chromosome, and therefore, a male bias in the offspring.

Spread and fixation of driving X chromosomes

Without resistance from the rest of the genome, a newly arising selfish genetic element is predicted to reach fixation (i.e. be found in all individuals in the population), even with a small transmission bias (Hamilton, 1967; Burt and Trivers, 2006; Lindholm *et al.*, 2016). Depending on the type of SGE and where it resides, this can mean different things for a population. For example, if a SGE resulted in the biased transmission of genes that produced a weak, or non-observable phenotype, it may reach fixation in a population without much of an impact on that species' overall fitness, and it could go unnoticed (Werren, 2011; Ågren and Clark, 2018). In these cases, one may only see evidence of a SGE when it is brought into a new genetic background, for example by crossing with a different population or closely related species (Hurst and Werren, 2001; Werren, 2011; Lindholm *et al.*, 2016). The observation of hybrid incompatibilities caused by SGEs has led to the suggestion that they could play an important role in speciation events (Frank 1991; Hurst and Pomiankowski 1991), perhaps showing mechanisms that act as reproductive barriers between species.

In sex chromosome drive, fixation of the driver would result in a local population extinction event (Hamilton, 1967; Carvalho and Vas, 1999; Lindholm *et al.*, 2016). When considering the strength of drive seen in some selfish X chromosome systems (~80-100% of offspring are females), it is easy to see how in only a few generations males could become extremely rare. If females were unable to find a mate, this could mean that the population would go extinct. Although a recent study reported a population collapse due to a selfish X chromosome (Pinzone and Dyer, 2013), it is hard to predict how often this happens in nature because we would never see all the extinctions that happened in the past. However, some selfish elements have been shown to persist for long periods of time in nature (Lindholm *et al.*, 2016). A major area of research is to understand the factors that determine their evolutionary persistence; this is thought to result from a combination of fitness costs and/or genes that can suppress their action (Jaenike, 2001; Burt and Trivers, 2006; Helleu *et al.*, 2015).

To further illustrate the population dynamics of X^d chromosomes we will consider the case of *Drosophila neotestacea*. In this system, there is a strong female-bias produced by male-carriers (~98% females) (Jaenike, 1996; Dyer, 2011). However, evidence suggests that this system is ~330,000-500,000 years old and has been relatively stable at frequencies (20-25%) at several sampling sites in North America (Pieper and Dyer, 2016). Sampling showed that one site had an increase in the frequency of individuals carrying the X^d chromosome (to 40-50%) and this was followed by a sharp decline in the number of flies caught at the following sampling point, with all of them being female carriers of X^d (Dyer, 2011; Pinzone and Dyer, 2013). The researchers hypothesized that they saw a population crash due to fixation of the driver, as the sites in surrounding areas have had stable frequencies for ~20 years.

Suppressor systems

Due to the destructive nature of selfish X chromosomes on Y-bearing sperm, there is direct pressure on the Y chromosome to resist their effects (Thomson, 1975; Jaenike, 2001; Burt and Trivers, 2006). This is because Y chromosomes that are sensitive to the effects of the driver will not be passed on to the following generation. Y chromosomes that can resist the effects of the selfish X chromosome will survive to the next generation. These resistant Y chromosomes are then expected to spread quickly in populations if they do not also result in negative fitness costs for the host (Clark, 1987).

Y-chromosome suppressors of drive have been found in many *Drosophila* species, including *D. simulans* (Cazemajor *et al.*, 1997; Tao *et al.*, 2007; Branco *et al.*, 2013), *D. affinis* (Unckless *et al.*, 2015), and *D. mediopunctata* (Carvalho and Klaczko, 1993; Carvalho *et al.*, 1997). *Drosophila simulans* harbours a large diversity of resistant Y chromosomes (Montchamp-Moreau *et al.*, 2001; Helleu *et al.*, 2016, 2019). This rapid evolution of different Y haplotypes that resist X chromosome drive highlights the pressure that the Y chromosome is under to suppress drive. This has led to the hypothesis that driving X chromosomes are an important force in Y chromosome evolution (Bachtrog, 2020), and perhaps in the evolution of sex chromosomes in general.

Autosomal suppressors are also selected for due to the extreme female bias caused by driving X chromosomes. This is because selection will favour resistant alleles that increase the frequency of the rarer sex (in this case, males) and return the sex ratio back to 1:1 (Hamilton, 1967; Wu, 1983). Autosomal suppressors have been found in many species of *Drosophila*, including *D. simulans* (Tao, *et al.*, 2007), and *D. testacea* (Keais *et al.*, 2020). To make things even more complicated, some species harbour both autosomal and Y-linked suppressors.

Complete suppression can restore sex ratios to 1:1; in these cases, both the selfish X chromosome and the suppressor are fixed in the population, leading to cryptic, or hidden, drivers (Helleu *et al.*, 2015). In other cases, a cycle of intragenomic conflict could lead to an arms race between the SGE and their suppressor. These systems can become increasingly complicated and involve numerous loci (Courret *et al.*, 2019). This is seen in *Drosophila simulans*, where different populations around the world are simultaneously fixed (i.e., at 100% prevalence) for one of three different cryptic selfish X chromosomes and their suppressors, meaning drive can only be detected through inter-population crosses (Cazemajor *et al.*, 2000; Tao, Araripe, *et al.*, 2007; Tao, Masly, *et al.*, 2007; Helleu *et al.*, 2016; Lin *et al.*, 2018).

Although suppressors are common, some driving X chromosomes have persisted for very long periods of time without having a suppressor present. One of the most intensively studied driving X chromosome systems, and one of the first to be discovered, is in *Drosophila pseudoobscura.* This driving X chromosome causes meiotic defects in Y-bearing sperm and an extreme female bias (~83-100% of offspring from males) (Sturtevant and Dobzhansky, 1936). This driving X chromosome has been stably persisting for at least 70 years at a frequency of ~25% in natural populations (Beckenbach, 1996; Price *et al.*, 2014). Sequence analysis has revealed that this driving X chromosome is very old, and is estimated

be up to 1 million years old (Kovacevic and Schaeffer, 2000). Despite intensive sampling, suppressors have never been found in this system, and it is thought that negative female fitness effects may be the reason for its stability (Wallace, 1948; Larner *et al.*, 2019; Price *et al.*, 2019)

Fitness consequences of selfish X chromosomes

Direct

Many selfish X chromosomes have been shown to reduce the fitness of individuals that carry them. In some cases, this reduced fitness is a direct consequence of the selfish action of the X chromosome. The destruction of Y-bearing sperm will result in only half of the amount of sperm being produced. Reduced male fertility, or reducing the likelihood of successful matings may offset a transmission bias, as wildtype males may produce more offspring (Lindholm et al., 2016). Additionally, males with a selfish X chromosome may not be able to produce sperm as quickly as wildtype (Price and Wedell, 2008; Wedell, 2013). This will be exacerbated in species where polyandry is common (Holman et al., 2015).

Indirect

Individuals that carry a selfish X chromosome may also have reduced fitness as a result of linked deleterious mutations. Selfish genetic elements often occur in areas of reduced recombination, such as inversions. This serves to maintain linkage of factors that are essential for element activity, such as drivers and enhancers of drive, or toxin and antidote genes. This may result in very large regions of suppressed recombination over long periods of time. For example, recombination has been suppressed over the entire length of the selfish X chromosome of *Drosophila recens* (Dyer *et al.*, 2007). However, the long term suppression of recombination also prevents the removal of deleterious mutations, and this can drastically lower fitness (Burt and Trivers, 2006; Dyer *et al.*, 2007; Lindholm *et al.*, 2016).

For example, female *D. recens* that are homozygous for the selfish X chromosome are sterile (Dyer *et al.*, 2007). Similarly in *D. pseudoobscura*, females that are homozygous for the driving X chromosome have reduced fertility and fecundity, producing almost half as many offspring and having a high occurrence of sterility (~23% of females) compared to wildtype and heterozygous drivers (Larner *et al.*, 2019). Both cases are presumed to be due to deleterious linked mutations. In addition to sterility, other negative fitness effects include

reduced adult survival, or egg to adult viability, as seen in the stalk-eyed fly, *Teleopsis dalmanni* (Finnegan *et al.*, 2019).

Recently, there has been an increased interest in how selfish X chromosomes affect fitness in both sexes. While past studies focused more on how disruption of Y-bearing sperm impacts male mating competitiveness, researchers are now finding that female fitness is also reduced, even if the transmission bias is working through male meiosis. There can also be a dosage effect, where females with two copies of the selfish X chromosome have more severe repercussions. For example, *D. recens* females carrying a single copy of the selfish X chromosome (i.e. heterozygous females) have a 16% reduction in fertility compared to homozygous wild-type flies, while females that are homozygous for the driver are sterile (Dyer and Hall, 2019). However, many systems are still lacking information on how females are impacted, and how this may contribute to the persistence and evolution of selfish X chromosomes.

A recently discovered X-chromosome drive system in Drosophila testacea

A selfish X chromosome system was recently discovered in the common Palearctic woodland fruit fly species, *Drosophila testacea* (Keais *et al.*, 2017) [note: an older paper describing a selfish X chromosome in *D. testacea* was actually about its relative *D. neotestacea* (James & Jaenike 1990)]. A line of flies was established from wild-caught flies that were collected near St. Sulpice, Vaud, Switzerland in summer 2012. The discovery of the sex ratio distortion was made unexpectedly during routine fly care, and the driving X line was isolated in 2014. Specifically, males that have the driving X (X^dY) produce heavily female-biased sex ratios (X^dX females), which is believed to result from spermatogenesis defects during production of Y bearing sperm. The strength of drive is quite strong, with most males having ~80-100% daughters. This is actually an underestimate of the strength of drive, because the few sons that were produced by X^dY males were mostly XO and therefore sterile. Specifically, it was shown that only 2 out of 38 sons of X^dY males had a Y chromosome and produced viable offspring. From field collections in 2016, it was found that approximately 10% of males from the St. Sulpice population carry the selfish X chromosome.

The X^d chromosome exhibits suppressed recombination and is highly divergent from the wildtype X chromosome. A previous phylogenetic analysis based on three conserved X-linked (*skpA, rpl36,* and *pdg*), found that the X^d chromosome diverged prior to the radiation of *D. testacea* and its closely related species *D. orientacea* and *D. neotestacea*, with the *skpA* gene on the X^d chromosome differing at 6 of 442 nucleotides (1.4%), including 2 nonsynonymous changes (Keais et al. 2017, 2020). This study estimated the X^d chromosome to have diverged ~920,000 years ago, suggesting that this X^d system has been existing as a stable, polymorphism for a very long time.

A previous master's student investigated whether there were any suppressors, or negative fitness consequences that could explain how this selfish X chromosome could persist for so long (Keais *et al.*, 2020). X^dY males showed sperm depletion, producing few offspring when mated multiply. Also, females homozygous for the driving X chromosome (X^dX^d) had fewer offspring. Finally, a few X^dY males collected in the wild had weakly distorted, or normal sex ratios, and controlled lab crosses showed that this is due to an autosomal suppressor acting against the driving X chromosome. It is believed that the combination of these direct and indirect fitness effects, along with the autosomal suppressor, acts to balance the strong transmission bias of the X^d chromosome in nature, perhaps explaining how it has persisted for so long.

Objectives

In this thesis, I characterize how a selfish X chromosome drive system in *Drosophila testacea* impacts females. My thesis has two components. The first part of my study compared fitness of females carrying zero, one, or two copies of the X^d chromosome. I first compared the fertility and fecundity of the different female genotypes through a series of egg hatching trials. Next, I examined whether I was able to detect any ovary pathologies, or differences in early egg stage development in adult female flies. Finally, I tested whether the presence of the X^d chromosome affected egg to adult development time.

In the second part, I performed experiments to ask whether there was a transmission bias of the X^d chromosome through females. Individual female flies that were heterozygous for the selfish X chromosome (i.e., X^dX genotype) were mated with males that carried a wildtype X chromosome (XY). Offspring resulting from these matings were individually collected and genotyped to determine whether heterozygous females were more likely to transmit the selfish X chromosome than the wildtype one. These were done as separate experiments for eggs, larvae, and adults.

Methods:

Fly stocks and maintenance:

The *Drosophila testacea* fly lines used in this study were founded from multiple, wild-caught, flies collected from St. Sulpice, Vaud, Switzerland in September 2012 (and supplemented with flies collected from the same place in July 2016), using store-bought *A. bisporus* mushrooms as baits. From these, lines were established that contained, or lacked, the selfish X chromosome (X^d). Flies are kept in the Perlman lab in plastic vials, and with Instant *Drosophila* medium (Carolina Biological, Burlington, NC, USA) and are supplemented with a small piece of *A. bisporus* mushroom. These are reared at 21.5°C on a 12-h light: dark cycle and 60% relative humidity.

Lines carrying a driving X chromosome (designated X^d) are maintained using a series of controlled crosses. Males that are X^dY can be identified by the extreme female sex ratio distortion in their offspring. Their daughters (all X^dX) are then mated to wildtype (XY) males. This will result in a mixture of wildtype (XY) and driving (X^dY) sons, as well as daughters. Single males are mated with a few wild-type (XX) females, and vials that result in heavy female bias are kept, and this cycle is repeated. As a result of this crossing scheme, our wildtype and selfish X chromosome lines have the same autosomes, Y chromosome, and mitochondria, and only differ in their X chromosome. All of the lines used in this study are free of suppressors of drive, and there is no recombination between X and X^d chromosomes in heterozygous females.

In early 2020, we isolated a spontaneous X-linked 'bright-eye' colour mutation (X^b), on an otherwise wildtype (i.e., not selfish) X chromosome. Individuals that are homozygous for this mutation have bright orange eyes that are easily distinguished from the typical dull dark red eye colour in *D. testacea*. As there is no recombination between selfish and nondriving X chromosomes, the bright-eye mutation greatly facilitates maintenance of the selfish X chromosome in the lab. For example, crossing bright-eye females (X^bX^b) with males carrying the selfish X chromosome (X^dY) will produce only heterozygous X^dX^b females. If these females are mated with bright-eye males (X^bY), they will produce offspring that can be easily distinguished: bright-eye males (X^bY), males with a selfish X chromosome and with dull dark eyes (X^dY), bright-eye females (X^bX^b), and heterozygous females with dull dark eyes (X^dX^b).

In addition, I succeeded in isolating a line that is homozygous for the selfish X chromosome (i.e., all females are X^dX^d). X^dX^d females from this line produce only X^dX^d

daughters when mated with selfish X^dY males. Mating X^dX^d females with wildtype (XY) males will produce X^dY sons and X^dX daughters.

DNA extraction:

PrepMan™ Ultra (ThermoFisher) protocols were used for DNA extraction of samples. Specimens were homogenized with a bead beater, in 1.5ml Eppendorf tubes with a mixture of beads and 50 uL of PrepMan™ for 15-30 seconds. Homogenized specimens were then incubated at 95°C for 10 minutes before cooling to room temperature. Extracted fly DNA was stored at -20°C. Samples were diluted 1:10 in dH2O prior to use.

For routine genotyping not requiring high quality DNA, squishing buffer was also used in place of PrepMan™ (9.8 mL dH20, 100 uL 1M Tris pH 8.0, 20 uL 0.5M EDTA, 50 uL 5M NaCl). Before use, 0.5 uL of 20 mg/mL proteinase K was added. Samples were incubated at 37°C for 20 minutes, and then 95 degrees for 2 minutes.

Genotyping:

The wildtype and selfish X chromosomes are highly divergent and do not recombine; recombination appears to be suppressed along the entire selfish X chromosome. As a result, we have developed a number of approaches that allow us to easily genotype flies. This is useful both for routine confirmation and stock maintenance, and for experiments. We use two approaches: a gene (related to *protamine*) that is only found on the selfish X chromosome, and genes (s*kpA* and *dynein*) that are found on both selfish and wildtype X chromosomes but that are divergent (Table 1).

The *protamine* qPCR primer set amplifies a portion of gene (called '*protamine*-like' because it shows homology to a *protamine*) that is only present on the X^d chromosome, and absent from the X chromosome. Successful amplification after qPCR indicates that the X^d chromosome is present in the sample. The gene and primer set were identified and designed by Ryan Gawryluk, a previous postdoc in the lab. The skpA-exact primer sets will amplify both the X^d and X chromosomes following a PCR reaction. Several SNPs within the s*kpA* region allows us to determine fly genotype after Sanger sequencing by comparing the resulting chromatograms (Keais et al. 2017, 2020). Finally, the dynein (dyn-q) primer set is based on an intron in a *dynein* gene that is found on both the X and X^d chromosomes. The intron length is quite different between X (89 nucleotides) and X^d (119 nucleotides) chromosome, which results in distinct melt temperatures for each genotype that appear as 'peaks' in the data readout. Homozygous wildtype flies, and driver flies have one peak at

different temperatures, and heterozygous individuals having two peaks (wildtype: 73.50°C, heterozygote: 72.60°C + 75.50°C, homozygote: 75.30°C). This difference and the primer sets were also identified and designed by Ryan Gawryluk.

For determining sex at egg and larval stages, primer sets are used that amplify a portion of *kl2*, a gene that is located on the Y chromosome (Dyer *et al.*, 2011, Keais *et al.*, 2017). Finally, as a DNA extraction control, in some cases I used primers called neoNa-q that amplify a *D. testacea* autosomal gene, instead of the *skpA* primer set. These primers were designed by Matt Ballinger, a previous postdoc in the lab.

Table 1. Primer sequences used for genotyping *Drosophila testacea*.

Primer name	Sequence (5' → 3')	Target amplified	Protocol used	Source
SkpA-F_exact	AACATGCCGACCATTAAGTTG	*skpA* gene (X/X^d chromosome)	Sanger sequencing	Keais *et al.*, (2020)
SkpA-R_exact	CTTCTCCTCACACCGTTCGTT			
Kl2q-F	AAGAACGCCTACGAAGCAA	*kl2* gene (Y chromosome)	qPCR (presence/absence)	Keais *et al.*, (2017)
Kl2q-R	TGAGATGCCTCCACTTGTTG			
protamine qFP	GCAGGATACTTCCCTTG	*protamine*-like gene (X^d chromosome)	qPCR (presence/absence)	courtesy of Dr. Ryan Gawryluk
protamine qRP	ATGCATTTCGAGGTTTCCTG			
dyn-qF	AAATTGAAGAAAACTCAATATCTTAGG	*dynein* gene ($XX/X^dX/X^dX^d$)	qPCR (high resolution melt curve)	courtesy of Dr. Ryan Gawryluk
dyn-qR	TGAAATATTTTAATGAAGGAAATACGA			
neoNA qF	GTCTCTGAGCCAGATTTCCAAAG	na-paleovirus (+ control)	qPCR (presence/absence)	courtesy of Dr. Matt Ballinger
neoNA qR	TACGCCTATTTTGCCTTGGG			

Female reproductive fitness

I compared hatch success and lifetime fecundity of wildtype (XX), heterozygous (X^dX), and homozygous driver (X^dX^d) females. To obtain experimental flies, I set up crosses to produce each female genotype (XX, X^dX, X^dX^d) using flies from both the line lacking the X^d chromosome and the homozygous driver line. Females emerging from these crosses were collected as virgins and were aged for one week prior to mating. Three groups of six females of each genotype were crossed to wildtype (XY) males. Flies were allowed to mate for 4 days, after which females were placed singly into vials containing an egg-laying medium (a mixture of agar, water, mushroom, sucrose, and mold inhibitor). Females were allowed to oviposit for 24 hours before being flipped into a new vial; this was repeated over four successive days. To confirm female genotype, their DNA was extracted using PrepMan™ Ultra (Thermofisher), and the skpA-exact marker gene was PCR-amplified and Sanger-sequenced (Sequetech, USA).

When the female was removed, I counted the total number of eggs and transferred the mushroom agar disc into a petri dish. Dental rolls saturated with distilled water were added to the petri dishes to retain humidity. Eggs were monitored for overall health but were otherwise left undisturbed for 96 hours. At this time point, it could be assumed that all eggs would have

enough time to hatch. I counted the number of unhatched and hatched eggs. I first investigated if there were differences in the average hatch rate depending on the genotype of the mother. I used a combination of Q-Q plots and Shapiro-Wilk's method to test for deviations from normality. Because the data was non-parametric, I used a Kruskal–Wallis test by ranks, followed by Mann–Whitney U tests for pairwise comparisons**.** I then tested whether female genotype affects the proportion of eggs hatched by fitting a generalized linear mixed effect model with a binomial error distribution, with hatched/unhatched as success/failure, and female genotype as a fixed dependent variable. Female ID was used as a random effect variable.

The total number of eggs hatched was used to estimate overall fecundity. To determine if there was a difference in the fecundity of each female genotype, I first compared the average number of eggs hatched by each female genotype. Fecundity was tested in three ways, on females that survived all four rounds, over one round, and all females regardless of survival. The homogeneity of variance assumption was violated in the model for females surviving all four rounds, so I used a Welsh's one-way test to relax this assumption. This was followed by pairwise t-tests with no assumption of equal variances. The model for all females, regardless of survival, violated the assumption of normality. In this case I used a Kruskal-Wallis test by ranks.

I next tested if the total number of eggs hatched was different between the female genotypes using a generalized linear model with a quasi-Poisson error distribution for count data to account for overdispersion in my data**.**

Ovary morphology

I compared the ovary morphology of wildtype (XX), heterozygous (X^dX), and homozygous driver (X^dX^d) females. In order to generate females with these different genotypes, I mated small groups of heterozygous (X^dX) females with individual driver (X^dY) or wildtype (XY) males for five days in uncrowded conditions. Upon emergence, virgin females were collected daily; only females emerging from the same vial and on the same day were kept together. After one week, females were sacrificed. Their heads were removed for genotyping (using dyn-q primer sets and high-resolution melt temperature peaks to determine genotype), and their ovaries were dissected. I photographed and measured ovary width and length under a dissecting scope measuring width and length. Individual ovarioles were teased apart to count developing eggs. I also recorded whether individual eggs were early (S9-11) or late (S12-E) stage.

I used a combination of Q-Q plots and Shapiro-Wilk's method to test for deviations from normality, and only ovary width was slightly non-normal. All of the data met the assumptions of independence and homogeneity of variance. I compared ovary width using a Kruskal–Wallis test by ranks. I then compared ovary length, total number of eggs, and eggs in late, and early stages of development using a one-way ANOVA, followed by Tukey post-hoc analysis tests for pairwise comparisons. Finally, I used a generalized linear model fit with a Poisson error distribution for count data to test if there were differences in the number of total eggs, and eggs at the early, and late-stage regions, between the three female genotypes.

Developmental time

My adult female transmission experiment (see below) also allowed me to test whether heterozygous (X^dX) females take longer to develop than wildtype (XX) ones. This experiment took advantage of a recessive X-linked 'bright' eye colour mutation (X^b). Individual heterozygous (X^bX^d) females were placed with two 'bright' eye (X^bY) males for 3-4 days. The female was then transferred to a new mushroom vial. She was subsequently transferred to a new mushroom vial 3-4 days later, and then again (i.e., a total of three vials per female). Emergent adult offspring were collected daily, sexed, and scored for eye colour. This allows one to distinguish between driving (X^dY) males, wildtype (X^bY) males, heterozygous (X^dX^b) females, and wildtype (X^bX^b) females. (Note that wildtype here refers to the fact that it is not driving.) To rule out the possibility that the bright-eye mutation affects development time or other components of fitness, XX^b females were mated with X^bY males, as controls. In order to compare the development time between X^dX^b and X^bX^b females, and X^dY and X^bY males, I used the two-sample unpaired t-test to compare the mean emergence date of the flies based on their genotype.

This experiment was performed by Steve Perlman; I analysed the data.

Xd chromosome transmission through females

To test whether the selfish X chromosome exhibits biased transmission in females, heterozygous (X^dX) females were mated with wildtype (XY) males, and the genotype of their offspring was determined. In separate experiments, offspring were genotyped as eggs, larvae, and adults.

In eggs

I mass mated heterozygous (X^dX) females with wildtype (XY) males. Flies were allowed to mate in vials with standard medium for three days before being transferred to new vials with a mushroom agar egg-laying medium. After 24 hours, females were transferred to individual vials with mushroom agar, and males were preserved in 95% ethanol. Females were allowed to oviposit on the mushroom agar medium for 24 hours before being preserved in 95% ethanol. Eggs were collected and their DNA was extracted immediately after collection using PrepMan^{TM} (Thermofisher). The samples were then diluted with dH20 (1:10) and stored at -20C until genotyping was completed. To genotype eggs and determine transmission of the X^d chromosome, I performed three qPCR reactions, using diagnostic primers that amplify a gene on the Y chromosome (*kl2*), a gene on the X^d chromosome (*protamine*-like gene, and *dynein*), and DNA extraction positive control primers (neoNa-q). The presence or absence of amplification allows me to distinguish between XY, X^dY, X^dX, and XX genotypes.

In larvae

Three to four, one-week old, virgin females (X^dX) were mated to individual wildtype (XY) males. Flies were allowed to mate for five days, flipping once during this time. Females were then isolated into individual vials for oviposition and were flipped into new vials every 24 hours over four days, prior to preservation in 95% ethanol. Once third instar larvae were present, all larvae were collected from each vial by flooding with dH20 and sifting through the substrate. These were stored in 95% ethanol until DNA extractions and genotyping was conducted. Larvae were genotyped individually to determine sex and driver status, as done for the egg X^d transmission stage.

In adults

The presence of an X-linked visible bright-eye colour (X^b) mutation on an otherwise wildtype (i.e., nondriving) X chromosome greatly facilitates quantifying biased X^d transmission through females. Crossing X^dX^b females with X^bY males will produce four possible genotypes, X^dXb, X^bXb, X^dY, X^bY, that can be distinguished by sex and eye colour. Biased transmission of the X^d chromosome would result in more dark eyed individuals (X^dX^b + X^dY) than bright-eyed (X^bX^b + X^bY) ones. These crosses are the same as in the development time experiment (see above), but individual females were transferred to new vials four times (development time was only analyzed for flies emerging from the first two

vials). To control for possible effects of the X^b mutation, control crosses between X^bX females and X^bY males were performed. This experiment was performed by Steve Perlman; I performed all the analyses.

Xd transmission data analysis:

Data was analysed in R (R core team 2020) using the packages: desctools (Signorell et al., 2020), car (Fox and Weisberg, 2019), GLMM (Knudson, 2020), ggplot2 (Wickham, 2016), and rcompanion (Mangiafico, 2020).

Fisher's exact test were first performed to determine whether the results were dependent on mother (vial effect), or the block, in situations where the dataset sample size was small enough to compute. If datasets were too large to get an exact measurement from a Fisher's exact test, a G-test, or Chi-square test was performed to get an approximation of independence.

To test whether the offspring genotype proportions were different from expected Mendelian ratios (from a heterozygous mother we would expect: ¼ XX, ¼ X^dX, ¼ XY, and ¼ X^dY), I fit a generalized linear model with a quasibinomial error distribution to account for overdispersion.

To test if offspring inherited the X^d chromosome preferentially over the X chromosome, I fit a generalized linear model with a binomial error distribution, where inheritance of the X^d was considered a success, and X chromosome inheritance was a failure.

Saturated models were fit and simplified in a stepwise method using a combination of AIC, Anova, and the compareGLM() (function in R- rcompanion package) to evaluate which model best fit the dataset. Overall, model fit was examined using QQ-plots and analysing residuals by plotting residuals versus fitted values.

Results:

Female fertility

I wanted to determine if females had reproductive fitness differences based on their X chromosome genotype. To do this, I evaluated the hatch rate of females carrying zero, one, or two X^d chromosomes to give me an estimate of female fertility. There were significant differences between female genotype hatch rates (Kruskal-Wallis chi-squared = 17.44, df = 2, p-value = 0.00016) (Figure 1). Females homozygous for the driving X chromosome had a significantly lower mean egg hatch (50.3% +/- 23.1 sd). The mean percentage hatched was comparable between the wildtype (65.3% +/- 23.7 sd) and heterozygous females (63.9% +/- 22.0 sd). This shows that homozygous (X^dX^d) driver females have reduced fertility compared to wildtype (XX) and heterozygous (X^dX) driver females.

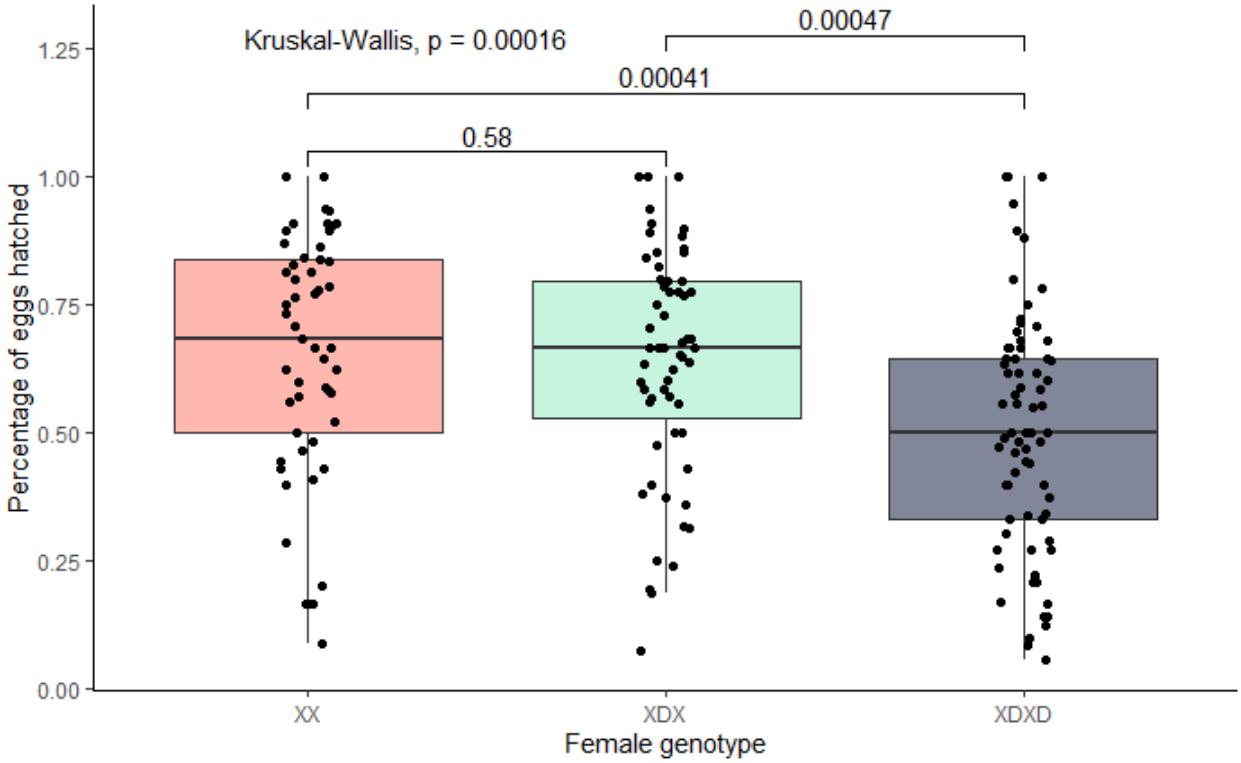


Figure 1. Average percentage of eggs hatched from individual females that carry zero, one, or two copies of a selfish X chromosome. Females of each genotype were mated to wildtype males, and resulting eggs were collected over 4 days. After 96 hours, unhatched eggs and empty egg casings were counted. Homozygous driver females (X^dX^d) have a lower percentage of their eggs hatch (~50%) than wildtype (~65%) or heterozygous driver females (~64%) (Kruskal-Wallis chi-squared = 17.44, df = 2, p-value = 0.00016). Wildtype and heterozygous females do not differ.

Female Fecundity

Next, I investigated whether X^d genotype resulted in difference in the overall lifetime fecundity of female flies. I used the number of eggs hatched by individual females over four blocks as a proxy for lifetime offspring production. I first considered only females that survived to end of block 4. Heterozygous females had the highest number of eggs hatch (82.56 +/- 27.04 s.d.) (Figure 2), followed by wildtype females (60.80+/- 276.52 s.d.), and X^dX^d females (44.30 +/- 9.01 s.d.). The difference between egg hatch of heterozygous and homozygous driver females (X^dX^d) was significantly ($p = 0.0043$, Wilcoxon pairwise comparison).

I also compared total egg hatch of all females, regardless of whether they survived to the final timepoint or not. X^dX females again have the highest egg hatch (51.90 +/- 25.50 s.d.), followed by wild type (XX) (41.85 +/- 27.11 s.d.), and then X^dX^d females (36.4 +/- 20.891 s.d.) (Supplemental Figure 1), with heterozygous and homozygous driver females being significantly different ($p = 0.0137$, Wilcoxon pairwise comparison). I saw the same pattern when excluding females did not survive past the first timepoint (XX: mean = 48.50 +/- 26.18 s.d., X^dX: mean = 53.83 +/- 25.52 s.d., X^dX^d: = 38.65 +/- 20.28 s.d.; X^dX vs X^dX^d $p = 0.018$ Wilcoxon pairwise comparison) (Supplemental Figure 2).

These results suggest the homozygous driver females have reduced fecundity (or, overall lifetime production of offspring) compared to wildtype and heterozygous driver females.

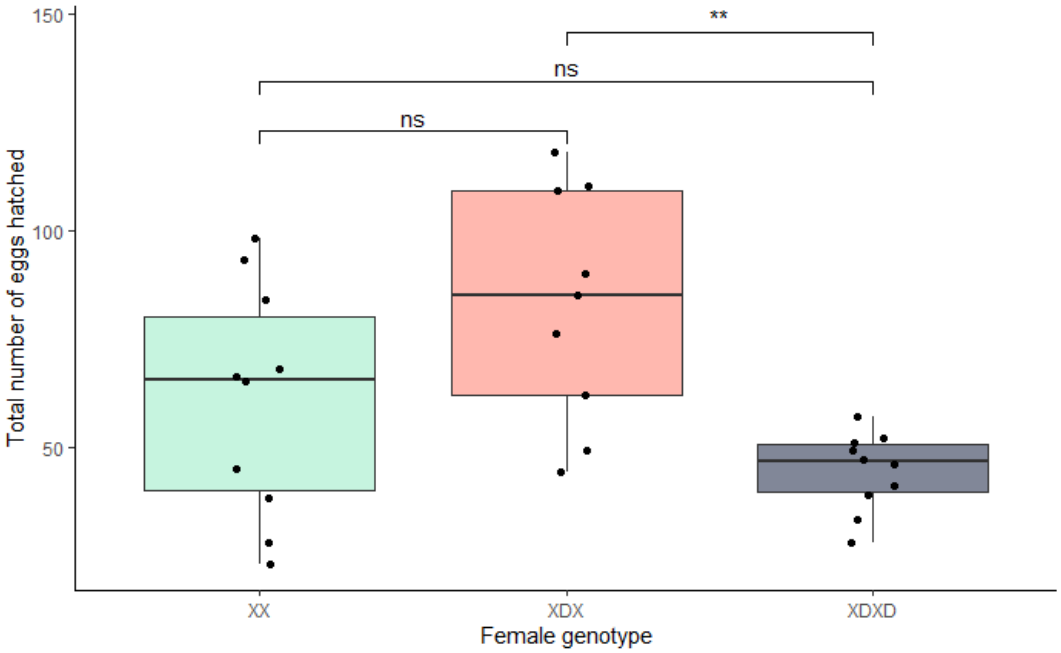


Figure 2. Total number of eggs hatched from individual females that carry zero, one, or two copies of a selfish X chromosome in *Drosophila testacea*. Females were mass mated to wildtype males, upon which they were allowed to oviposit individually in vials for 24 hrs, and then transferred to new vials three more times. After 96 hours, unhatched eggs and empty egg casings (hatched) were counted. Total hatched eggs were tallied for all surviving females (i.e., that had laid eggs over 4 consecutive days). These were used as a proxy for lifetime fecundity (total offspring produced). Heterozygous driver females produced the most offspring, with this being statistically significant from the numbers produced by homozygous driver females ($p = 0.0043$, Wilcoxon pairwise comparison) (X^dX^d).

Ovary morphology

Due to reduced reproductive success in X^dX^d females, as well as spermatogenesis defects in X^dY males, I wanted to investigate whether there was any ovarian pathology associated with the X^d chromosome. There were no significant differences seen in ovary morphology between the different genotypes (Figure 3, Supplemental Table 1), although X^dX females had a large range of variability between values seen for ovary size (801 µm min, 2310 µm max for ovary length; and 538 µm min, 1820 µm max for width). In some cases, the ovaries of heterozygous females appeared normal and fully developed, while in other cases, they appeared extremely undeveloped and contained no eggs (Figure 4).

Wildtype females had the most early-stage eggs, followed by heterozygous, and then X^dX^d females, with the effect of genotype approaching statistical significance (ANOVA, F = 3.17, p-value 0.05), although none of the pairwise comparisons were significantly different, perhaps due to small sample size. This suggest that overall ovarian morphology between females X chromosome genotype is similar.

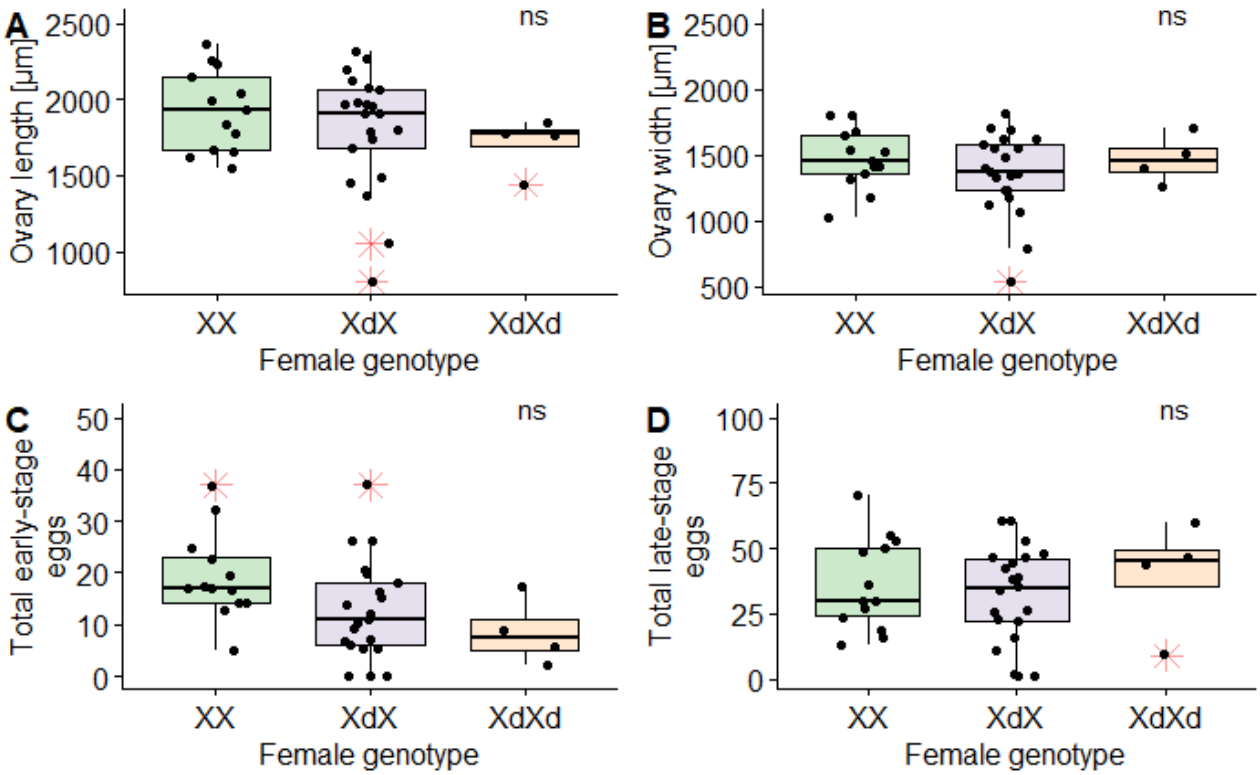


Figure 3. Comparison of A) ovary width, B) length, and numbers of developing eggs in C) early (S9-11) and D) late stages (S12-Egg) in individual ovarioles of one week old, virgin flies that were: wildtype (XX), heterozygous (X^dX), or homozygous (X^dX^d) for a driving X chromosome. No significant differences were seen between the three genotypes; however, a large amount of variation was seen in ovary size in heterozygous X^dX females.

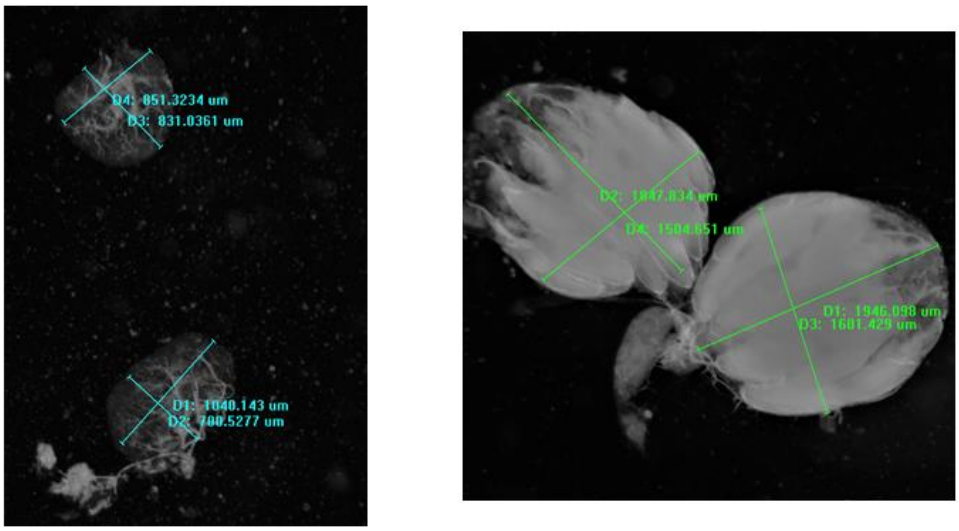


Figure 4. Examples of variation in ovary phenotypes present in heterozygous females. Often ovaries would be large and well-developed, similar to wildtype flies (*right panel*). However, several individuals had smaller than average ovaries that appeared to be under-developed, or abnormal, and containing no eggs (*left panel*).

Egg-adult development time

Larval defects are often seen in post-zygotic embryo killer systems through females. As well, mutations in genes regulating embryogenesis may be present due to the accumulation of deleterious mutations on the X^d chromosome. To test if development of offspring was impacted by X chromosome genotype, I evaluated the time it took for newly laid eggs to emerge as adults. Homozygous bright-eye (X^bX^b) and heterozygous driver/bright eye (X^dX^b) females took the same time to develop (X^bX^b: mean = 4.82 emergence day +/- 2.92 s.d., X^dX^b: mean = 4.79 emergence day +/- 2.55 s.d., p-value =0.93; two sample t-test) (Figure 5). Similarly, there was no difference between male X^bY and X^dY average emergence date (X^bY: 4.82 +/- 2.77 s.d., X^dY: 4.69 +/- 2.93 s.d., p = 0.70; two sample t-test) (Figure 6).

In addition, control matings show that the bright-eye mutation does not affect development time, as there were no differences between males (X^bY: mean = 3.56 +/- 1.77 s.d.; XY: mean = 3.77 +/- 1.71 s.d., p-value = 0.61, two sample t-test) (Supplemental Figure 3), or females (X^bX^b: mean = 3.85 +/ 1.69, XX^b: mean = 3.50 +/ 1.68 s.d., p-value = 0.37; two sample t-test) genotypes (Supplemental Figure 4).

This suggest that development time is similar between wildtype flies (with a bright-eye mutation) and flies that carry a X^d chromosome.

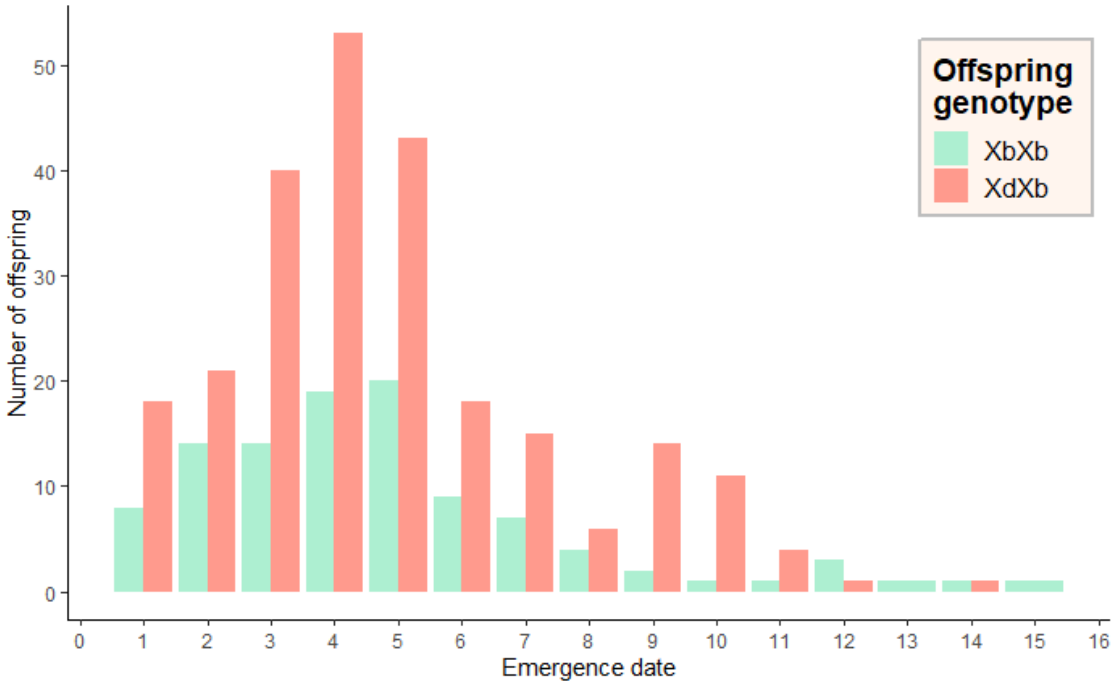


Figure 5. X^dX^b and X^bX^b females have similar adult emergence time. (Note the greater number of X^dX^b females.)

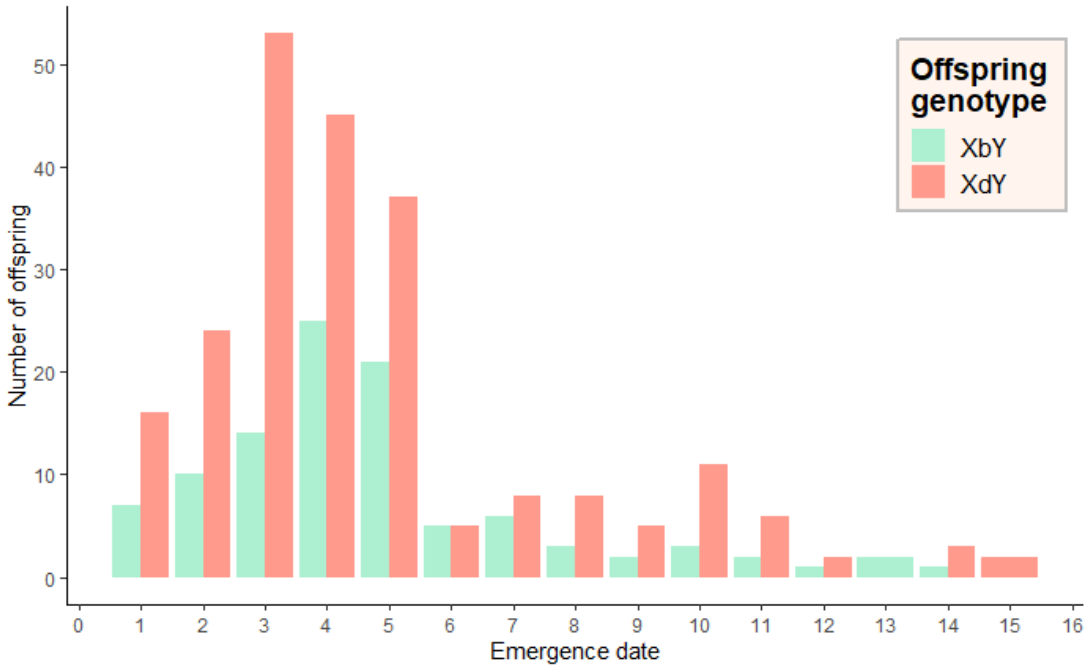


Figure 6. X^dY and X^bY males have similar adult emergence time. (Note the greater number of X^dY males.)

Xd chromosome transmission through females

In eggs

I was interested in seeing if there was a bias transmission of the X^d chromosome from females to their offspring. To determine if this was happening during chromosomal segregation, I tested whether a bias was seen in eggs laid by heterozygous driver females. Eggs laid by individual X^dX females (mated to wildtype males) were collected and individually genotyped to determine offspring genotype. The expected proportions for each genotype resulting from this cross is ~ 25% (¼ XX, ¼ XY, ¼ X^dX, ¼ X^dY). There is a significant difference between our offspring genotype proportions and our expected Mendelian ratios (1:1:1:1) (X-squared = 18.74, df = 3, p-value = 0.00031, chisq.test) (Figure 7). To further explore which genotypes have deviated from expected values, each genotype proportion was tested with an exact binomial test. We see that all genotypes deviated from expected proportions, with XX (~11%) and XY (~16%) frequency being lower, and X^dX (~37%) and X^dY (~37%) higher than ~25%.

There was a significant deviation from the expected 1:1 ratio of X^d chromosome inheritance in the offspring of each heterozygous mother (Figure 8). Inheritance of the X^d chromosome was significantly greater than 50% compared to the wild-type X chromosome (p-value = 1.62e-05; 95% CI= 0.64, 1.00; exact binomial test) in pooled data from all three heterozygous mothers, with about 73% of offspring inheriting the X^d chromosome (p-value = 3.23e-05; 95% CI= 0.62, 0.82; exact binomial test), suggesting that there is a biased transmission of the X^d chromosome in eggs laid by X^dX females.

In larvae

I also wanted to determine if there was a detectable transmission bias in larvae from heterozygous mothers. All larvae from single X^dX females, mated to XY males, were individually genotyped for the X^d chromosome. We see that there is a difference in genotype proportions from pooled larval data (X-squared = 16.67, df = 3, p-value = 0.00083, chisq.test) (Figure 7), with wildtype males showed a significant decrease from the expected ~25% frequency (~13% of offspring, p-value = 2.051e-05, 95% CI = 0.086, 0.18, exact binomial test). There is a significant transmission bias of the X^d chromosome compared to the X chromosome (p-value = 0.023; 95% CI= 0.51, 1.00; exact binomial test) (Figure 8). Offspring inherited the X^d chromosome about 57% of the time (p-value = 0.045; 95% CI = 0.50, 0.64; exact binomial test). This suggests that the transmission bias seen at the egg stage is also seen in larvae.

In adults

To test whether biased X^d transmission was detectable in adult offspring, matings were set up between individuals with X^d and/or X chromosomes with a bright-eye mutation, which allows us to easily genotype offspring by eye. Offspring genotypes showed a significant departure from Mendelian expectations (X-squared = 125.46, df = 3, p-value < 2.2e-16) (Figure 7). All genotypes deviated from expected frequencies with X^bX^b, X^bY, X^dX^b, and X^dY making up about 18%, 15%, 36% and 31% of offspring proportions, respectively. Mother B3 had significantly different genotype proportions compared to the other mothers, with ~ 45% being X^dX^b and 36% X^dY (expected= ~25% each).

There is also a significant transmission bias of the X^d chromosome compared to the X chromosome seen in the adult offspring of heterozygous mothers (p-value < 2.2e-16; 95% CI= 0.64, 0.70; exact binomial test), with 67% inheriting the X^d chromosome (Figure 8). Mother B3 has significantly more offspring (81%) inheriting the X^d chromosome, compared to the other mothers, suggesting that the initial transmission bias in eggs is carried through until the offspring reach adulthood.

Control crosses showed that the bright-eye mutation did not affect transmission, as X^bX females passed the bright-eye mutation to half of their offspring (p-value = 0.42; 95% CI = 0.45, 0.62; exact binomial test).

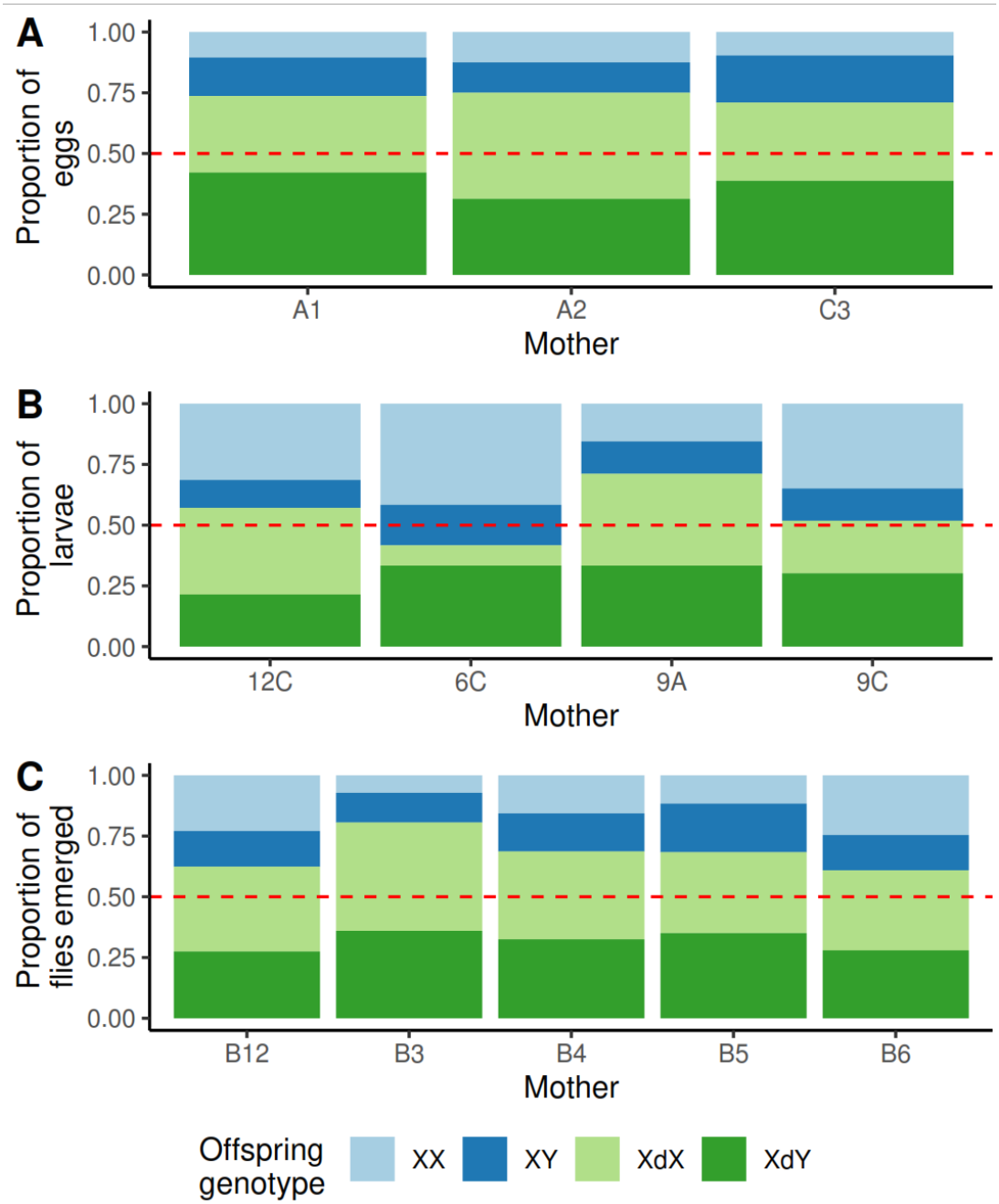


Figure 7. Genotype proportions measured at the A) egg, B) larval, and C) adult stages of offspring of heterozygous (X^dX^b) females mated with wildtype (X^bY) males. Observed genotypes showed significant deviations from expected 1:1:1:1 genotype proportions, at all life-stages.

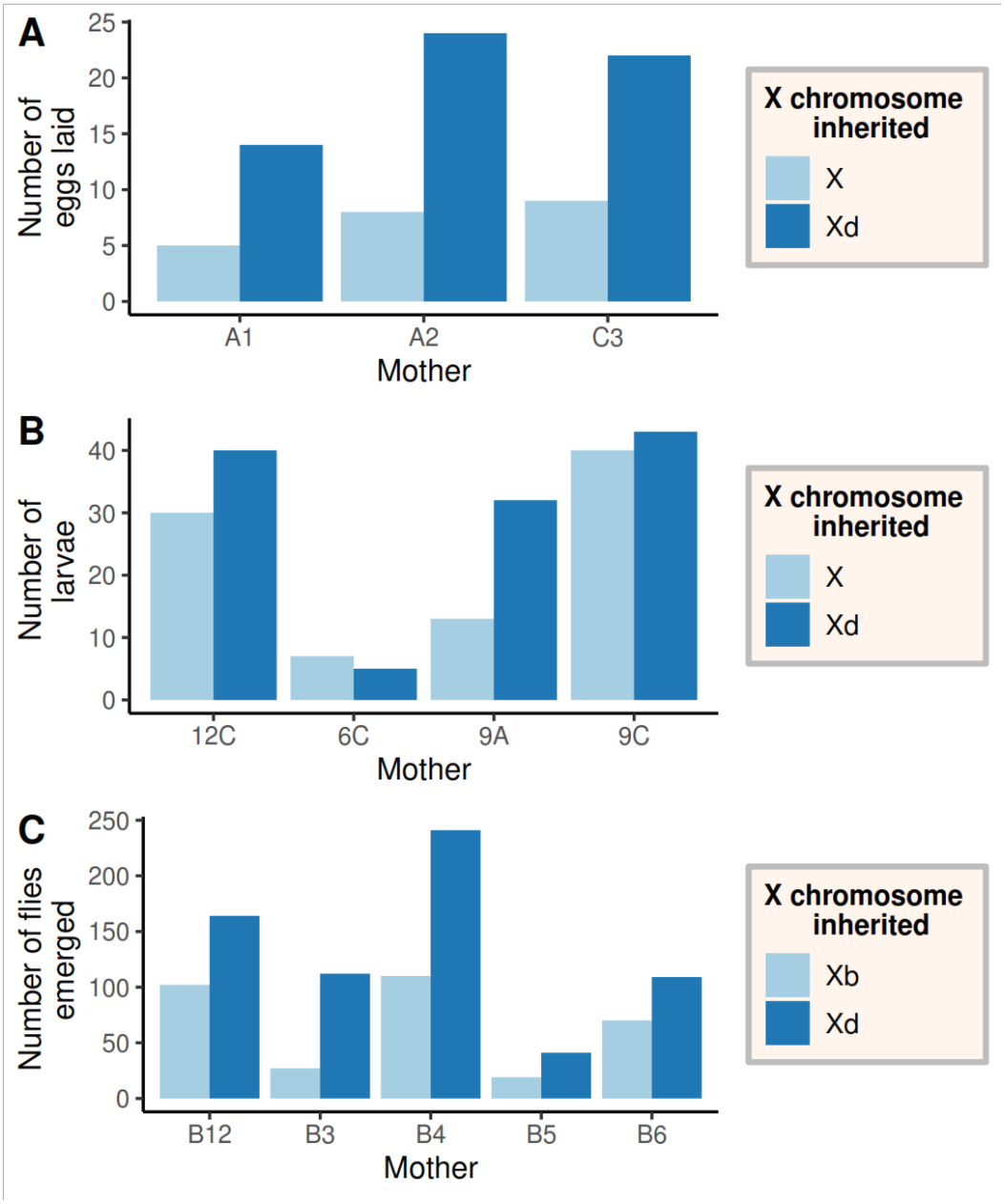


Figure 8. X^d inheritance in offspring from individual heterozygous (X^dX^b) females mated with wildtype (X^bY) males, at A) egg, B) larval, and C) adult stages. Offspring are more significantly more likely to inherit the X^d chromosome than the X^b chromosome from their mother.

Discussion

A new X chromosome drive system was recently discovered in *Drosophila testacea* (Keais *et al.*, 2017). Males carrying a driving X chromosome sire almost exclusively daughters because they almost always transmit the X chromosome. In this thesis, I further characterize how this selfish X chromosome in *Drosophila testacea* impacts females. The first part of my study compared fitness of females carrying zero, one, or two copies of the X^d chromosome. I found that females that are homozygous for the driving X chromosome (X^dX^d) produce fewer offspring than heterozygous or wildtype females. There did not appear to be any difference in ovary phenotype, or egg to adult development time.

In the second part, I performed experiments to ask whether there was a transmission bias of the X^d chromosome through females. To elucidate this possibility, I did a series of experiments where I genotyped all offspring of X^dX mothers mated to wildtype males. This was done at egg, larval, and adult stages. I found increased transmission of the X^d chromosome over the X chromosome across all life stages, with ~55-75 % of offspring inheriting the X^d chromosome. My findings help contribute to our understanding of this unique selfish X chromosome system.

Homozygous driver females have reduced reproductive fitness

Here I show that homozygous driver females (X^dX^d) have reduced reproductive fitness compared to heterozygous and wildtype females. Females that were homozygous for the selfish X chromosome had a ~15% lower egg hatch compared to the other genotypes (XX and X^dX). Additionally, X^dX^d females have a lower overall lifetime production of offspring. This may explain why it has been difficult to maintain a homozygous X^d line in the laboratory. An earlier study found that X^dX^d produced fewer offspring (Keais *et al.*, 2020), but that study could not disentangle whether this was because X^dX^d females were less likely to mate, had reduced egg hatch, or whether their offspring had increased mortality. Also, that study did not include wildtype females.

A reduction in female fitness appears to be a common pattern of X-chromosome drive systems in flies. For example, we see female fitness consequences for heterozygous and homozygous carriers in *Drosophila recens* (Jaenike, 1996; Dyer and Hall, 2019) and *Drosophila pseudoobscura* (Larner *et al.*, 2019). In these X-drive systems, female flies that are homozygous for the driver were found to have a higher likelihood of sterility, and this was especially pronounced in *D. recens*. Additionally, fertile females that carry drivers have reduced reproductive fitness, with fewer eggs hatching and offspring reaching adulthood.

This is very similar to what we see in *D. testacea*, with there being an increased cost to homozygous female carriers.

Why eggs laid by X^dX^d females have reduced hatch success is not known, but this is likely because of deleterious mutations in an X-linked gene involved in oogenesis. These deleterious mutations would be unable to be removed from a driving X chromosome that does not recombine. It will be interesting to compare ovarian transcriptomes of XX, X^dX, X^dX^d females, as well as sequencing the X^d and X chromosomes, to look for inactivating mutations associated with oogenesis genes. It would also be interesting to perform more detailed histological studies to look for identifiable egg defects in X^dX^d females. As far as I am aware, the genetic basis of reduced female fitness has not been identified in any selfish X system, including *D. pseudoobscura* or *D. recens*.

The X^dX^d fitness difference is an important factor in determining the maintenance of the X^d chromosome in *D. testacea*. This is because, as the X^d chromosome spreads in the population, there will be increasing numbers of X^dX^d females. We know that many these females would be sterile, and the rest would have reduced reproductive fitness. This may be offset the transmission bias and allow this system to remain polymorphic. If we revisit the X-chromosome drive system in *D. pseudoobscura*, we saw that there were more severe reproductive consequences for the X^dX^d mothers (Larner *et al.*, 2019). In this case, these fitness differences are enough to maintain X^d at a relatively high prevalence (~25%), and this is without the presence of any suppressors. Given that we already know the *D. testacea* system has an autosomal suppressor, it is quite plausible that these two factors are contributing to the longevity of this X-drive system.

Reproductive fitness of wildtype females and heterozygous females appears to be similar in the *D. testacea* X-drive system. This result contrasts with what we have seen in other studies where there appears to be a dosage effect resulting from the X^d chromosome (e.g., *D. recens* in Dyer *et al.*, 2019). However, in *D. pseudoobscura* we see a similar result to ours, where the X^dX females appear to be reproductively healthy. This may be because having both an X and X^d chromosome together provides some sort of genetic advantage (or, hybrid vigour); perhaps laboratory strains of the wildtype line are also slightly inbred.

I did not observe any other fitness effects. There were no differences seen in ovary morphology or development time, although my development time experiment did not include X^dX^d females. In addition to including crosses in the future that would allow me to measure and compare X^dX^d development time, it might be interesting to examine development time under differing environmental conditions, as all the experiments in this study were

maintained at constant larval density, temperature, and humidity. For example, we know that changes in larval density can impact emergence time and size in *Drosophila* (Mrnsua and Moya, 1990; Joshi *et al.*, 1998**)**. So, it may be beneficial to see if varying larval density and/or food availability affects development time between fly genotypes.

The selfish X chromosome shows biased transmission through both males and females in D. testacea

In this thesis, I show that heterozygous females (X^dX) are more likely to pass on the X^d chromosome to their offspring. I found this bias at all offspring developmental life stages: egg, larva, and adult. This is in addition to the transmission bias of the X^d chromosome through male meiosis (i.e., via destruction of Y-bearing sperm). This study is the first to identify a selfish genetic element that acts to bias transmission of a X chromosome through both male and female meiosis. In fact, I am not aware of any strong examples where meiotic drive is occurring through both sexes. This is highly unusual, as male, and female meiosis are very different processes and this has major implications for how this system evolved, its general mechanism, and how it has been maintained for so long; at the very least, biased X^d transmission through females, further tilts the balance towards increased X^d in the population. The only other study I am aware of suggesting transmission distortion in both sexes examined 1302 single nucleotide polymorphisms in *Taeniopygia guttata* zebra finches (Knief *et al.*, 2015). The authors found 3 linked SNPs on an autosome that were weakly but significantly overrepresented through both males (57.5%) and females (56.0%). The authors suggest that this could be due to a drive system, although more work is needed to determine what is going on.

The timing of SGE action can give us clues as to how it may be cheating Mendelian inheritance. Seeing the X^d transmission bias in eggs suggests that the process is occurring during meiosis. Perhaps the X^d chromosome is able to bias its orientation into the egg pole and away from the polar body. If we observed transmission bias in larvae and adults, but not in eggs, then this might suggest that the distortion is post-embryonic, such as the Medea maternal killers in *Tribolium* beetles (Beeman et al., 1992), but this is not the case.

Female X^d distortion was stronger in the egg and adult experiments than in the larval one. This may be due to slight differences in the experimental design between experiments that led to differing larval densities. There may have been a higher density and/or more intense competition in the larval transmission experiment. If the larvae that carry an X^d chromosome are poorer competitors, this would have affected my results. Additionally, the

different genotypes may have fitness differences that affect them at various life-stages because developmental genes could be expressed at different times and tissues. It may also be possible that some of the larvae were missed while sifting through substrate in the vial.

There are fewer examples of female meiotic drive than drive through males, and it is generally weaker than what is seen in male X chromosome drive systems. For example, the well-known case of female centromere drive in *Mimulus* monkeyflowers exhibits a ~55-60% transmission bias in interspecies crosses (Fishman and Saunders, 2008). Compared to male drive (~80-100% transmission), this is quite weak. A large sample size would be required to distinguish female drive from normal deviations from the expected 1:1 ratio. Additionally, biased transmission of the X chromosome through females would not produce the same dramatic sex-ratio distortion seen in male meiotic drive. This is because we are seeing a transmission bias of one X chromosome over another, instead of one sex over the other. Given both the weak transmission and the lack of sex-ratio distortion, it is hard to say how often female drive occurs. In addition, weak but statistically significant centromeric drivers are still expected to spread, and once they have become fixed in the population, we would no longer see their action.

I am only aware of one case of female meiotic drive in *Drosophila*, in *D. americana* (Stewart *et al.*, 2019). This species is polymorphic for a chromosomal fusion that was found to have a transmission bias of ~57% over its homologous chromosome. This transmission bias was also seen in hybrids between *D. americana* and its sister species, *D. novamexicana*. The authors speculate that the chromosomal fusions result in differing stability of kinetochore attachments, allowing for preferential segregation into the egg.

There are a few possibilities that could result in X chromosome drive occurring through both male and female meiosis. Since, male meiotic drivers are common in *Drosophila*, it seems plausible that a male meiotic driver arose first in *D. testacea*. It could then be possible that a second SGE could evolve independently of the male meiotic driver, through the accumulation of mutations on the driving X chromosome. We already know that the *D. testacea* driving X chromosome is extremely divergent from its wildtype counterpart. Additionally, centromeres and pericentromeres often reside in areas of low recombination and they tend to consist of large stretched of repeating DNA sequence (satellite repeats) (Iwata-Otsubo *et al.*, 2017; Shatskikh *et al.*, 2020). Structural changes to these satellite repeats can influence alignment at metaphase plate and create differential attachment strengths to spindles, making centromeres an effective target for selfish genetic elements to manipulate gametogenesis. It is possible that this SGE has resulted in the accumulation of

duplicated satellite repeats within, or in the surrounding regions, of the centromere. This could eventually have impacted X chromosome alignment during meiosis, thereby determining which chromosome is retained in the egg.

Alternatively, perhaps both male and female drive are caused by the same element. In one possible model, a mutation results in the duplication of satellite repeats in the centromeric region of the X^d chromosome. This expanded centromere region could have resulted in differential attachment of kinetochore proteins during meiosis and a segregation bias into the egg. Although literature is sparse regarding centromere drive in *Drosophila*, we do see some evidence that suggests there is genetic conflict occurring during centromere segregation. For example, we see significant differences (expansions and contractions) in centromere satellite repeats between *Drosophila* species (Bracewell *et al.*, 2019). Additionally, centromere proteins (CID and CAL1) show evidence of positive selection and strong divergence between species, resulting in hybrid incompatibilities (Malik and Henikoff, 2001; Beck and Llopart, 2015; Rosin and Mellone, 2016; Kursel and Malik, 2017). This has been suggested to be evidence of pressures related to centromere drive, as it would be expected that genes related to meiosis should be conserved due to their important cellular function, yet they undergo rapid evolution.

Over time, the large-scale changes in the X^d chromosome also impacted how the chromosomes aligned and/or paired during male meiosis (McKee and Lindsley, 1987; McKee *et al.*, 1998), with there being an increased chance of improper pairing or misalignment (Peacock, 1965; Baker and Carpenter, 1972; Peacock *et al.*, 1975; McKee and Lindsley, 1987; Mckee *et al.*, 1998). Misalignments during male meiosis can lead to non-disjunction events. In these cases, chromosomes are unable to properly go through meiosis and often cannot separate at anaphase. If non-disjunction is more likely to happen during X-Y pairings, then this could perhaps result in biased X chromosome transmission.

Earlier studies into meiotic mutants in *Drosophila* melanogaster showed that there were some overlapping common themes between meiotic drive and chromosome misalignment (Peacock, 1965; Baker and Carpenter, 1972; McKee and Lindsley, 1987; McKee *et al.*, 1998). These studies looked at flies that had X-linked mutations that negatively impacted meiosis (e.g., inversions on X chromosome, or deletions of genetic material). Interestingly, these studies often found that these meiotic mutants produced unexpected sex ratios, with males be underrepresented in progeny. Additionally, XO males were more often produced than XY males in crosses between driver males and wildtype females, and spermatogenesis defects were found to result from non-disjunction during meiosis. It was

determined that when chromosomes do not properly separate during meiosis, they migrate to one pole in a non-random matter. The other pole does not receive the male's sex chromosome and ends up being XO, while the improperly joined chromosomes are discarded. It could be possible we are seeing a centromere drive in *D. testacea* that also results in the mispairing of the XY chromosomes in male meiosis. If there have been structure changes to X-Y pairing sites, or perhaps, multiple inversions close to centromeric regions, then this could explain the similar patterns in the *D. testacea* system, with males having spermatogenesis defects, a sex bias in offspring, and more XO sons than XY.

Some follow-up experiments might provide more insight into how this selfish X chromosome in *D. testacea* behaves during meiosis. Using FISH (Fluorescence in situ hybridization) to follow X and Y chromosomes during male and female meiosis, would allow us to see if there is preferential segregation of the X^d chromosome into the egg. This would also determine whether non-disjunction is occurring during meiosis in X^dY males. Karyotyping, via polytene chromosome squashes, along with long-read DNA sequencing, would allow us to identify inversions, centromeres, or other areas of low recombination. Additionally, it would be interesting to see if the autosomal suppressor that suppresses male drive (Keais et al., 2020) can also suppress against female drive. This would further help to elucidate whether this system is a product of one, or two SGEs, and additionally, if two-which one came first?

Supplemental Information

Supplemental Table 1. Summary statistics of ovary data.

	XX (N=13)	**X^dX (N=21)**	**X^dX^d (N=4)**	**Kruskal-Wallis chi-squared**	**df**	**p-value**
Ovary length [µm]						
Mean (SD)	1930 (267)	1800 (388)	1710 (181)	1.9825	2	0.3711
Median [Min, Max]	1930 [1550, 2360]	1910 [801, 2310]	1770 [1440, 1850]			
Ovary width [µm]						
Mean (SD)	1470 (229)	1360 (310)	1470 (188)	1.0865	2	0.5809
Median [Min, Max]	1450 [1020, 1810]	1380 [538, 1820]	1450 [1260, 1710]			
Total eggs						
Mean (SD)	55.5 (17.3)	44.8 (22.9)	48.3 (16.7)	1.7122	2	0.4248
Median [Min, Max]	55.0 [30.0, 87.0]	51.0 [1.00, 75.0]	50.5 [26.0, 66.0]			
Late stage eggs						
Mean (SD)	36.2 (17.6)	32.1 (18.5)	39.8 (21.7)	0.72914	2	0.6945
Median [Min, Max]	30.0 [13.0, 70.0]	35.0 [1.00, 60.0]	45.0 [9.00, 60.0]			
Early stage eggs						
Mean (SD)	19.2 (8.38)	12.6 (9.61)	8.50 (6.35)	5.9665	2	0.05063
Median [Min, Max]	17.0 [5.00, 37.0]	11.0 [0, 37.0]	7.50 [2.00, 17.0]			

Supplemental Table 2. Summary statistics for female reproductive fitness.

Female genotype	Total eggs laid	Total hatched	Total unhatched	Mean percentage hatched	n	sd
XX	2130	1145	986	0.653	53	0.237
X^dX	1575	993	583	0.639	59	0.21
X^dX^d	3444	1401	2052	0.503	73	0.231

Supplemental Table 3. Summary statistics for female development time

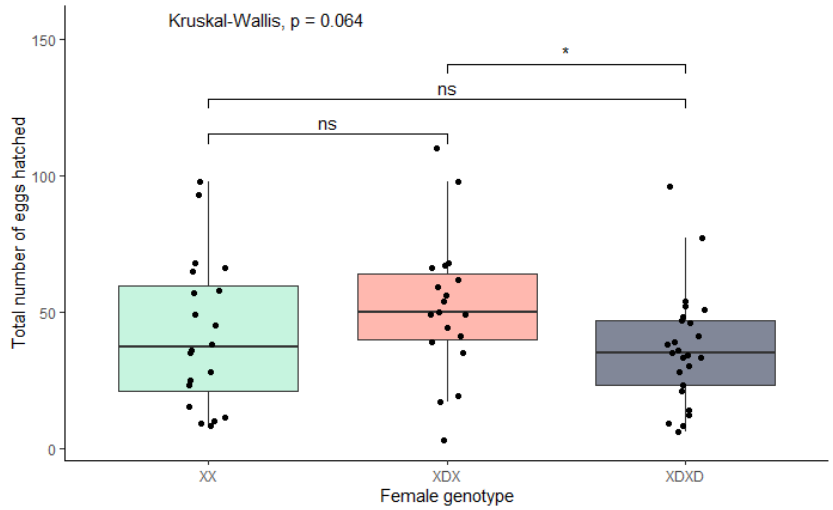
Cross	Offspring genotype	N	mean emergence day	sd	se	t-test	df	p-value
B3	X^bX^b	7	3.71	1.98	0.75	-0.86796	52	0.3894
B3	X^dX^b	47	4.59	2.57	0.38			
B4	X^bX^b	40	6.13	3.88	0.61	0.94334	127	0.3473
B4	X^dX^b	89	5.52	3.14	0.33			
B5	X^bX^b	7	4.57	1.4	0.53	1.0083	25	0.323
B5	X^dX^b	20	4.05	1.1	0.25			
B6	X^bX^b	14	4.43	2.24	0.6	-1.013	34	0.3182
B6	X^dX^b	22	5.14	1.91	0.41			
B12	X^bX^b	37	3.81	1.49	0.24	-0.75827	102	0.45
B12	X^dX^b	67	4.07	1.8	0.22			

Supplemental Table 4. Proportions of genotypes pooled from each developmental stage that were inherited from heterozygous females mated to wildtype males. Expected proportions for each genotype would be equal, at 25% each (or, 1:1:1:1). [Note- for adult stage XX = XX^b* (carries bright-eye mutation), and XY= X^bY**]

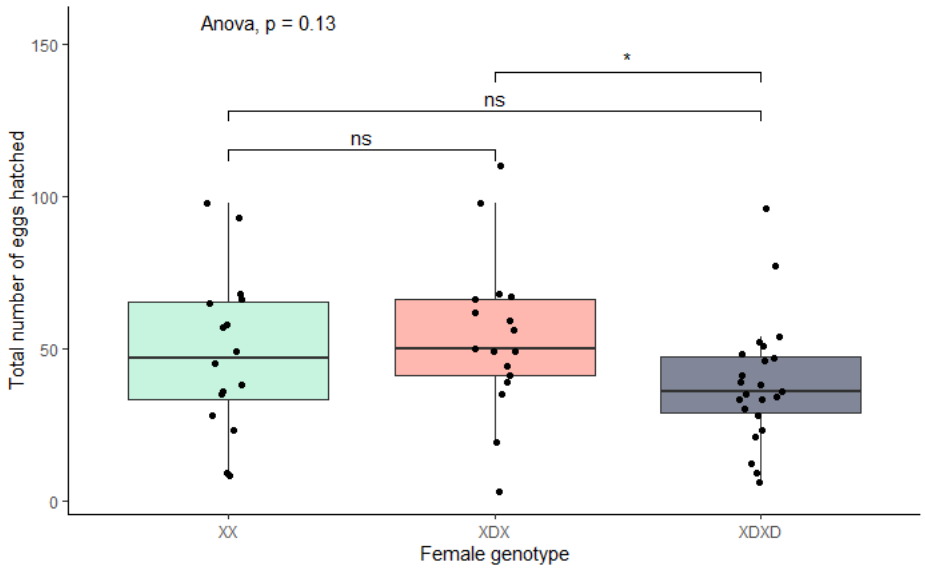
Stage	Prop XX	Prop XY	Prop X^dX	Prop X^dY	Chi-sq (1:1:1:1)	df	p-value
Egg	0.11	0.16	0.37	0.37	18	3	4.40E-04
Larvae	0.30	0.13	0.29	0.28	16.67	3	8.28E-04
Adult	0.18*	0.15**	0.36	0.31	125.46	3	2.20E-16

Supplemental Table 5. Proportion of offspring inheriting the X^d vs X chromosome from heterozygous females mated to wild type males. Expected proportions for each X chromosome would be equal, at 50% each (or, 1:1). [Note- for adult stage X chromosome = X^b* (carries bright-eye mutation)]

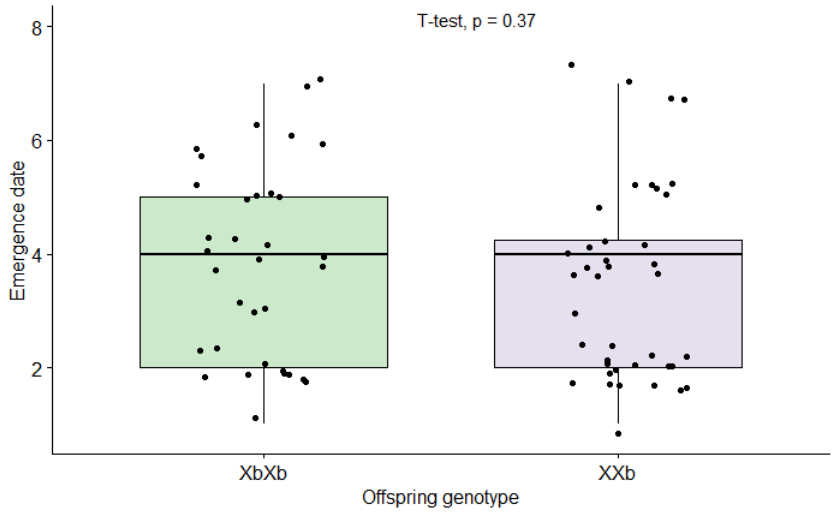
Stage	Prop X^d	Prop X	Chi-sq (1:1)	df	p-value
Egg	0.73	0.27	17.61	1	2.72E-05
Larvae	0.57	0.43	4.29	1	0.04
Adult	0.67	0.33*	115.5	1	2.20E-16



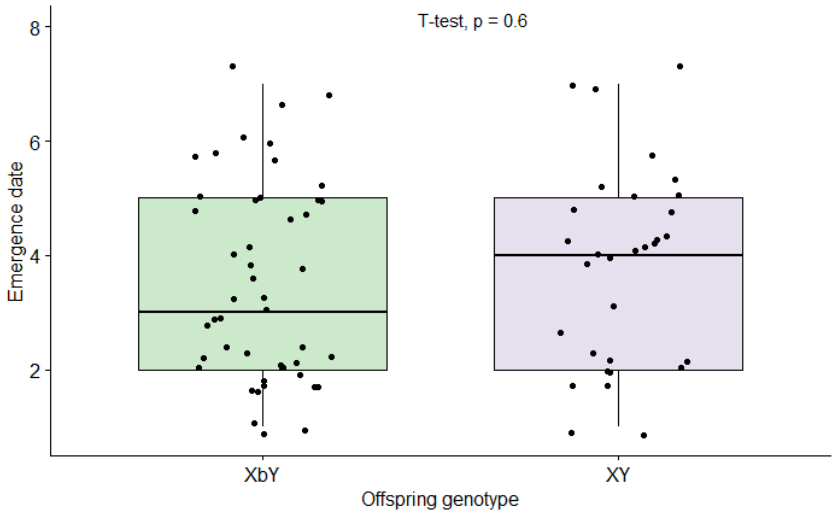
Supplemental Figure 1. Total number of eggs hatched from individual females that carry zero, one, or two copies of a selfish X chromosome. Females were mass mated to wildtype males, upon which they were allowed to oviposit individually in vials for 24 hrs, and then transferred to new vials three more times. After 96 hours, unhatched eggs and empty egg casings (hatched) were counted. Total hatched eggs were tallied for all females, regardless of whether they survived over 4 days of egg-laying. These were used as a proxy for lifetime fecundity (total offspring produced). Heterozygous driver females produced the most offspring, with this being statistically significant from the numbers produced by homozygous driver females (X^dX^d).



Supplemental Figure 2. Total number of eggs hatched from individual females that carry zero, one, or two copies of a selfish X chromosome. Females were mass mated to wildtype males, upon which they were allowed to oviposit individually in vials for 24 hrs, and then transferred to new vials three more times. After 96 hours, unhatched eggs and empty egg casings (hatched) were counted. Total hatched eggs were tallied for females that survived at least one round of egg-laying. These were used as a proxy for lifetime fecundity (total offspring produced). Heterozygous driver females produced the most offspring.



Supplemental Figure 3. X^bX and XX females have similar adult emergence time.



Supplemental Figure 4. XY and X^bY males have similar adult emergence time.

Bibliography

Ågren, J. A. and A. G. Clark. **2018**. Selfish genetic elements. *PLoS Genet.* **14**: e1007700.

Akera, T., L. Chmátal, E. Trimm, K. Yang, C. Aonbangkhen, D. M. Chenoweth, C. Janke, R. M. Schultz and M. A. Lampson. **2017**. Spindle asymmetry drives non-mendelian chromosome segregation. *Science* **358**: 668–672.

Akhverdyan, M. and K. Fredga. **2001**. EM studies of female meiosis in wood lemmings with different sex chromosome constitutions. *J. Exp. Zool.* **290**: 504–516.

Angélica, M., B. Núñez, N. L. Nuckolls and S. E. Zanders. **2018**. Genetic villains: killer meiotic drivers. *Trends Genet.* **34**: 424–433.

Bachtrog, D. 2020. The Y chromosome as a battleground for intragenomic conflict. *Trends Genet.* **36**: 510–522.

Baker, B. S. and A. T. C. Carpenter. **1972**. Genetic analysis of sex chromosomal meiotic mutants in *Drosophila melanogaster*. *Genetics* **71**: 255–286.

Bauer, H., S. Schindler, Y. Charron, J. Willert, B. Kusecek and B. G. Herrmann. **2012**. The nucleoside diphosphate kinase gene *Nme3* acts as quantitative trait locus promoting non-Mendelian inheritance. *PLoS Genet.* **8**: e1002567.

Bauer, H., N. Véron, J. Willert and B. G. Herrmann. **2007**. The *t*-complex-encoded guanine nucleotide exchange factor *Fgd2* reveals that two opposing signaling pathways promote transmission ratio distortion in the mouse. *Genes Dev.* **21**: 143–147.

Bauer, H., J. Willert, B. Koschorz and B. G. Herrmann. **2005**. The t complex–encoded GTPase-activating protein Tagap1 acts as a transmission ratio distorter in mice. *Nat. Genet.* **37**: 969–973.

Beck, E. A. and A. Llopart. **2015**. Widespread positive selection drives differentiation of centromeric proteins in the *Drosophila melanogaster* subgroup. *Sci. Rep.* **5**: 17197.

Beckenbach, A. T. 1996. Selection and the "*Sex-Ratio*" polymorphism in natural populations of *Drosophila pseudoobscura*. *Evolution* **50**: 787–794.

Beeman, R. W., K. S. Friesen and R. E. Denell. **1992**. Maternal-effect selfish genes in flour beetles. *Science* **256**: 89–93.

Bracewell, R., K. Chatla, M. J. Nalley and D. Bachtrog. **2019**. Dynamic turnover of centromeres drives karyotype evolution in *Drosophila*. *eLife* **8**: e49002.

Branco, A. T., Y. Tao, D. L. Hartl and B. Lemos. **2013**. Natural variation of the Y chromosome suppresses sex ratio distortion and modulates testis-specific gene expression in *Drosophila simulans*. *Heredity* **111**: 8–15.

Brown, J. D. and R. J. O'Neill. **2010**. Chromosomes, conflict, and epigenetics: chromosomal speciation revisited. *Annu. Rev. Genomics Hum. Genet.* **11**: 291–316.

Burt, A. and R. Trivers. **2006**. *Genes in conflict: the biology of selfish genetic elements*. The Belknap Press of Harvard University Press, United States of America.

Carvalho, A. and L. B. Klaczkoi. **1993**. Autosomal suppressors of *sex-ratio* in *Drosophila mediopunctata*. *Heredity.* **71**: 546–551.

Carvalho, A. and S. Vas. **1999**. Are *Drosophila SR* drive chromosomes always balanced? *Heredity.* **83**: 221–228.

Carvalho, B., S. C. Vaz and L. B. Klaczko. **1997**. Polymorphism for Y-linked suppressors of sex-ratio in two natural populations of *Drosophila mediopunctata*. *Genetics* **146**: 891–902.

Cazemajor, M., D. Joly and C. Montchamp-Moreau. **2000**. *Sex-ratio* meiotic drive in *Drosophila simulans* is related to equational nondisjunction of the Y chromosome. *Genetics* **154**: 229–236.

Cazemajor, M., C. Landre and C. Montchamp-Moreau. **1997**. The *Sex-Ratio* trait in *Drosophila simulans*: genetic analysis of distortion and suppression. *Genetics* **147**: 635–642.

Charlesworth, B. and D. Charlesworth. **2000**. The degeneration of Y chromosomes. *Philos. Trans. R. Soc. B Biol. Sci.* **355**: 1563–1572.

Chesley, P. and L. C. Dunn. **1936**. The inheritance of taillessness (anury) in the house mouse. *Genetics* **21**: 525–536.

Chmátal, L., R. M. Schultz, B. E. Black and M. A. Lampson. **2017**. Cell biology of cheating-transmission of centromeres and other selfish elements through asymmetric meiosis. In: *Centromeres and Kinetochores: Discovering the Molecular Mechanisms Underlying Chromosome Inheritance* (B. E. Black, ed), pp. 377–396. Springer International Publishing, Cham.

Chmátal, L., K. Yang, R. M. Schultz and M. A. Lampson. **2015**. Spatial regulation of kinetochore microtubule attachments by destabilization at spindle poles in meiosis I. *Curr. Biol.* **25**: 1835–1841.

Clark, A. G. 1987. Natural selection and Y-linked polymorphism. *Genetics* **115**: 569–577.

Courret, C., C.-H. Chang, K. H.-C. Wei, C. Montchamp-Moreau and A. M. Larracuente. **2019**. Meiotic drive mechanisms: lessons from *Drosophila*. *Proc. R. Soc. B Biol. Sci.* **286**: 20191430.

Courret, C., P. R. Gérard, D. Ogereau, M. Falque and C. Montchamp-Moreau. **2019**. X-chromosome meiotic drive in *Drosophila simulans*: a QTL approach reveals the complex polygenic determinism of *Paris* drive suppression. *Heredity (Edinb).* **122**: 906–915.

Crespi, B. and P. Nosil. **2013**. Conflictual speciation: species formation via genomic conflict. *Trends Ecol. Evol.* **28**: 48–57.

De Villena, F. P. M. and C. Sapienza. **2001**. Female meiosis drives karyotypic evolution in mammals. *Genetics* **159**: 1179–1189.

Dyer, K. A. 2011. Local selection underlies the geographic distribution of *sex-ratio* drive in *Drosophila neotestacea*. *Evolution.* **66**: 973–984.

Dyer, K. A., B. Charlesworth and J. Jaenike. **2007**. Chromosome-wide linkage disequilibrium as a consequence of meiotic drive. *Proc. Natl. Acad. Sci.* **104**: 1587–1592.

Dyer, K. A. and D. W. Hall. **2019**. Fitness consequences of a non-recombining sex-ratio drive chromosome can explain its prevalence in the wild. *Proc. R. Soc. B Biol. Sci.* **286**: 20192529.

Eickbush, M. T., J. M. Young and S. E. Zanders. **2019**. Killer meiotic drive and dynamic evolution of the *wtf* gene family. *Mol. Biol. Evol.* **36**: 1201–1214.

Finnegan, S. R., N. J. White, D. Koh, M. F. Camus, K. Fowler, A. Pomiankowski, A. Pomiankowski, A. Lindholm and T. Price. **2019**. Meiotic drive reduces egg-to-adult viability in stalk-eyed flies. *Proc. R. Soc. B Biol. Sci.* **286**: 20191414.

Fishman, L. and A. Saunders. **2008**. Centromere-associated female meiotic drive entails male fitness costs in monkeyflowers. *Science.* **332**: 1559–1562.

Fouvry, L., D. Ogereau, A. Berger, F. Gavory and C. Montchamp-Moreau. **2011**. Sequence analysis of the segmental duplication responsible for *Paris Sex-Ratio* drive in *Drosophila simulans*. *G3* **1**: 401–410.

Fredga, K. and M. G. Bulmer. **1988**. Aberrant chromosomal sex-determining mechanisms in mammals, with special reference to species with XY females [and discussion]. *Philos. Trans. R. Soc. B Biol. Sci.* **322**: 83–95.

Fredga, K., A. Gropp, H. Winking and F. Frank. **1976**. Fertile XX- and XY-type females in the wood lemming *Myopus schisticolor*. *Nature* **261**: 225–227.

Gershenson, S. 1928. A new sex-ratio anormality in *Drosophila obscura*. *Genetics* **13**: 488–507.

Gileva, E. A. 1975. Chromosomal diversity and an aberrant genetic system of sex determination in the arctic lemming, *Dicrostonyx torquatus* pallas (1779). *Genetica* **52/53**: 99–103.

Gileva, E. A. 1987. Meiotic drive in the sex chromosome system of the varying lemming, *Dicrostonyx torquatus* Pall. (Rodentia, Microtinae). *Heredity* **59**: 383–389.

Gileva, E. A., I. E. Benenson, L. A. Konopistseva, V. F. Puchkov and I. A. Makaranets. **1982**. XO females in the varying lemming, *Dicrostonyx torquatus*: reproductive performance and its evolutionary significance. *Evolution* **36**: 601–609.

Gonzalez-Candelas, F., J. L. Mensua and A. Moya. **1990**. Larval competition in *Drosophila melanogaster*: effects on development time. *Genetica* **82**: 33–44.

Hamilton, W. D. 1967. Extraordinary sex ratios. *Science* **156**: 477–488.

Helleu, Q., C. Courret, D. Ogereau, K. L. Burnham, N. Chaminade, M. Chakir, S. Aulard and C. Montchamp-Moreau. **2019**. *Sex-Ratio* meiotic drive shapes the evolution of the Y chromosome in *Drosophila simulans*. *Mol. Biol. Evol.* **36**: 2668–2681.

Helleu, Q., P. R. Gérard, R. Dubruille, D. Ogereau, B. Prud and B. Loppin. **2016**. Rapid evolution of a Y-chromosome heterochromatin protein underlies sex chromosome meiotic drive. *Proc. Natl. Acad. Sci.* **113**: 4110–4115.

Helleu, Q., P. R. Gerard and C. Montchamp-moreau. **2015**. Sex chromosome drive. *Cold Spring Harb. Perspect. Biol.* **7**: a017616.

Henikoff, S., K. Ahmad and H. S. Malik. **2001**. The centromere paradox: stable inheritance with rapidly evolving DNA. *Science* **293**: 1098–1102.

Herrmann, B. G. and H. Bauer. **2012**. The mouse *t*-haplotype: a selfish chromosome-genetics, molecular mechanism, and evolution. In: *Evolution of the House Mouse* (M. Macholán, S. Baird, P. Munclinger, and J. Piálek, eds), pp. 297–314. Cambridge: Cambridge University Press.

Herrmann, B. G., B. Koschorz, K. Wertz, K. J. Mclaughlin and A. Kispert. **1999**. A protein kinase encoded by the t complex responder gene causes non-mendelian inheritance. *Nature* **402**: 141–146.

Holman, L., T. A. R. Price, N. Wedell and H. Kokko. **2015**. Coevolutionary dynamics of polyandry and sex-linked meiotic drive. *Evolution* **69**: 709–720.

Horváth, B. and A. T. Kalinka. **2016**. Effects of larval crowding on quantitative variation for development time and viability in *Drosophila melanogaster*. *Ecol. Evol.* **6**: 8460–8473.

Hu, W., Z.-D. Jiang, F. Suo, J.-X. Zheng, W.-Z. He and L. Du. **2017**. A large gene family in fission yeast encodes spore killers that subvert Mendel's law. *eLife.* **6**: e26057.

Hurst, G. D. D. and J. H. Werren. **2001**. The role of selfish genetic elements in eukaryotic evolution. *Nat. Rev. Genet.* **2**: 597–606.

Iwata-Otsubo, A., J. M. Dawicki-Mckenna, T. Akera, S. J. Falk, L. Chmátal, K. Yang, B. A. Sullivan, R. M. Schultz, M. A. Lampson and B. E. Black. **2017**. Expanded satellite repeats amplify a discrete CENP-A nucleosome assembly site on chromosomes that drive in female meiosis. *Curr. Biol.* **27**: 2365–2373.

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. Syst.* **32**: 25–49.

John, A., K. Vinayan and J. Varghese. **2016**. Achiasmy: male fruit flies are not ready to mix. *Front. Cell Dev. Biol.* **4**: 75.

Joshi, A., W.-P. Wu and L. D. Mueller. **1998**. Density-dependent natural selection in *Drosophila*: adaptation to adult crowding. *Evol. Ecol.* **12**: 363–376.

Keais, G. L., M. A. Hanson, B. E. Gowen and S. J. Perlman. **2017**. X chromosome drive in a widespread Palearctic woodland fly, *Drosophila testacea*. *J. Evol. Biol.* **30**: 1185–1194.

Keais, G. L., S. Lu and S. J. Perlman. **2020**. Autosomal suppression and fitness costs of an old driving X chromosome in *Drosophila testacea*. *J. Evol. Biol.* **33**: 619–628.

Knief, U., H. Schielzeth, H. Ellegren, B. Kempenaers and W. Forstmeier. **2015**. A prezygotic transmission distorter acting equally in female and male zebra finches *Taeniopygia guttata*. *Mol. Ecol.* **24**: 3846–3859.

Kovacevic, M. and S. W. Schaeffer. **2000**. Molecular population genetics of X-linked genes in *Drosophila pseudoobscura*. *Genetics* **156**: 155–172.

Kruger, A. N. and J. L. Mueller. **2021**. Mechanisms of meiotic drive in symmetric and asymmetric meiosis. *Cell. Mol. Life Sci.* 10.1007/s00018-020-03735–0.

Kursel, L. E. and H. S. Malik. **2017**. Recurrent gene duplication leads to diverse repertoires of centromeric histones in *Drosophila* species. *Mol. Biol. Evol.* **34**: 1445–1462.

Kursel, L. E. and H. S. Malik. **2018**. The cellular mechanisms and consequences of centromere drive. *Curr. Opin. Cell Biol.* **52**: 58–65.

Kusano, A., C. Staber and B. Ganetzky. **2001**. Nuclear mislocalization of enzymatically active RanGAP causes segregation distortion in *Drosophil*a. *Dev. Cell* **1**: 351–361.

Larner, W., T. Price, L. Holman and N. Wedell. **2019**. An X-linked meiotic drive allele has strong, recessive fitness costs in female *Drosophila pseudoobscura*. *Proc. R. Soc. B Biol. Sci.* **286**: 20192038.

Larracuente, A. M. and D. C. Presgraves. **2012**. The selfish *Segregation Distorter* gene complex of *Drosophila melanogaster*. *Genetics* **192**: 33–53.

Lin, C. J., F. Hu, R. Dubruille, J. Vedanayagam, J. Wen, P. Smibert, B. Loppin and E. C. Lai. **2018**. The hpRNA/RNAi Pathway Is Essential to Resolve Intragenomic Conflict in the *Drosophila Male* Germline. *Dev. Cell* **46**: 316-326.

Lindholm, A. K., K. A. Dyer, R. C. Firman, L. Fishman, W. Forstmeier, L. Holman, H. Johannesson, U. Knief, H. Kokko, A. M. Larracuente, *et al.* 2016. The ecology and evolutionary dynamics of meiotic drive. *Trends Ecol. Evol.* **31**: 315–326.

Mackintosh, C., A. Pomiankowski and M. F. Scott. **2021**. X-linked meiotic drive can boost population size and persistence. *Genetics* **217**: iyaa018.

Malik, H. S. and S. Henikoff. **2001**. Adaptive evolution of *Cid*, a centromere-specific histone in *Drosophila*. *Genetics* **157**: 1293–1298.

Malik, H. S. and S. Henikoff. **2009**. Major evolutionary transitions in centromere complexity. *Cell* **138**: 1067–1082.

Mckee, B. D., K. Wilhelm, C. Merrill and X. Ren. **1998**. Male sterility and meiotic drive associated with sex chromosome rearrangements in *Drosophila*: role of X-Y pairing. *Genetics* **149**: 143–155.

McKee, B. and D. L. Lindsley. **1987**. Inseparability of X-heterochromatic functions responsible for X:Y pairing, meiotic drive, and male fertility in *Drosophila melanogaster*. *Genetics* **116**: 399–407.

Merrill, C., L. Bayraktaroglu, A. Kusano and B. Ganetzky. **1999**. Truncated RanGAP encoded by the *segregation distorter* locus of *Drosophila*. *Science.* **283**: 1742–1745.

Montchamp-Moreau, C., V. Ginhoux and A. Atlan. **2001**. The Y chromosomes of *Drosophila simulans* are highly polymorphic for their ability to suppress sex-ratio drive. *Evolution* **55**: 728–737.

Montchamp-Moreau, C., D. Ogereau, N. Chaminade, A. Colard and S. Aulard. **2006**. Organization of the sex-ratio meiotic drive region in *Drosophila simulans*. *Genetics* **174**: 1365–1371.

Newton, M. E., R. J. Wood and D. I. Southern. **1976**. A cytogenetic analysis of meiotic drive in the mosquito, *Aedes aegypti* (L.). *Genetica* **46**: 297–318.

Nuckolls, N. L., M. A. B. Nuñez, M. T. Eickbush, J. M. Young, J. J. Lange, J. S. Yu, G. R. Smith, S. L. Jaspersen, H. S. Malik and S. E. Zanders. **2017**. *wtf* genes are prolific dual poison-antidote meiotic drivers. *eLife* **6*:*** e26033.

Nuñez, M. A. B., S. I. M, M. T. Eickbush, Y. Liang, J. J. Lange, A. M. Kent and S. E. Zanders. **2020**. Dramatically diverse *Schizosaccharomyces pombe wtf* meiotic drivers all display high gamete-killing efficiency. *PLoS Genet.* **16**: e1008350.

Pardo-Manuel De Villena, F. and C. Sapienza. **2001**. Nonrandom segregation during meiosis: the unfairness of females. *Mamm. Genome* **12**: 331–339.

Peacock, W. J. 1965. Nonrandom segregation of chromosomes in *Drosophila* males. *Genetics* **51**: 573–583.

Peacock, W. J., G. L. G. Miklos and D. J. Goodchild. **1975**. Sex chromosome meiotic drive systems in *Drosophila melanogaster* I. Abnormal spermatid development in males with a heterochromatin-deficient X chromosome (sc^4sc^8). *Genetics* **79**: 613–634.

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

Pieper, K. E., R. L. Unckless and K. A. Dyer. **2018**. A fast-evolving X-linked duplicate of *importin-α2* is overexpressed in sex-ratio drive in *Drosophila neotestacea*. *Mol. Ecol.* **27**: 5165–5179.

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

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

Powers, P. A. and B. Ganetzky. **1991**. On the components of *Segregation Distortion* in *Drosophila melanogaster*. V. Molecular analysis of the Sd locus. *Genetics* **129**: 133–144.

Price, T. A. R., R. Verspoor and N. Wedell. **2019**. Ancient gene drives : an evolutionary paradox. *Proc. R. Soc. B Biol. Sci.* **286**: 20192267.

Price, T. A. R. and N. Wedell. **2008**. Selfish genetic elements and sexual selection: their impact on male fertility. *Genetica* **132**: 295–307.

Rhoades, M. M. 1942. Preferential segregation in maize. *Genetics* **27**: 395–407.

Rosin, L. and B. G. Mellone. **2016**. Co-evolving CENP-A and CAL1 domains mediate centromeric CENP-A deposition across *Drosophila* species. *Dev. Cell* **37**: 136–147.

Sandler, L., Y. Hiraizumi and I. Sandler. **1958**. Meiotic drive in natural populations of *Drosophila melanogaster*. I. The cytogenetic basis of segregation-distortion. *Genetics* **44**: 233–250.

Shatskikh, A. S., A. A. Kotov, V. E. Adashev, S. S. Bazylev, L. V Olenina and A. M. Larracuente. **2020**. Functional significance of satellite DNAs: insights from *Drosophila*. *Front. Cell Dev. Biol.* **8**: 312.

Stewart, N. B., Y. H. Ahmed-Braimah, D. G. Cerne and F. Bryant. **2019**. Female meiotic drive preferentially segregates derived metacentric chromosomes in *Drosophila*. *bioRxiv*, doi: https://doi.org/10.1101/638684.

Sturtevant, A. H. and T. H. Dobzhansky. **1936**. Geographic distribution and cytology of "*sex-rati*o" in *Drosophila pseudoobscura* and related species. *Genetics* **21**: 473–490.

Sweeny, T. L. and A. R. Barr. **1978**. Sex ratio distortion caused by meiotic drive in a mosquito, *Culex pipiens* L. *Genetics* **88**: 427–446.

Tao, Y., L. Araripe, S. B. Kingan, Y. Ke, H. Xiao and D. L. Hartl. **2007**. A sex-ratio meiotic drive system in *Drosophila simulans.* II : an X-linked distorter. *PLoS Biol.* **5**: e293.

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

Temin, R. G., B. Ganetzky, P. A. Powers, T. W. Lyttle, S. Pimpinelli, P. Dimitri, C.-I. Wu and Y. Hiraizumi. **1991**. Segregation distortion in *Drosophila melanogaster*: genetic and molecular analyses. *Am. Nat.* **137**: 287–331.

Thomson, G. J. 1975. Population genetics of modifiers of meiotic drive:* IV. On the evolution of *Sex-Ratio* distortion. *Theor. Popul. Biol.* **8**: 202–211.

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

Wallace, B. 1948. Studies on "*sex-ratio*" in *Drosophila pseudoobscura*. I. Selection and "sex-ratio." *Evolution* **2**: 189–217.

Wedell, N. 2013. The dynamic relationship between polyandry and selfish genetic elements. *Philos. Trans. R. Soc. B Biol. Sci.* **368**: 20120049.

Werren, J. H. 2011. Selfish genetic elements, genetic conflict, and evolutionary innovation. *Proc. Natl. Acad. Sci.* **108**: 10863–10870.

Winking, H., A. Gropp and K. Fredga. **1981**. Sex determination and phenotype in wood lemmings with XXY and related karyotypic anomalies. *Hum. Genet.* **58**: 98–104.

Wood, R. J. 1991. Sex-ratio distortion caused by meiotic drive in mosquitoes. *Am. Nat.* **137**: 379–391.

Wu, C.-I. 1983. The fate of autosomal modifiers of the *Sex-Ratio* trait in *Drosophila* and other sex-linked meiotic drive systems. *Theor. Popul. Biol.* **24**: 107–120.

Yoshida, K. and J. Kitano. **2012**. The contribution of female meiotic drive to the evolution of neo-sex chromosomes. *Evolution* **66**: 3198–3208.

Zanders, S. E., M. T. Eickbush, J. S. Yu, J.-W. Kang, K. R. Fowler, G. R. Smith and H. S. Malik. **2014**. Genome rearrangements and pervasive meiotic drive cause hybrid infertility in fission yeast. *eLife* **3**: e02630.

Zedek, F. and P. Bureš. **2016**. CenH3 evolution reflects meiotic symmetry as predicted by the centromere drive model. *Sci. Rep.* **6**: 1–6.