The effect of *sex-ratio* meiotic drive on sex, survival, and size in the Malaysian stalk-eyed fly, *Teleopsis dalmanni*

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I, Sam Ronan Finnegan, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.
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Abstract

Meiotic drive is a type of selfish genetic element that, in heterozygous males, disables or destroys non-carrier sperm in order to bias its own transmission. Meiotic drive genes are predicted to spread rapidly due to this transmission advantage and even, if sex-linked, cause population extinction due to the loss of one sex. Despite this, many meiotic drive genes are found at low/moderate, stable frequencies, which implies their carriers must bear unknown costs that balance this transmission advantage. Such costs may come about as direct or pleiotropic effects of the meiotic drive gene(s) themselves, or because drive is often associated with inversions that are expected to accumulate deleterious mutations. The theme of the thesis has been to search for costs of meiotic drive in the Malaysian stalk-eyed fly, *Teleopsis dalmanni*. First, I assess whether the expected low quality of meiotic drive males has impacted the strength of their mate preference for high quality females. I find that male mate preference does not depend on drive status but does depend on eyespan. Male eyespan is a sexually-selected ornamental trait and drive males typically have small eyespan. I also show that drive males are unable to mate as frequently as non-drive males. Second, I study the effect of meiotic drive on the egg-to-adult viability of stalk-eyed flies. I show that drive reduces the viability of both sexes, with drive males and homozygous drive females showing the greatest loss of
viability compared to their non-drive counterparts. Third, I study the effect of larval food stress on the drive-associated reduction in male eyespan. I find that drive males and females have reduced eyespan, but the magnitude of this reduction does not increase under high stress. This implies that the small eyespan of drive males is unlikely to be a condition-dependent effect of the expected increased mutation load. I discuss how small eyespan may instead be part of a suite of adaptations to maintain high fertility in the face of the destruction of half of all sperm. Finally, I use experimental evolution to track the frequency of meiotic drive in cage populations and find considerable heterogeneity. While one cage is driven extinct due to the loss of males, drive frequency generally declines in other populations, disappearing entirely from some after six generations.
Impact statement

Populations of many species carry meiotic drive – selfish genetic elements that bias their own transmission by killing or disabling sperm carrying the alternative chromosome. Due to this transmission advantage, meiotic drive elements are expected to spread rapidly. It has long been recognised that the rapid transmission of drive elements could have large impacts on populations that carry them. In the case of sex-linked meiotic drive, strongly biased transmission of sex chromosomes has the potential to cause population extinction due to a lack of one of the sexes. Despite this, many meiotic drive systems are found at a low/moderate, stable frequency. This represents somewhat of an evolutionary puzzle. In this thesis, I make contributions towards understanding this puzzle. In chapter 2, I test for the first time whether meiotic drive affects the mating preferences of carrier males. This work was published in Behavioral Ecology this year and was presented at the 16th congress of the European Society of Evolutionary Biology in Groningen in 2017. In chapter 3, I show that male and female Teleopsis dalmannii stalk-eyed flies carrying meiotic drive have reduced egg-to-adult viability. Viability selection acting against drive carriers must partially explain why meiotic drive in this species does not spread. This work was published in Proceedings of the Royal Society B: Biological Sciences in 2019. Through results obtained in chapter 4, and building on previous work, I suggest that the reduced size of a sexual ornament in males carrying meiotic drive may represent part of a suite of adaptations to compensate for the loss
of sperm caused by meiotic drive. This may hopefully encourage future researchers to consider compensatory adaptations in drive systems. In chapter 5, I established a number of experimental evolution lines in order to track the frequency of meiotic drive in the laboratory. Future work on these lines may provide further insights into why drive does not spread in this species. The work in chapter 5 was motivated by observations made on flies collected during a field sampling trip carried out during the course of this PhD. Through this field work I also collected *T. whitei*, another species of stalk-eyed fly that is closely related to my study species and also carries meiotic drive. This enabled me to establish a laboratory stock of this species that can be used by future members of UCL stalk-eyed fly research group to understand how meiotic drive has evolved in these species.

More broadly, the work presented in this thesis may have relevance to the population control of vectors of human disease. In recent years there has been considerable interest in the use of artificial gene drives as a means of causing the collapse of populations of insect vectors. In many cases these artificial gene drives are based on or inspired by sperm-killer meiotic drive systems. The parallels between artificial and naturally occurring meiotic drive systems make it likely that the development of synthetic gene drives may be usefully informed by efforts made here to understand why many natural meiotic drive systems do not spread.
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Chapter 1

General introduction
1.1 Meiotic drive

Meiotic drive, also called transmission ratio distortion or segregation distortion, refers to the subversion of fair Mendelian segregation by a selfish genetic element so that this element is transmitted to more than half of the gametes (Sandler and Novitski 1957). While the term meiotic drive was originally coined to refer to the preferential movement into the egg and away from the polar bodies at female meiosis (Sandler and Novitski 1957), it is now used more generally (Burt and Trivers 2006). Indeed, the most intensively studied group of meiotic drivers are the so-called “sperm-killers”. These are elements that, in a heterozygous male, kill or otherwise disable sperm carrying the homologous chromosome to ensure that the chromosome on which they reside is preferentially transmitted, at up to 100% frequency (Lindholm et al. 2016). This phenomenon also exists in other kingdoms, such as the spore-killers in fungi (Turner and Perkins 1979) and the pollen-killers in plants (Cameron and Moav 1956; Taylor 1994; Yang et al. 2012), but here I focus on sperm-killers in animals. Examples of such meiotic drive systems have been found on the autosomes (Sandler et al, 1959; Silver 1985), and both the X (Dermitzakis et al. 2000; Tao et al. 2001; Merçot et al. 1995; Presgraves et al. 1997) and Y (Wood and Newton, 1991) sex chromosomes in a variety of species (Jaenike 2001). Sex chromosome meiotic drive appears to be more common than autosomal drive. This may reflect observational bias, as sex chromosome meiotic drive is more easily detectable due to the production of extremely female- or male-biased broods.
through the destruction of Y- or X-bearing sperm respectively. Autosomal drive is likely to go unnoticed unless the drive genes are linked to a conspicuous marker, as is the case in the \( t \)-haplotype in mice (Silver, 1993). Alternatively, sex chromosome meiotic drive may evolve more easily than autosomal drive (Frank 1991; Hurst and Pomiankowski 1991; Lyttle 1991). Meiotic drive systems typically require the action of at least two loci. For example, in the autosomal segregation distorter (\( SD \)) system in \textit{Drosophila melanogaster}, the driving \( SD \) locus targets the \textit{Responder} (\( Rsp \)) locus on the homologous chromosome, a region of satellite DNA consisting of repeats of a 240bp motif. The drive haplotype itself carries a \( Rsp \) variant with fewer repeats that is insensitive to the action of \( SD \) (Larracuente and Presgraves 2012). Tight linkage is required between drive and insensitive target loci to prevent the production of suicidal chromosomes through recombination. As such, drivers are often found in regions of low recombination such as in inversions or near the centromere (Hammer et al. 1989; Johns et al. 2005; Dyer et al. 2007; Larracuente and Presgraves 2012). In the case of sex chromosome drive, there is already no recombination between heteromorphic sex chromosomes in the heterogametic sex so there is no risk of the sensitive target site moving onto the drive chromosome. This is likely to explain the preponderance of sex chromosome drive (Frank 1991; Hurst and Pomiankowski 1991; Lyttle 1991). However, X-linked meiotic drivers can undergo recombination in females. Many X-linked drivers are associated with inversions (Wallace 1948; Stalker 1961; Johns et al. 2005; Dyer et al. 2007; Pieper and Dyer. 2016; but see Cazemajor et al. 1997). This association
appears to have arisen to maintain linkage between the drive gene(s) and other loci that modify or enhance drive (Wu and Beckenbach 1983). X-chromosome drive is considerably more common than Y-chromosome drive, which has been detected only in *Aedes* and *Culex* mosquitoes (Wood and Newton 1991). The rarity of Y-chromosome drive may be because driving Y-chromosomes are always transmitted from male to male and are therefore expressed every generation, in contrast to X-chromosome drive, where driving X chromosomes are found in males only in every other generation. This means that Y-chromosome drive is expected to spread more quickly and is associated with a higher extinction risk (Hamilton 1967). Alternatively, Y-chromosome drive may be less likely to evolve as, in species with well-differentiated sex chromosomes, the Y usually has comparatively few genes (Helleu et al. 2015). Similarly, the Y chromosome may offer more possible target sites than does the X chromosome, as target loci in drive systems are often repetitive sequences (Jaenike 2001). In mosquitoes, the X and Y chromosomes are morphological similar and the Y has not degenerated (Newton et al. 1974), lending support to these ideas.

Meiotic drive may be a potent force shaping the evolution of genomes. This is particularly true of sex-linked meiotic drivers. The selfish action of drive genes is expected to cause considerable conflict over meiotic transmission rates. Most obviously, the Y chromosome is under strong selection to evolve resistance to the action of an X-linked driver locus. However, as X-linked drivers produce strongly female-biased broods, they also generate conflict
with the rest of the genome over the population sex ratio (Fisher, 1930).
Once suppressed, a driver may evolve neutrally and begin to decay, as may be the case for the cryptic (i.e. completely suppressed) Winters drive system in *Drosophila simulans* (Kigan et al. 2010). Alternatively, enhancers of drive may evolve to escape suppression and restore the drive phenotype. Indeed, many drive systems are associated with multiple enhancers and suppressors (Jaenike 2001; Lindholm et al. 2016). It has been suggested that this recurrent conflict over the transmission of sex chromosomes has contributed to fundamental evolutionary patterns and processes, such as the genetic silencing of sex chromosomes in meiosis and the finding that genes that contribute to hybrid sterility are often found on the X (Hurst and Pomiankowski 1991; Meiklejohn and Tao 2010; Rice 2013).

The transmission ratio advantage of meiotic drivers is expected to have large, potentially rapid, impacts on populations carrying them, broadly leading to three possible fates. First, a drive haplotype may spread rapidly and fix. Once fixed, there are no longer sensitive haplotypes to drive against. In the case of autosomal drivers, this may have no obvious, observable phenotype. However, the spread of a sex-linked meiotic drive gene would have drastic effects on the population sex ratio and may lead to extinction due to the loss of one of the sexes (Hamilton, 1967). This has been demonstrated in an elegant experiment by Lyttle (1979), who translocated the *Drosophila melanogaster* autosomal driver *SD* onto the Y chromosome, causing extinction of laboratory populations within 7 generations. Evidence
for meiotic drive causing extinction in wild populations is, however, limited to
one putative anecdotal example (Pinzone and Dyer 2013). Of course, this
may be due to the inherent difficulties of studying population extinction –
extinct populations leave behind no trace (Lindholm et al. 2016). It is
therefore difficult to determine just how common this phenomenon may be.

A second possible fate of meiotic drive systems is complete suppression of
drive. As stated above, conflict over the transmission ratio strongly selects for
genes on the target chromosome or elsewhere in the genome to suppress
the action of meiotic drive. Indeed, several meiotic drive systems have been
serendipitously discovered in inter-specific or inter-population crosses, where
meiotic drivers have been crossed into a novel genetic background lacking
suppressors that are fixed in their native background (Dermitzakis et al.
2000; Tao et al. 2001; Orr and Ivring, 2005). The spread of drive and
suppressor genes can be remarkably rapid, as evidenced in the X-linked
Paris sex-ratio (SR) drive system, one of three known drive systems in
Drosophila simulans (Bastide et al. 2011; Bastide et al. 2013). The Paris
driver has undergone huge shifts in frequency in this cosmopolitan species
as it has spread throughout Africa, the Indian Ocean, and the Middle East in
the last ~25 years. For example, in Israel, Paris SR rose from <5% frequency
in 2000 to >70% in 2006, and in Egypt, Paris increased from 6% frequency in
2007 to >60% in 2012 (Bastide et al. 2013). This spread of SR was shortly
followed by a similar increase in suppression frequency over a similar and
remarkably short timescale (Bastide et al. 2013). In contrast, drive frequency
in Kenya, Madagascar, and Mayotte – the presumed ancestral origin of *D. simulans* (Kopp et al. 2006; Schöfl and Schlötterer 2006) – is now in decline following complete suppression (Bastide et al. 2011).

Perhaps the least well understood fate of drive systems is the one studied in the most detail: a balanced polymorphism. Despite the strong transmission advantage, many drivers are found at low to moderate frequencies (Ardlie and Silver 1996; Huang et al. 2001; Wilkinson et al. 2003; Dyer 2012; Price et al. 2014). Some systems, such as the X-linked sex-ratio in *Drosophila pseudoobscura* are believed to be of the order of millions of years old (Silver 1993; Babcock and Anderson 1996; Kovacevic and Schaeffer 2000). Repeated sampling efforts across decades suggest that the frequency of drive in these systems is stable over wide geographic areas and relatively long timescales, even in systems that lack genetic suppressors of drive (Dyer 2012; Price et al. 2014). These low frequencies suggest that there must be additional costs to carrying meiotic drive that offset the large transmission advantage. The search for these negative fitness consequences is a common goal in meiotic drive research and a central theme of this thesis.

### 1.2 The costs of meiotic drive

The costs of drive may come about due to pleiotropic effects of the drive gene(s) itself, or due to the action of deleterious alleles that have accumulated in drive-associated inversions. As meiotic drivers cause the
destruction or disablement of up to half of all sperm, it is intuitive to imagine that this may have a significant impact on the fertility of carrier males. Additionally, many drive systems are associated with large chromosomal inversions (Jaenike 2001; Lindholm et al. 2016). Such large inversions constitute a large mutational target and the lack of recombination between inverted and non-inverted chromosomes prevents the removal of deleterious mutations by recombination (Navarro et al. 1997). At low drive frequency, recessive mutations accumulate as the effective population size is low and selection weak (Kirkpatrick 2010). The accumulation of deleterious mutations is expected to lead to a general reduction in genetic quality, as mutations could feasibly impact on any trait influenced by factors within the inverted region, which may extend across large portions of the chromosome (Dobzhansky 1939; Silver et al. 1993; Johns et al. 2005), or even its entire length (Dyer et al. 2007). Here, I detail the known costs in a number of prominent sperm-killer drive systems, focussing on deficits to fertility, sperm competitive ability when females mate multiply, viability, female fecundity, and attractiveness to mates.

1.2.1 Fertility

Reduced fertility of drive males under non-competitive conditions has been reported in *Drosophila melanogaster* (Hartl et al. 1976), *D. pseudoobscura* (Beckenbach 1978; Wu 1983a,b), *D. subobscura* (Hauschteck-Jungen 1983), *D. quinara* (Jaenike 1996), *D. recens* (Jaenike, 1996), *D. simulans* (Atlan et
al. 2004; Angelard et al. 2008), *T. dalmanni* (Wilkinson et al. 2006; but see Meade et al. 2020), *Mus musculus* (Manser et al. 2011), and *D. affinis* (Unckless et al. 2015). However, the magnitude of these reductions in fertility vary considerably by species and appear to be influenced by experimental conditions. For example, when *D. simulans* males carrying Paris drive are housed continuously with one or two females, they do not produce fewer offspring over their lifetime than standard males kept under the same conditions (Mercot et al. 1995; Atlan et al. 2004). However, when males and females are separated after a single mating, the lifetime reproductive output of females mated to drive males is lower than that of females mated singly to standard males (Atlan et al. 2004), suggesting that *D. simulans* drive males transfer fewer sperm per mating than standard males, as do *D. pseudoobscura* (Policansky and Ellison 1970). In *D. pseudoobscura*, drive male fertility may depend on age (Beckenbach 1978) and temperature (Wu 1983a; Price et al. 2012a).

One finding that is consistent across species is that the fertility of drive males depends on the mating rate (Wu 1983b; Janike 1996; Atlan et al. 2004; Wilkinson et al. 2006; Pinzone and Dyer 2013). In *D. recens*, for example, drive males suffer slight reductions in fertility after a single mating, but their fertility falls to less than half that of standard males in subsequent matings (Jaenike 1996). In several species, drive and standard males do not differ in their fertility after a single mating (Jaenike 1996; Wu 1983a; Wilkinson et al. 2006) but as males perform successive copulations, drive male fertility
generally declines faster than standard male fertility. For example, in *D. pseudoobscura*, virgin drive and standard males who mate once a day do not differ in their fertility, but when males mate three times successively, drive males show a 35% reduction in fertility in their third mating (Wu 1983a). As drive and standard males have similar fertility in the first two matings, this means that the cumulative reduction in fertility across all three copulations is considerably less than 35% and further factors are therefore needed to explain why drive does not spread. In *D. simulans* and *D. neotestacea*, drive males suffer a reduction in fertility after a single mating, and drive male fertility declines to less than half that of standard males when tasked with mating with multiple females over a short timescale (Atlan et al. 2004; Pinzone and Dyer et al. 2013). Both these studies used a design where males who mated successfully with a single female were subsequently kept together with 10 females for 24 hours. These 10 females were then separated and allowed to oviposit individually. This allowed an estimation of the number of females that males successfully mated with. In *D. simulans*, standard males mated with 8.4 females on average, while drive males mated with only 5.5 (Atlan et al. 2004). In *D. neotestacea*, the mating rate of both types of male was considerably lower. Standard males mated with an average of 2.6 females and drive males mated with 1.3. Interestingly, of the *D. neotestacea* females who do successfully produce offspring, the number of offspring sired by drive and standard males does not differ (Pinzone and Dyer 2013). This suggests that *D. neotestacea* drive males transfer similar
numbers of sperm per mating but cannot maintain the same mating rate as standard males.

What drives these differences in fertility when males mate multiply? One possibility is that males are simply becoming sperm depleted. While there is remarkable variation in sperm morphology and number across species (Immler et al. 2011), males typically produce sperm in excess of that required to fertilise all of a female’s eggs (Lefevre and Jonsson 1962; Wood and Newton 1991). For example, Lefevre and Jonsson (1962) showed that *D. melanogaster* females can only store about 20% of the sperm produced in a single ejaculate. Even with a 50% reduction in sperm number, a drive male may still produce sperm in excess of a female’s storage capacity, which likely explains why drive and standard males have similar fertility under single mating conditions in many species. However, as the mating rate increases, drive males may be less able to replenish sperm numbers and so their fertility declines faster than standard males.

An alternative explanation that has received comparatively little attention is that drive males may suffer deficits in the non-sperm component of the ejaculate. *D. melanogaster* males that have mated exhaustively have depleted accessory glands, the organs that produce the non-sperm components of the ejaculate, and no longer transfer sperm, even though significant numbers of fully motile sperm remain in the seminal vesicles (Lefevre and Jonsson 1962). The studies in *D. simulans* and *D. neotestacea*
above highlight the importance of mating rate in drive male fertility. In *D. melanogaster* and the stalk-eyed fly *Teleopsis dalmanni*, the mating rate is correlated with accessory gland size, not testis size (Bangham et al. 2002; Rogers et al. 2005). *T. dalmanni* drive males transfer the same number of sperm per ejaculate as standard males, and may have similar fertility to standard males, even at high mating rates (Meade et al. 2019; Meade et al. 2020 but see Wilkinson et al. 2006). Drive males are able to maintain high fertility despite the loss of half of all sperm as they have comparatively large testes. However, this increased investment in testes production is apparently traded off with investment in the accessory glands, which are reduced in drive males, who are unable to mate at the same frequency as standard males (Wilkinson et al. 2003; Finnegan et al. 2020; Meade et al. 2020).

Further studies should seek to determine the impact of meiotic drive on the accessory gland products in other species.

For deficits in fertility alone to restrict the spread of meiotic drive, drive male fertility must drop to *less than half* that of non-drive (standard) males (Jaenike 1996) in order to offset its transmission advantage. This is assuming females only mate with one male, and that male fertility is not affected by female behaviour, which may be unrealistic (Wedell 2013; Taylor et al. 2014). The effect of sperm competition and multiple mating in females on drive male fertility is discussed further down the page. If male fertility is linearly dependent on the number of available sperm, one might expect, *at most*, a 50% reduction in drive male fertility when drive is successful in killing
100% of sperm carrying the homologous chromosome. One reason for a
greater than two-fold decline in fertility is that drive males may not only
produce fewer sperm, but also sperm of lower quality (Angelard et al. 2008;
Sutter and Lindholm 2015). Such reduced quality may come about as a by-
product of the action of drive. For example, in the mouse t-haplotype, several
trans-acting loci impair flagellar function in all sperm by over-activating the
sperm motility kinase SMOK1, but motility is rescued in sperm that inherit the
t-haplotype through localised expression of a dominant-negative version of
this gene (Herrmann et al. 1999; Bravo Nuñez et al. 2018). If this rescue
mechanism is not completely fine-tuned, then t-haplotype bearing sperm may
still suffer some reduction in quality (Sutter and Lindholm 2015).

Alternatively, as males of many species produce sperm in considerable
excess, the dysfunction of up to half of all sperm may not lead to large
effective reductions in fertility, as has been reported for the Y-linked driver in

A caveat of the majority of the work detailed above is that male fertility is
typically estimated by counting the number of adult offspring produced by a
cross. This measure is a product of male fertility, female fecundity, and egg-
to-adult viability. The effect of female fecundity can be minimised by
standardisation of females used in crosses, but this measure may be
confounded if the offspring of drive and standard males have differential
viabilities. Drive is known to affect larval viability in D. pseudoobscura
(Wallace 1948; Curtsinger and Feldman 1980) and T. dallmanni (Finnegan et
In T. dalmanni, Wilkinson et al. (2006) found that drive males had reduced fertility under multiple mating when estimated through offspring counts. Meade et al. (2020), however, found no reduction in fertility under multiple mating when fertility was estimated through counts of the numbers of fertilised and unfertilised eggs laid by mated females, which controls for the effects of differential viabilities. While this difference could be due to the specific mating conditions of these two experiments (8 females for 24h in Wilkinson et al. (2006) versus 5 females for 10h in Meade et al. (2020)), it is also possible that the apparent fertility cost measured by Wilkinson et al. (2006) is slightly inflated by a greater proportion of offspring of drive males not surviving to adulthood. The effect of meiotic drive on viability is discussed in more detail further down the page.

In summary, it is intuitive to imagine that drive causes the loss of half of all sperm, and therefore drive males should transfer half the sperm of standard males per mating and suffer a loss of fertility. However, while it is true that drive males of some species do indeed transfer half the sperm of standard males (Policansky and Ellison 1970), drive males in other species do not (Meade et al. 2019). For reduced fertility alone to prevent the spread of drive drive male fertility would need to fall to less than half that of standard male males (Jaenike 1996). This condition is not met under single mating conditions for most species (but see Angelard et al. 2008) but may be met under multiple mating in D. simulans (Atlan et al. 2004), D. recens (Jaenike 1996), and D. neotestacea (Pinzone and Dyer 2013). For the reduced fertility
of drive males to be sufficient to prevent the spread of drive in these species, the mating rate in the wild would need to regularly be high enough for this cost to reveal itself. In several other species (*D. pseudoobscura, D. subobscura, M. musculus, T. dalmani*), the fertility of drive males does not fall below the necessary threshold even under multiple mating. Further costs are therefore needed to explain polymorphism in these species.

### 1.2.2 Sperm competition

Thus far I have discussed how drive males may suffer reduced fertility when they are the sole male that mates with a female. However, this may be unrepresentative of natural conditions as polyandry is widespread and common across diverse taxa (Taylor et al. 2014). To better understand the dynamics of meiotic drive in the wild, the fertility of drive males should therefore be considered when competing against sperm from other males. To understand how meiotic drive affects the sperm competitive ability of its carriers, some consideration should be given to how sperm competition affects paternity under normal conditions. If, for example, paternity is determined by a fair raffle, then paternity is expected to be proportional to the number of sperm a male delivers (Parker 1990). Drive males would therefore be expected to do poorly under sperm competitive conditions when they transfer fewer sperm per mating (Policansky and Ellison 1970; Atlan et al. 2004), but not when they do not (Meade et al. 2019). Alternatively, paternity may be strongly influenced by a male’s position to mate. For example, in
mice, the majority of offspring are sired by the male that is first to mate (Manser et al. 2017), while in *Drosophila*, the majority of offspring are typically sired by the male who mates last (Simmons 2001). The expected success of a male under these types of systems is considerably more complicated to predict (Parker and Pizzari 2010) but will depend, among other things, on his ability to dislodge stored sperm from males that have previously mated with a female and/or resist these attempts from subsequent males (Wigby and Chapman 2004). These traits are expected to be dependent on sperm quantity and quality (Wigby and Chapman 2004; Rowe and Pruett-Jones 2011). It is possible that the same mechanisms that make drive-bearing sperm good intra-ejaculate competitors (killing or disabling rival sperm) may make them bad at competing against the sperm from non-drive males (Manser et al. 2011; Bravo Nuñez et al. 2018).

For example, in the stalk-eyed fly, *Teleopsis dalmanni*, the paternity of offspring produced by multiply mated females is determined by a fair raffle model with sperm mixing (Corley et al. 2006). Under this model, and assuming drive males have half as many sperm as standard males, we would expect them to sire 33% of the offspring of a female mated to both types of male. Instead, the paternity share is closer to 25% (Wilkinson et al. 2006). Meade et al. (2020) recently showed that drive and standard males actually transfer similar numbers of sperm per ejaculate. As the fertilisation success of drive males is lower than expected based on the number of sperm transferred alone (Wilkinson and Fry 2001; Wilkinson et al. 2006), this
suggests that sperm quality is significantly reduced in this species. In the
*Drosophila* genus, there is typically second-male precedence, meaning that
when a female mates twice, it is the second male that typically sires the
majority of offspring (range: 0.5-0.9; Simmons 2001). The proportion of
offspring sired by the second male is commonly referred to as P2. In *D.
simulans*, Atlan et al. (2004) found that drive males who mate after a
standard male have a P2 of 0.51, while standard males who mate after a
drive male have a P2 of 0.90. The reduction in sperm competitive ability is
even greater in *D. pseudoobscura*. P2 in this species has been previously
estimated at around 0.8 in double matings with standard males (Cobbs 1977;
Turner and Anderson 1984). However, when a drive male mated after a
standard male, Price et al. (2008a) estimated P2 for drive males to be as low
as 0.14. In these species, the reduced P2 of drive males may be because
drive males transfer comparatively few sperm which are unable to dislodge
previously-stored sperm, or drive sperm may be of lower quality, or a
combination of both. Reduced sperm competitive ability has also been
inferred in mice (Manser et al. 2011). The fertility of drive males under
conditions of sperm competition has not been investigated in other species,
though reduced sperm competitive ability appears to be a general feature of
drive systems (Price and Wedell 2008).

1.2.3 Polyandry as a defence against the costs of drive
The weak sperm competitive ability of drive males provides an avenue for females to reduce the likelihood of their eggs being fertilised by drive sperm. By increasing their mating rate, females can foster sperm competition and therefore bias fertilisation success away from drive males. This behaviour is likely to be adaptive as drive is often associated with costs to organismal fitness (Jaenike 2001). Additionally, in the case of X-linked meiotic drive, the cost of mating with a drive male increases as drive spreads and the population sex ratio becomes female-biased. This is because copulations with drive males produce few or no sons, who would have a higher reproductive value in a female-biased population (Fisher 1930). The idea that females might increase their mating rate in response to drive was first put forward by Haig and Bergstrom (1995) and expanded upon by Zeh and Zeh (1996; 1997). Price et al. (2008b) elegantly tested this theory using experimental evolution. They set up several selection lines that had similar initial remating rates but differed in whether or not drive was present. They found strong evidence that the presence of drive led to increased mating rates in their populations, with females from drive lines doubling their likelihood of remating at first opportunity compared to females from lines where drive was absent. The mean time to remating also fell from 3.25 days to 2.75 days. An interesting additional finding from this experiment was that the frequency of drive in polyandrous lines had fallen from an initial 30% to less than 5% by the time the assays were performed 10 generations later. This finding was explored further in an additional experimental evolution study (Price et al. 2010). The authors established 48 populations, each
consisting of 60 males and 60 females, with a drive frequency of approximately 30%. Twelve of these populations were assigned to a monandrous treatment where males and females were mixed for only four hours, during which time females will not remate. The remaining 36 populations were assigned to treatments with varying degrees of polyandry, allowing two, three or six total mating opportunities. The frequency of drive did not change in the monandrous populations, but declined in all polyandrous treatments. Remarkably, the monandrous populations were also associated with increased extinction risk. After 15 generations, 5 of 12 monandrous populations had been driven extinct. Drive is implicated in these extinction events as the generation preceding extinction had a large population size, but the population was extremely female-biased. In contrast, none of the 36 polyandrous populations went extinct. Just one additional mating opportunity was enough to prevent the spread of drive, reduce its frequency, and reduce the risk of drive-mediated population extinction.

Several studies have now investigated the relationship between drive frequency and polyandry in the wild (Wilkinson et al. 2003; Manser et al. 2011; Pinzone and Dyer 2013; Price et al. 2014). First, Wilkinson et al. (2003) found that the frequency of multiple mating was higher in stalk-eyed fly species that have meiotic drive (*Teleopsis dalmanni* and *T. whitei*) than in a species that does not (*T. quinqueguttata*) but does not differ with drive frequency among *T. dalmanni* and *T. whitei* populations. Manser et al. (2011) studied a free-living population of the house mouse, *Mus musculus*, that
carries the autosomal driver \( t \)-haplotype. Despite a transmission ratio advantage of 90% and an increased adult lifespan of female \( t \)-haplotype heterozygotes, drive frequency declined in this population over a 5.5 year period. Many \( t \)-haplotypes carry recessive lethals (Silver 1993), but the low natural frequency of drive in mice (Huang et al. 2001; Carroll et al. 2004; Manser et al. 2011) cannot be explained by the effect of recessive lethal alleles alone – a condition commonly referred to as the “\( t \) frequency paradox”. Manser et al. (2011) found that the rate of multiple paternity in their population was fairly high (0.3), though this may be an underestimate of the true degree of polyandry as double matings where the sperm from only one male succeeds in fertilisation would not produce multiple paternity litters. Using stochastic simulations, they showed that the decline in drive frequency could only be explained by a scenario where the females often mate multiply and drive sperm is disadvantaged in sperm competition. Further support that polyandry is involved in reducing drive frequency in this species comes from experimentally evolving selection lines (Manser et al. 2017). Eight selection lines were maintained, half of which were kept under forced monandry, and half of which had a polygamous mating system (Firman and Simmons 2010). The frequency of drive remained at its moderately high initial frequency in monandrous lines over 20 generations but declined in polygamous lines. In both \( D. \, pseudoobscura \) and \( D. \, neotestacea \), \( X \)-linked meiotic drive exists along a latitudinal cline in north America, reaching moderate frequencies (~30%) in the southern parts of these species ranges and being rare or absent in the north. These clines have remained stable across time, including
across multiple sampling trips as much as 80 years apart in the case of *D. pseudoobscura* (Sturtevant and Dobzhansky 1936; James and Jaenike 1990; Dyer 2012; Price et al. 2014). In both species there is also a cline in the frequency of multiple mating, which is negatively correlated with drive frequency. Polyandry is rare in the south where drive is at moderate frequency and common in the north where drive is rare (Pinzone and Dyer 2013; Price et al. 2014). *D. neotestacea* and *D. pseudoobscura* are distantly related and drive has evolved independently in these species (Price et al. 2014). As similar patterns are found in these and other species, it suggests that polyandry may be a general phenomenon that protects against the spread of meiotic drive in nature. However, while the frequency of polyandry is correlated with the frequency of drive and there is evidence that polyandry can evolve in response to drive (Price et al. 2008b), it is unlikely that drive explains the natural cline in polyandry in *D. neotestacea* and *D. pseudoobscura*. This is for two reasons. Firstly, if drive is the only factor influencing polyandry in the wild then we would see a positive correlation between polyandry and drive, but we see a negative one (Pinzone and Dyer 2013; Price et al. 2014). Second, polyandry and drive would be expected to fluctuate in time. As drive invades a population, polyandry would be selected for. Once polyandry has become common, drive would decline. But as multiple mating has a cost (Fowler and Partridge 1989; Wigby and Chapman 2005), polyandry should then decrease, which would allow drive to invade again, and so on. Instead, we see find that the frequency of
drive is stable across decades (Price et al. 2014). Other factors are therefore needed to explain the natural cline in polyandry.

The relationship between X-linked meiotic drive and polyandry was explicitly modelled by Holman et al. (2015). These authors find that polyandry alone cannot prevent the spread of meiotic drive. This is because the benefit of polyandry is at its strongest when the frequency of drive is 50%. At low frequencies, the probability of mating with both a drive and a standard male is low. At high frequencies, polyandry provides increased benefits when it occurs as it increases the likelihood of producing sons in an increasingly female-biased population (Fisher 1930), but is less effective at checking the spread of drive because probability of mating with both a drive and a standard male is low as standard males (and males in general) become rare (Taylor and Jaenike 2002). However, if drive is prevented from fixing by further frequency-dependent costs, such as decreased fitness in homozygous females, then polyandry can be selectively maintained and has large impacts on the evolutionary dynamics of drive.

1.2.4 Viability

As previously stated, meiotic drive is often associated with inversions that cover appreciable portions of the chromosome (Jaenike 2001; Lindholm et al. 2016), which restrict recombination (Kirkpatrick 2010) and may therefore be prone to the accumulation of deleterious alleles. One way the increased
mutation load of the drive chromosome may impact the fitness of carriers is through reduced viability. In X-linked drive systems, deficits to male viability are unlikely to have a stabilising effect on drive frequency as male viability selection would operate in a frequency-independent manner (Edwards 1961; Carvalho and Vaz 1999). In females, the effect of drive on viability will depend on the dominance of drive-associated mutations. Recessive costs to viability and fecundity (discussed below) are expected because they will not be exposed to selection until the frequency of drive is high enough for homozygotes to be common. Such fitness costs provide a possible source of frequency-dependent selection against drive. This is because as drive increases in frequency, homozygotes become more common and selection against drive increases exponentially as its frequency increases.

The best known and most extreme example of costs to drive homozygotes is seen in the autosomal $t$-haplotype system in mice. $t$-haplotypes are variants of chromosome 17 that are transmitted to ~90% of the progeny of heterozygotes (Silver 1985). These chromosomes are associated with 4 inversions that cover approximately 40Mb and contain several semi-lethal alleles that are common to all $t$-haplotype variants (Silver 1993). Many $t$-haplotypes also carry fully lethal recessive alleles, split into 16 “complementation groups” (Klein et al. 1984). Mice homozygous for the same $t$-haplotype die in utero without exception (Silver 1985), while those homozygous for different but complementing $t$-haplotypes have greatly reduced litter sizes (Silagi 1962). Of those offspring that survive to parturition,
few survive to adulthood, and surviving males are usually sterile (Silver 1985). However, as previously mentioned, the observed frequency of t-haplotypes is considerably lower than expected based on the reduced fitness of homozygotes (Bruck 1957, Lewontin 1968; Manser et al. 2011), so other costs such as reduced sperm competitive ability (discussed above) or non-random mating (discussed below) are required to resolve this so-called t-frequency paradox.

Deleterious mutations associated with drive have been studied in insects by measuring the reduction in egg-to-adult viability. This is empirically feasible to measure via a comparison of drive frequency at developmental stages from eggs through to the eclosion of adults. It is surprising how rarely this has been explicitly tested. When the effect of meiotic drive viability has been explicitly tested, the results have been somewhat inconsistent and appear to depend on experimental conditions. In D. pseudoobscura, Wallace (1948) found that the effect of drive on viability was temperature dependent. At 16C, female drive homozygotes had a viability of 0.62, relative to standard homozygotes, but this fell to 0.15 at 25C. He also reported that drive heterozygotes had higher viability than either type of homozygote. On the other hand, Curtsinger and Feldman (1980) found that the effect of drive in females is additive, with standard and drive homozygotes being the most and least fit respectively. Both of these studies also found reduced viability of drive males. Beckenbach (1983) suggests that these effects may be strongly density-dependent. He reported a decline in viability in drive males, as did...
two previous studies, but only under high density conditions. Beckenbach (1983) also did not find any reduction in drive homozygote female viability, though his design was less sensitive to the detection of these effects than those of Wallace (1948) and Curtsinger and Feldman (1980). Wong and Holman (2020) recently measured the fitness effects of three different variants of the autosomal segregation distorher (SD) in *D. melanogaster*. They found that each affected viability differently. In one haplotype, embryos homozygous for SD die before hatching. In another haplotype homozygous larvae hatch but none survive until adulthood. In the third haplotype, viability was not significantly reduced. In addition, a few studies have indirectly measured the viability of drive carriers. For example, Stalker (1961) studied the prevalence of X-linked drive in *D. paramelanica*. In a cross that was expected to give an equal proportion of drive and standard males, only 71/199 drive males were recovered, suggesting that viability of drive males is reduced. Varandas et al. (1997) used a model developed by Edwards (1961) to understand drive frequency dynamics in *D. mediopunctata*. The model that best fit the data assumed a reduction in the viability of drive males, a reduction in viability in drive homozygous females and a slight overdominance in heterozygous females. The only other insect group that has been investigated is stalk-eyed flies. Wilkinson et al. (2006) did not detect any genotypic deviation from expectation in several relatively large-scale crosses of *T. dalmanni*. But in this thesis (Chapter 3) I report a reduction in male and female viability of approximately 20%. Given the
variability of the estimates of viability costs in laboratory studies of these species, it is difficult to predict what effect drive has on viability in the wild.

1.2.5 Fecundity

The accumulation of recessive deleterious mutations could also be reflected in the reduced fecundity of homozygous females. An extreme example of this is seen in *D. recens*, where the driving X chromosome carries a complex set of overlapping inversions that spans its entire length and is fixed for a recessive mutation that causes female sterility (Dyer et al. 2007). On the other hand, female drive homozygotes in *D. neotestacea* show no reduction in fertility or viability, despite the presence of inversions (Dyer 2012; Pieper and Dyer 2016). Drive also does not cause a reduction in fecundity in *T. dalmanni* stalk-eyed flies (Wilkinson et al. 2006). In *D. melanogaster*, fecundity is reduced in one haplotype of the autosomal SD drive system, but not in two others (Wong and Holman 2020). A series of studies have investigated the effect of meiotic drive on fecundity in *D. pseudoobscura* (reviewed in Price et al. 2019). Wallace (1948) and Beckenbach (1983) found that drive homozygote females had reduced fecundity. Wallace (1948) additionally found overdominance for fecundity, with heterozygotes laying more eggs than either type of homozygote. Curtsinger and Feldman (1980) repeated this second finding but did not detect a reduction in fecundity in drive homozygotes. As with the effect of drive on viability, these differences arise from variation in experimental design, and probably depend on the
density at which flies are reared (Price et al. 2019). Recently, Larner et al. (2019) found that drive homozygotes produced fewer than half the total progeny of standard homozygotes and were also more likely to experience complete reproductive failure. They did not find evidence of overdominance for fecundity, in contrast to previous work (Wallace 1948; Curtsinger and Feldman 1980; Beckenbach 1983). Instead, offspring production did not differ between heterozygotes and standard homozygotes (Larner et al. 2019). These estimates are likely to be more reliable than those from previous work as offspring production was measured for individual females, allowing estimates of mean and standard error, while previous work simply reports the pooled egg counts of multiple females kept together (Wallace 1948). In this study, however, only total progeny production was measured, which incorporates differences in sperm delivery, female fecundity, and the differential viability of larval genotypes. While it is likely that increased mortality of drive males had some impact on total progeny production, as assessed by a female-biased sex ratio, it unlikely that differential viability alone explains the large reduction in progeny production. Further, as much as it might be enlightening to know how much these different factors contribute to the reduction in progeny production of drive homozygotes, it is on their combined effects that selection acts. Interestingly, in a model parametrised by these relative fitness values, Larner et al. (2019) find that the frequency of drive in the wild closely matches the frequency predicted by their model under a realistic range of polyandry values. This suggests that the reduced fitness of drive homozygotes, combined with the reduced sperm
competition of drive males and clinal variation in multiple mating, may be sufficient to explain the cline in drive frequency in wild populations of this species.

1.2.6 Mate choice

In previous sections I have discussed how females can reduce the risk of siring offspring from males carrying meiotic drive by mating multiply and fostering sperm competition. Another strategy is simply to avoid mating with drive males in the first place. This idea was first investigated in the mouse $t$-haplotype system. Levine et al. (1980) found that when wildtype (+/+) laboratory females were placed in cages that allowed competition between heterozygote (+/$t$) and +/+ males, +/+ males sired significantly more offspring. Unfortunately, this study did not attempt to measure actual female preference, nor was it able to disentangle the effects of male dominance or sperm competition in multiple paternities. Subsequent studies from Sarah Lenington and colleagues (Lenington 1983; Lenington and Egid 1985; Coopersmith and Lenington 1990; Coopersmith and Lenington 1992) attempted to explore these effects further. In these studies, females were placed in a test area with one +/$t$ and one +/+ male, or with the bedding of these males that had acquired their odour. The time spent near these males or their odour was used as a proxy for preference. In all studies, +/$t$ females showed a significant preference for +/+ males over +/$t$ males. +/+ females, however, showed a preference for +/+ males in some studies but not in
others (reviewed in Lenington et al 1992). Additionally, Coopersmith and Lenington (1990) demonstrated that +/t females specifically chose to mate with +/t males from a different complementation group to their own when given the choice between the same and dissimilar t haplotypes, presumably to avoid homozygous lethality. More recently, Lindholm et al. (2013) examined mate choice in wild mice carrying the t-haplotype. They found that +/t males sired significantly more offspring with +/+ females than they did with +/t females. As the frequency of +/t males in the population increased, they attained a higher paternity share with +/+ but not with +/t females. This suggests that +/+ females mated randomly, but +/t females did not. In a recent study employing a simple no-choice design, Sutter and Lindholm (2016) found no evidence of discrimination against +/t males either by +/+, or even by genetically incompatible +/t females.

A confounding effect of the mouse model is that male dominance rank is an important factor in determining male mating success (Franks and Lenington 1986), and this effect overrides the t-haplotype effect, with +/t females exerting preferences for dominant males, even if they are +/t. It is only when females can choose amongst males of equal (or undetermined) dominance rank that the preference with respect to t-haplotype is exposed (Coopersmith and Lenington, 1992). The effect that the t genotype may have on dominance rank remains unclear. Lenington et al. (1996) found that +/t males were more dominant than +/+ males in arena experiments but Carroll et al. (2004) found the opposite result in a semi-natural context.
It has also been suggested that mate choice may play a role in the dynamics of drive in the stalk-eyed fly. In this family, the eyes of males and females are displaced laterally by hypertrophic extensions of the head capsule (Wilkinson and Dodson 1997). In many species of this family, there exists considerable sexual dimorphism in this feature, with males exhibiting significantly longer eyestalks than females (Burkhardt and de la Motte 1985). Females in these sexually dimorphic species prefer to mate with males with longer eyestalks (Wilkinson and Reillo 1994; Cotton et al. 2010), likely as a form of “good genes” sexual selection (Zahavi, 1975). In the two most intensively studied species, *T. dalmanni* and *T. whitei*, meiotic drive is known to exist at reasonably high frequencies (≈10-18% and ≈30% respectively) in the wild (Wilkinson et al. 2003). Wilkinson et al. (1998) first suggested that meiotic drive might be important in the well-known mate choice dynamics of these species. Wilkinson et al. (1998) artificially selected male *T. dalmanni* for large and small eyespan in replicate lines and found a correlation between eyespan size and brood sex ratio, with smaller eyespan males producing more female-biased broods and larger eyespan males producing more male-biased broods. The authors concluded that male-biased broods occurred due to the presence of a Y-linked suppressor (Ym) that, when paired with the driving X chromosome, produces approximately 63% male progeny (Presgraves et al. 1997). They further concluded that female preference for large eyespan in these flies may have evolved because large male eyespan signals the genetic quality due to suppression of drive.
However, subsequent work has not confirmed the presence of suppressors on either the Y or the autosomes (Paczolt et al. 2017). Furthermore, modelling suggest that preference for Y-linked suppression cannot evolve (Reinhold et al. 1999). This is because Y-linked suppressors must come at a cost, or else they would spread to fixation and drive would be undetectable. At equilibrium, the benefit of producing more male offspring in a female-biased population is offset by the viability cost of these sons, and there is no selection for the drive-resistant allele. In fact, the Y-linked suppressor is likely to be deleterious, as males carrying the Ym chromosome produce fewer offspring than Y+ males (Pomiankowski and Hurst 1999). A more theoretically favourable possibility is therefore that females should simply choose to mate with males that do not carry the driving X chromosome. This would require tight linkage between loci that contribute to large eyespan and the non-driving X chromosome in a region of low recombination such as near the centromere or in an inversion, otherwise recombination would be expected to quickly break up this relationship and choice would not be expected to evolve (Lande and Wilkinson 1999). Intriguingly, attempts to map the driving X chromosome in *T. dalmanni* revealed just such a relationship (Johns et al. 2005). Johns et al. crossed lines artificially selected for long and short eyespan and genotyped F2 flies at 24 microsatellite loci. They found that female-biased broods segregated with a specific X haplotype at four microsatellite loci (ms54, ms125, ms244 and ms395) that is not broken up by
recombination. These loci were found to be 33cM apart in control crosses, suggesting a paracentric inversion may keep these loci locked together on the driving X haplotype. Additionally, an X-linked QTL that explains 36% of the variation in male eyespan was found within this region (Johns et al. 2005). More recent work (Cotton et al, 2014) has found that this relationship holds true in the wild, with one of the previously identified microsatellite loci (ms395) showing a particularly strong relationship with both meiotic drive and male eyespan, such that individuals with large ms395 alleles had small eyespan and female-biased broods. It is possible that females are therefore able to use male eyespan as an indicator of meiotic drive status, and this may be an important factor in the evolution of their mating preferences.

Apart from these two examples, there exists little evidence of female choice against drive- carrying males. Indeed, in Drosophila pseudoobscura, females do not prefer standard males over males carrying a driving X chromosome, although there would be considerable benefit in doing so (Price et al. 2012b). As previously mentioned, this mate discrimination requires the genetic linkage between meiotic drive and some phenotypically observable quality lie in an area of low recombination (Pomiankowski and Hurst, 1999). This may be the case for both eyespan in stalk-eyed flies (Johns et al. 2005) and odour in mice (Lenington et al. 1992) but these conditions are likely to be rare (Lande and Wilkinson 1999).

1.3 Study species
Stalk-eyed flies (order: Diptera, family: Diopsidae) are characterised by hypercephalic elongation of the head capsule into long stalks, with the eyes and antennae located at the end of these stalks (Baker et al. 2001). There are several hundred species of stalk-eyed fly, the majority of which are found in the African and South East Asian tropics (Wilkinson and Dodson 1997). While head elongations and antlers are present in other Dipteran species (Wilkinson and Dodson 1997), the diopsid family is unique in that hypercephaly is present in both sexes (Baker et al. 2001). Many of these species are sexually monomorphic, which is believed to be the ancestral state (Baker and Williamson 2001). The initial evolution of eyestalks may have been driven by selection for an increase in visual acuity. The number of ommatidia (individual optical components of the compound eye) increases with eyespan and a greater number of ommatidia is associated with increased binocular field of view (Burkhardt and de la Motte 1983). However, a large number of diopsid species show sexual dimorphism (Wilkinson and Dodson 1997; Baker et al. 2001), a condition that has independently evolved at least four times (Baker and Williamson 2001). In all sexually dimorphic species, males have larger eyespan than females, both in absolute terms and relative to their body size. The exaggerated eyespan of males in sexually dimorphic species is likely driven by male-male competition (Panhuis and Wilkinson 1999; Small et al. 2009) and female choice (Wilkinson and Reillo 1994; Cotton et al. 2010). During the day, stalk-eyed flies feed on mould, yeast, and fungus from decaying plant matter in their forest habitat. Eggs are
laid in this decaying vegetation, and larvae develop there (Burkhardt and de la Motte 1983: Wilkinson and Dodson 1997). At night, dimorphic species aggregate in a lek-style mating system (Wilkinson and Dodson 1997; Cotton et al. 2010). In two closely-related Malaysian stalk eyed flies, *T. dalmanni* and *T. whitei*, this occurs on exposed root hairs overhanging the banks of rainforest streams (Burkhardt and de la Motte 1983; Cotton et al. 2010). At dusk, males arrive before females and select a roosting site. Males guard these sites, competing aggressively with other males. In these contests, the male with the largest eyespan typically wins (Burkhardt and de la Motte 1983; Lorch et al. 1993; Small et al. 2009). Females arrive later and choose where to roost, and with whom to mate, on the basis of male eyespan (Wilkinson and Reillo 1994; Cotton et al. 2010). The number of females in a male’s harem is correlated with his eyespan (Burkhardt and de la Motte 1983; Cotton et al. 2010). The majority of mating occurs at dawn the following morning. Males mate multiply during this period in both the laboratory (Meade et al. 2020) and the wild (Cotton et al. 2015). Females mate multiply in the laboratory (Baker et al. 2001; Wilkinson et al. 2003), but their mating rate has not been measured in the wild.

X-chromosome meiotic drive has been reported in at least two species of stalk-eyed flies (*Teleopsis* (formally *Cyrtodiopsis*) *dalmanni* and *T. whitei*) in southeast Asia (Presgraves et al. 1997). Meiotic drive in this species, as in many other X-chromosome drive systems, is referred to as *sex-ratio* or SR. Drive males in these species sire broods with >90% female offspring. In *T.*
*dalmanni*, spermatids mature in bundles where, in SR males, the heads of Y-bearing sperm fail to elongate (Presgraves et al. 1997). Populations of *T. dalmanni* and *T. whitei* carry sex-ratio meiotic drive at frequencies of approximately 20% and 15% respectively (Wilkinson et al. 2003), and these frequencies are stable over collections made more than 20 years apart (Paczolt et al. 2017). However, historic estimates of drive frequency in *T. dalmanni* are complicated by the more recent finding that these populations actually consist of two sympatric clades that exhibit little phenotypic divergence but complete reproductive isolation (Christianson et al. 2005; Rose et al. 2014), and so likely represent cryptic species (Paczolt et al. 2017). As these species have not been formally described, I refer to them as *T. dalmanni*-1 and *T. dalmanni*-2, following Paczolt et al. (2017). Strongly female-biased broods have been reported in *T. dalmanni*-1, but not *T. dalmanni*-2, suggesting that meiotic drive is either at too low a frequency to be detected, completely suppressed, or completely absent in this species (Paczolt et al. 2017). All work in this thesis is carried out using *T. dalmanni*-1 as a study species.

The meiotic drive gene(s) in *T. dalmanni* are located within at least one large inversion (Johns et al. 2005; Paczolt et al. 2017). There is little to no recombination between the drive and standard chromosomes (Johns et al. 2005; Paczolt et al. 2017); they show considerable divergence (Reinhardt et al. 2014; Paczolt et al. 2017). The drive chromosome is at least 500,000 years old and appears to be evolving independently from the standard
chromosome in an otherwise shared genetic background (Paczolt et al. 2017). There are 955 fixed genetic differences between the drive and standard X chromosomes, compared with only 11 on the autosomes, and there are over 500 differentially expressed transcripts expressed in the testes (Reinhardt et al. 2014).

As previously stated, *T. dalmanni* males carrying meiotic drive have reduced eyespan compared to standard males. While large eyespan was initially attributed to Y-linked suppression (Wilkinson et al. 1998), it is now known that the males carrying the drive X chromosome simply have smaller eyespan (Johns et al. 2005; Cotton et al. 2014). The relationship between meiotic drive and eyespan is explored further in chapter 4 of this thesis.

The large inversion(s) on the drive X chromosome, as well as the lack of recombination, are expected to have led to the accumulation of deleterious mutations. To what extent has this caused reduced fitness of the drive chromosome? Wilkinson et al. (2006) found no evidence for reduced adult lifespan in drive carriers, nor do drive females suffer reduced fecundity. To the contrary, Wilkinson et al. (2006) actually report weak overdominance for fecundity. *T. dalmanni* drive males do not suffer reduced fertility at low mating rates (Wilkinson et al. 2006), despite producing half the number of normal sperm bundles (Presgraves et al. 1997). At high mating rates (8 females in 24 hours), Wilkinson et al. (2006) found that drive males were less fertile than standard males. This mating rate is considerably higher than
males would usually encounter in the wild (Lorch et al. 1993; Cotton et al. 2015), and it is unclear whether this reduction in fertility is because drive males transfer fewer sperm, or because they mate less often (Wilkinson et al. 2003; Finnegan et al. 2020; Meade et al. 2020). Furthermore, Wilkinson et al. (2006) measured male fertility by counting the number of adult progeny sired by drive males, but this measure is confounded by potential differences in the egg-to-adult viability of the progeny of drive and standard males. In chapter 3 of this thesis, I explicitly test for such differences. Meade et al. (2019) found that drive and standard males do not differ in their sperm transfer per mating. Subsequently, Meade et al. (2020) measured male fertility through counts of fertilised and unfertilised eggs and found no reduction in drive male fertility even under high mating rates (5 females for 10 hours). However, as stated previously, T. dalmanni drive males do particularly poorly under conditions of sperm competition (Wilkinson et al. 2006), suggesting that while drive males do not transfer fewer sperm per mating (Meade et al. 2019), their sperm is likely of lower quality.

1.4 Structure of the thesis

This thesis is comprised of seven chapters. The first (this chapter) is a general introduction to meiotic drive and its known costs in a variety of species. In chapter 2, I explore whether meiotic drive incurs costs relating to male mate preference and in chapter 3, I then examine costs relating to egg-
to-adult viability. In chapter 4, I explore how drive affects the condition-dependence of stalk-eyed fly eyespan. Chapter 5 describes an experimental evolution study that tracks the frequency of drive in cage populations that are maintained on different larval food treatments. Chapter 6 is a general discussion and chapter 7 is a collection of various appendices and includes full copies of published first-author papers.

1.4.1 Chapter 2

Male mate preferences have been demonstrated across a wide array of taxa. In this chapter, I explore whether meiotic drive impacts male mate preference in the stalk-eyed fly *Teleopsis dalmanni*. The expression of mate preference is expected to be associated with a cost. As drive is associated with a low-frequency inversion that causes reduced organismal fitness, drive males may be less able to bear this cost. Drive males may also experience weaker selection for preference maintenance if they are avoided by females. Using binary choice trials, across two experiments, I confirmed male preference for large (fecund) females but found no evidence that the strength of male preference differs between drive and standard males. I show that large eyespan males displayed strong preference for large females, whereas small eyespan males showed no preference. Taken together, these results suggest that, even though meiotic drive is associated with lower genetic quality, it does not directly interfere with male mate preference among available females. The laboratory work in this chapter was carried out with the
assistance of a summer student (Leslie Nitsche), funded by an award from the Association for the Study of Animal Behaviour (UK). Genotyping assistance was provided by a postdoctoral researcher (M. Florencia Camus) and a technician (Matteo Mondani). This chapter was published in *Behavioral Ecology* in 2019: Finnegan SR, Nitsche L, Mondani M, Camus MF, Fowler K, Pomiankowski A. 2020. Does meiotic drive alter male mate preference? *Behavioral Ecology* **31**: 194-201. (Appendix D)

### 1.4.2 Chapter 3

Meiotic drive is found in a stable polymorphism at moderate frequency in several species, including the stalk-eyed fly *T. dalmanni*. This suggests there must be strong frequency-dependent selection resisting its spread. One possible source of this type of selection would be a reduction in viability of female homozygotes, as has been suggested in other species. In this chapter, I use large-scale controlled crosses to study the effect of meiotic drive on egg-to-adult viability and estimate the strength of selection against drive in males and females. I find that drive reduces egg-to-adult viability in both sexes. In females, homozygous females experience a greater reduction in viability ($S_f = 0.242$) and the deleterious effects of SR are additive ($h = 0.511$). The male deficit in viability ($S_m = 0.214$) is not different from that in homozygous females. The laboratory work in this chapter was carried out with the assistance of two fourth-year undergraduate students (Nathan White and Dixon Koh) and genotyping support was provided by a postdoctoral
researcher (M Florencia Camus). This chapter has been published in 2019:
Finnegan SR, White NJ, Koh D, Camus MF, Fowler K, Pomiankowski A.

1.4.3 Chapter 4

The handicap principle of sexual selection proposes that ornamental traits are an honest signal of underlying genetic quality. This suggests that the ornamental trait in stalk-eyed flies, eyespan, should therefore show a heightened condition-dependent response to stress. The meiotic drive chromosome in stalk-eyed flies is associated with a low-frequency inversion which is expected to have accumulated deleterious mutations that cannot easily be removed by recombination. Male carriers of meiotic drive also have smaller eyespan than those with a standard X chromosome. It is predicted that reduced genetic quality of the drive chromosome should be reflected in the condition-dependent expression of eyespan, with the eyespan of drive males declining more sharply with increased stress. In this chapter, I test this prediction by rearing male and female larvae under benign and high stress larval environments. I find that drive adult males and adult females have smaller eyespan than standard adult males and females under both stress treatments, but this difference does not increase under high stress. This suggests that the reduced eyespan of drive males is not a consequence of the increased mutation load. Instead, it is likely that the drive chromosome is
simply fixed for small eyespan alleles. This is perhaps part of a set of adaptions that maintain fertility in the face of drive-mediated destruction of sperm.

1.4.3 Chapter 5

*Sex-ratio* meiotic drive (SR) is often predicted to spread rapidly through populations that carry it, potentially even causing extinction due to a lack of males. Despite this, drive in many species is found at a moderate frequency in the wild and is stable across time. In the Gombak valley, the frequency of drive in the stalk-eyed fly *T. dalmanni* is stable over time, but varies considerably between populations, where it is negatively correlated with population size. In order to better understand these findings, I established 24 cage populations of stalk-eyed flies with drive at a moderately high initial frequency. Larvae in these populations were reared on either high or low quality food. This work is still ongoing, but in this chapter I discuss preliminary findings. I find that the frequency of drive has declined in laboratory cage populations, being lost altogether from some after only six generations. SR was maintained at a higher frequency in populations where larvae were fed on low quality food. I discuss several possible explanations for these findings. I also find evidence that one cage has been driven extinct by drive, suggesting that small populations are vulnerable to perturbations in population sex ratio and SR frequency.
1.4.5 Chapter 6

In this chapter I give a recapitulation of the main findings and general discussion from previous chapters. I also highlight further avenues of research that could be explored to better understand the evolution and maintenance of meiotic drive in natural populations.

1.4.6 Chapter 7

This chapter consists of appendices containing supplementary information for previous chapters, as well as copies of published papers. Appendix A is the supplementary information for chapter 2 and consists of model tables, effect size estimates, and a supplementary figure. Appendix B is the supplementary information for chapter 3 and contains model tables, effect size estimates, and a supplementary table. Appendix C is the supplementary information for chapter 4 and consists of model tables, effect size estimates, and a supplementary figure. Appendix D is a copy of the published version of chapter 2, published in *Behavioral Ecology* in 2020. Similarly, Appendix E is a copy of the published version of chapter 3, published in *Proceedings of the Royal Society B: Biological Sciences* in 2019.
1.5 References


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Does meiotic drive alter male mate preference?
2.1 Abstract

Male mate preferences have been demonstrated across a range of species, including the Malaysian stalk-eyed fly, *Teleopsis dalmanni*. This species is subject to SR, an X-linked male meiotic driver, that causes the dysfunction of Y-sperm and the production of all-female broods. While there has been work considering female avoidance of meiotic drive males, the mating decisions of drive-bearing males have not been considered previously. Drive males may be less able to bear the cost of choice as SR is associated with a low frequency inversion that causes reduced organismal fitness. Drive males may also experience weaker selection for preference maintenance if they are avoided by females. Using binary choice trials, across two experiments, I confirm male preference for large (fecund) females but find no evidence that the strength of male preference differs between drive and standard males. I show that large eyespan males display strong preference for large females while small eyespan males show no preference. Taken together, these results suggest that even though meiotic drive is associated with lower genetic quality it does not directly interfere with male mate preference among available females. However, as drive males tend to have smaller eyespan (albeit only ~5% on average), this will to a minor extent weaken their strength of preference.
2.2 Introduction

Despite a historical narrative of indiscriminate males attempting to mate with choosy females (Bateman 1948), male mate preference is a widespread phenomenon (Bonduriansky 2001; Edward and Chapman 2011). It has even been observed in diverse lekking species, where males only provide sperm, including flies (Shelly et al. 2012), birds (Sæther et al. 2001) and fish (Werner and Lotem 2003). Several conditions have been identified for selection to favour the evolution of male mate preference (Bonduriansky 2001). The first is that mating must be costly or it would not pay males to be choosy (Bonduriansky 2001). Costs may arise if sampling of females leads to higher predation risk, greater disease transmission or simply requires more time (Parker 1983; Pomiankowski 1987). There are also opportunistic costs to males since the duration of a mating inevitably reduces the time available to search for and mate with other females (Bonduriansky 2001). In addition, sperm production is costly (Dewsbury 1982) and limits the mating capacity of individual males. So, males need to allocate their ejaculate strategically among females, a form of cryptic male preference (Wedell et al. 2002). On the other hand, there must be variation in female quality, so that male choice among females yields a benefit (Parker 1983). An obvious benefit for males arises from variation in female fecundity (Bonduriansky 2001) generated by current or future egg production, female age and mating status (e.g. virgin vs. mated, time since last mating, degree of sperm competition). Also, females may vary in genetic quality or genetic compatibility. Overall, to promote the
The evolution of male mate preference the costs of assessing potential mates should be low enough that they do not outweigh the benefits of preference (Nakahashi 2008), as with the evolution of female preference (Pomiankowski 1987).

The Malaysian stalk-eyed fly, *Teleopsis dalmanni*, fulfils these general conditions for the evolution of male mate preference. In the wild, male stalk-eyed flies establish lek sites at dusk which attract females. Most mating occurs in a short period (~20-30 minutes) at dawn the next day (Burkhardt and de la Motte 1985; Chapman et al. 2005). The majority of leks contain a single male with an average of two females (range 1-7; Cotton et al. 2010), providing males with the opportunity to mate selectively. The direct cost of male preference is likely to be small as a male can easily compare females that settle on his lek. In addition, in the dawn period there is typically no competition for mating, as only the harem male mates. However, there may be costs related to the mating rate. Mating is associated with a temporary reduction in accessory gland size, and these organs do not recover to pre-mating size for around 24 hours (Rogers et al. 2005). In a study of the correlates of mating frequency, the majority of males (76.1%) presented with six females were unable to mate with all of them within an hour (Rogers et al. 2005), considerably longer than the early morning period of mating in the field (Cotton et al. 2010). These data suggest that males suffer limits to their daily mating capacity, which probably extends across days. In addition, females are observed to fly off leks during the dawn period, whether they
have mated or not (A Pomiankowski, personal observation). A male pre-occupied mating with one female loses the opportunity to mate with others. Males are likely to benefit from exercising mate preference because females vary in fecundity. In the wild and the laboratory, female fecundity is positively correlated with body size and nutritional status (David et al. 1998; Cotton et al. 2010, 2015). Female eyespan is a likely target trait for male preference. In field samples, female eyespan is predictive of fecundity even after controlling for body size, with which it strongly covaries (Cotton et al. 2010). Indeed, male mate preference for large eyespan and high fecundity has been reported in this species under both laboratory and field conditions (Cotton et al. 2015). Together this evidence suggests that females vary in reproductive quality in ways that will affect male fitness and the costs of male preference are unlikely to outweigh the potential benefits.

Here we investigate the effect of sex-ratio (SR), X-linked meiotic drive, on male mate preference in T. dalmanni. SR systems are common in flies, causing male carriers to produce female-biased broods (Jaenike, 2001; Lindholm et al. 2016). In stalk-eyed flies, the SR chromosome (XSR) exists at moderate frequencies (~20%; Wilkinson et al. 2003; Cotton et al. 2014; Paczolt et al. 2017). The gene(s) controlling meiotic drive are located in a large paracentric inversion covering most of the XSR chromosome (Johns et al. 2005; Paczolt et al. 2017). Low frequency inversions are associated with reduced recombination rates and are subject to weaker natural selection and the accumulation of deleterious mutations (Hoffmann and Rieseberg 2008;
Kirkpatrick 2010). In several drive systems, this results in reduced viability (Curtsinger and Feldman 1980; Beckenbach 1996; Larracuente andPresgraves 2012; Sutter and Lindholm 2015). Reinhardt et al. (2014) showed that there are almost a thousand fixed differences between SR and ST (the wild-type X chromosome) X-linked genes in *T. dalmanni*, but only 11 for autosomal genes, consistent with mutation accumulation on X\textsuperscript{SR}. There is some evidence for reduced genetic quality of X\textsuperscript{SR}. Males and females carrying the X\textsuperscript{SR} chromosome have reduced egg-to-adult viability (Finnegan et al. 2019), even though adult longevity is not affected (Wilkinson et al. 2006). In addition, SR males have repeatedly been shown to have reduced eyespan both in laboratory (Wilkinson et al. 1998; Johns et al. 2005; Meade et al. 2020) and wild populations (Cotton et al. 2014). This association probably arises because male eyespan is highly condition-dependent and reflects environmental (David et al. 1998; Cotton et al. 2004) and genetic quality (David et al. 2000; Bellamy et al. 2013).

Previous work has not investigated whether meiotic drive affects sexual preference. The X chromosome is likely to be a favourable location for the evolution of preference genes (Kirkpatrick and Hall 2004) and there is some evidence that sex-linked preferences are common (Muralidhar 2019). In *T. dalmanni*, differences in mate preference between SR and ST bearers are expected to be influenced by X-linked factors because their autosomes are freely recombining. A number of arguments lead to the prediction that SR males will show weaker mate preference than ST males. Female mate
preferences are often costly condition-dependent traits, with the highest quality females showing the strongest preference for the most attractive males (Cotton et al. 2006). For example, female three-spined sticklebacks \((Gasterosteus aculeatus)\) from high condition families display strong preference for male red throat coloration while females from low condition families do not (Bakker et al. 1999). In \(T. dalmanni\), if male mate preference is costly, low condition SR males may be less able to bear this cost, leading to weaker SR male preferences for high value females (Howie and Pomiankowski 2018). A more direct association may arise due to linkage of preference alleles to the \(X^{SR}\) inversion. Given greater mutational decay on the \(X^{SR}\) chromosome, SR males would be expected to display weaker preferences for high quality females. A third possibility arises from the association of SR with reduced male eyespan. Theoretical work suggests that visual perception improves as eyespan increases (Burkhardt and de la Motte 1983). Small eyespan may limit the ability of males to discriminate among females. Mate preference in female stalk-eyed flies shows an association between eyespan and visual discrimination (Hingle et al. 2001a), and this may well extend to males. A final possibility is that since females prefer to roost and mate with males of large eyespan (Wilkinson and Reillo 1994; Wilkinson and Dodson 1997; Hingle et al. 2001a; Cotton et al. 2010), SR males on average attract fewer females to their leks. This could result in weaker selection for mate preference among SR males if they have less opportunity for choice. A potential example of this is the two-spotted goby, \(Gobiusculus flavescens\), where large attractive males prefer to mate with
colourful females, but small less attractive males express no preference, despite equal courtship effort (Amundsen and Forsgren 2003).

To assay male mate preference, I used simple binary choice trials (Cotton et al. 2015) to measure the strength of male mate preference in stalk-eyed flies. In two experiments, SR and ST males were presented with two females, one large and one small, and allowed to mate freely during a short time period. Two females is the mean number observed in the wild on male-female leks (Cotton et al. 2010). The design aimed to mimic, under controlled conditions, the sex ratio and time-frame under which male preference is expressed in the wild. In the first experiment, focal male eyespan was constrained to lie within a narrow range of trait values to test whether the genotypic differences between SR and ST males cause differences in mating behaviour independent of male eyespan. In the second experiment, focal male eyespan was unconstrained and drawn from its natural distribution to determine the direct effect of eyespan and its association with genotype (SR and ST) on mate preference.

2.3 Methods

2.3.1 Source populations

A stock population was obtained from Ulu Gombak in Malaysia (3°19’N 101°45’E) in 2005 (by Sam Cotton and Andrew Pomiankowski). It is
maintained at 25˚C on a 12:12 hour light:dark cycle at high population density. This population’s males are only standard (i.e. wildtype), and it is designated the ST stock, as it does not contain individuals carrying the X_{SR} drive chromosome.

In 2012 a further collection was made of male flies from the same location (by Alison Cotton and Sam Cotton) and used to create a SR stock population that maintains the X_{SR} chromosome, following a standard protocol (Presgraves et al. 1997; Meade et al. 2019). Briefly, individual males from the SR population are housed with three ST stock females and mate freely. Their offspring sex ratio is scored. Males siring female-biased broods (>90% female offspring, >15 total offspring) are designated SR (X_{SR}/Y), and their female progeny are therefore carriers of the SR chromosome (X_{SR}/X^{ST}). Progeny from other males, which are likely to be ST, are discarded. The resulting heterozygous females are then mated with ST stock males (X^{ST}/Y), producing SR (X_{SR}/Y) and ST (X^{ST}/Y) males in an expected 1:1 ratio. These males are crossed to three ST stock females, and the process is repeated (i.e. keeping the progeny of X_{SR}/Y males and discarding those of X^{ST}/Y males). The regular crossing with ST stock males and females homogenises the autosomes, Y chromosome, wildtype ST chromosome and mitochondrial genes across the two stock populations. In other respects, the SR and ST stocks were kept under similar conditions.

2.3.2 Experimental flies
Experimental males were collected from egg-lays, petri dishes containing moistened cotton wool and ~15g pureed sweetcorn, placed into SR stock cages. The petri dishes were removed after 3 days and subsequently the eclosed adults were collected after 3-4 weeks. Eyespan was measured as the distance between the outermost edges of the eye bulbs (Cotton et al. 2004), using ImageJ (v1.5.0). In the first experiment, males were standardised to a narrow range of eyespan (7.5-8.5 mm) to minimise any potential effect of variation in male eyespan on female behaviour. Males were housed in large cages (35cm x 22cm x 20cm) with a similar number of stock females for them to mate at a normal rate prior to the mating assay. Experimental females were collected from the ST stock population and their eyespan measured. Females used in the experiment were defined as large (eyespan ≥ 5.8 mm) or small (eyespan ≤ 5.4 mm), following Rogers et al. (2006) and Cotton et al. (2015). Intermediate size females were discarded. Large adult females were fed high quality food consisting of 100% pureed corn. Small adult females were fed low quality food consisting of 20% pureed corn and 80% sugar solution (25% sugar w/v), with the addition of an indigestible bulking agent (3% carboxymethylcellulose w/v) to make the viscosity similar to that of the high quality food (Rogers et al. 2008; Cotton et al. 2015). The two diets were used to amplify differences in fecundity between the size classes of experimental female (Cotton et al. 2015). Previous work with more extreme dietary differences shows that diet does
not affect the rate of female mating (Hingle et al. 2001b). The two classes of female were housed with stock males to allow them to mate at a normal rate.

In the second experiment, males were reared from egg-lays collected from SR stock cages with variable amounts of corn (between 1.5 – 15g) to generate size variation in eyespan and thorax. Otherwise the procedures used were similar to the first experiment. One exception was that both types of female, large and small, were fed the same high quality food as adults. This ensured that the assays of male preference were independent of any differences in fecundity brought about by dietary manipulation.

2.3.3 Male mating assays

Male flies were presented with a choice of large and small females in mating chambers (Figure 1; Cotton et al. (2015)). Mating chambers were set up in the afternoon prior to each assay. Males were placed in the top compartment, with one large and one small female placed in the bottom compartment. Interactions between males and females were prevented during this period by a cardboard partition placed between the compartments. At dawn on the assay day, the partition was removed and the mating chambers were observed for 30 minutes. The number of copulations with each size class and the order of mating were recorded. A successful copulation was defined as intromission lasting more than 30 seconds, as copulations shorter than this duration do not result in spermatophore transfer
(Rogers et al. 2006). Males that attempted to mate but were unsuccessful were presented with a different set of one large and one small female and observed for an additional 30 minutes. After completion of the assay, focal males were frozen and stored in ethanol. Females were isolated in individual 500ml pots for two days before being returned to population cages, ensuring that no females were used in assays on consecutive days.

2.3.4 Genotyping

The experimenters were blind to the genotype of experimental males, as this was inferred post-hoc by genotyping. DNA was extracted using a standard protocol. In experiment 1, half the thorax of each fly was added to individual 1.5ml Eppendorf tubes containing 10μl Proteinase K (10mg.ml⁻¹) and 250μl of DIGSOL at 55°C. Subsequently, 300μl of 4M ammonium acetate was added and samples spun down for 10 minutes at 13,000RPM. The supernatant was then aspirated into new tubes containing 1ml 100% ethanol and spun for 10 minutes at 13,000RPM to precipitate DNA. DNA was then washed in 70% ethanol to remove excess salt and left to air-dry for 45 minutes before being eluted in 30ul Low-TE (1mM Tris-HCl pH8, 0.1mM EDTA). DNA was PCR-amplified on a 2720 Thermal Cycler (Applied Biosystems) in 96-well plates with each well containing 1μl of dried DNA, 1μl of QIAGEN Multiplex PCR Mastermix (Qiagen), and 1μl of Primer mix (consisting of the forward and reverse primers for ms395 and comp162710 each at a concentration of 0.2μM). A drop (10μl) of mineral oil was added to
limit evaporation. Fragment length analysis was carried out using an ABI3730 Genetic Analyzer (Applied Biosystems) with a ROX500 size standard. Microsatellite allele sizes were assigned using Fragman package v. 1.07 (Covarrubias-Parazan et al. 2016) in R v. 3.2.3 (R Development Core Team, 2018) and checked using GENEMAPPER 4.0. Two markers were used to distinguish SR and ST males. Microsatellite \textit{ms395} has a bimodal distribution where large (>218bp) alleles are strongly associated with SR (Johns et al. 2005; Cotton et al. 2014; Meade et al. 2019; Paczolt et al. 2017). \textit{Comp162710} is an indel marker with a small allele (201bp) found in SR males, and a large allele (286bp) found in ST males (GS Wilkinson, personal communication), which has been used previously as a SR marker (Meade et al. 2019). Males with large \textit{ms395} alleles and small \textit{comp162710} alleles were classed as SR. Where markers gave conflicting signals, genotype was assigned on the basis of \textit{comp162710} allele size.

In experiment 2, the same procedure was used to extract DNA, but a different PCR protocol was used. Each well consisted of 1μl of DNA, 0.1μl of 5x Phusion Taq polymerase (New England BioLabs), 0.2μl of dNTPs, 6.2μl UltraPure water, and 0.5μl each of the 10μM forward and reverse primers for \textit{comp162710}. \textit{Comp162710} fragment lengths were assayed by gel electrophoresis on a 3% agarose gel with a 0.5x TBE buffer.
2.3.5 Statistical analysis – Genotype and male preference

All statistical analyses were carried out in R (R Core Team 2016) Model outputs are reported in the Supplementary Information. In the first experiment, I analysed the effect of genotype on the number of copulations with each size class of female using logistic regression, weighted by the total number of copulations carried out by each male, with a quasi-binomial error structure to account for over-dispersion. The intercept term in this model determines whether males show preference for either size class of females. The data was also split by genotype and the same model was run to determine if SR and ST males preferred large females. For comparison with earlier work (Cotton et al. 2015), mate preference for each individual male was assessed using an index based on the proportion of total copulations with the large female, \( Pref = (C_L - C_S) / (C_L + C_S) \), where \( C_L \) and \( C_S \) are the number of copulations with the large and small females respectively. Preference values range ±1 and are symmetric about zero. For an individual male, a value greater than zero indicates preference for large females, and less than zero indicates preference for small females. Preference in each consecutive mating was assessed using binomial tests on the number of copulations with large and small females, on the pooled dataset, and SR and ST males separately. The effect of genotype on the number of copulations with large and small females was analysed for each consecutive mating using generalised linear models with quasi-binomial error distributions.
2.3.6 Statistical analysis – Eyespan and male preference

The second experiment allowed me to consider whether male eyespan had an effect on mating preference and its interaction with male genotype. First, the effects of male eyespan, genotype and their interaction were modelled for the number of copulations with each size class of female in a generalized linear model, weighted by the total number of copulations carried out by each male, with a quasi-binomial error structure. Then, males were split into three eyespan categories: small (eyespan < 6.0mm), medium (eyespan 6.0mm - 7.5mm) and large (eyespan > 7.5mm). The effect of eyespan category, genotype, and their interaction on the number of copulations with each size class of female was analysed in a generalised linear model with a quasi-binomial error distribution. The difference in mean preferences of each size group was assessed using the glht function of the multcomp package in R. The effect of genotype on thorax length and eyespan was analysed in a linear model. Other tests were carried out as in the first experiment.

2.3.7 Statistical analysis – Mating frequency

The effect of genotype on mating frequency in the first experiment was reported previously (Meade et al. 2020). Here we combined data across both experiments to examine how the total number of matings by each male was affected by genotype in generalised linear models with Poisson error distribution. We then analysed the effect of eyespan on mating frequency
using data from the second experiment, in which there was variation in male eyespan.

2.4 Results

2.4.1 Genotype and male preference

In the first experiment, males showed a preference for large females when genotypes were pooled ($Pref \text{ mean } \pm \text{ SE} = 0.3637 \pm 0.056; t = 6.287, P < 0.0001, n = 162$). Males preferred large females in their first ($Pref \text{ mean } \pm \text{ SE} = 0.4321 \pm 0.0711, P < 0.0001, n = 162$), second ($Pref \text{ mean } \pm \text{ SE} = 0.3030 \pm 0.0832, P = 0.0006, n = 132$) and third mating ($Pref \text{ mean } \pm \text{ SE} = 0.4257 \pm 0.0904, P < 0.0001, n = 101$). For subsequent matings there was no male preference for large females, in large part reflecting the reduced sample size (fourth mating: $Pref \text{ mean } \pm \text{ SE} = 0.1803 \pm 0.1269, n = 61, P = 0.2000$; fifth mating: $Pref \text{ mean } \pm \text{ SE} = 0.2593 \pm 0.1894, n = 27, P = 0.2478$).

The preference of SR and ST males did not differ from each other (GLM: $t = 0.150, P = 0.8808, n = 157$). Preference was for large eyespan females in both SR ($Pref \text{ mean } \pm \text{ SE} = 0.3970 \pm 0.080, t = 4.959 P < 0.0001, n = 81$) and ST males ($Pref \text{ mean } \pm \text{ SE} = 0.3367 \pm 0.0806, t = 4.098, P = 0.0001, n = 76$; Figure 2). Across consecutive copulations, SR and ST males preferred large females in the first (SR $Pref \text{ mean } \pm \text{ SE} = 0.5062 \pm 0.0964, P < 0.0001, n = 81$; ST $Pref \text{ mean } \pm \text{ SE} = 0.3684 \pm 0.1073, P = 0.0018, n = 76$),
second (SR Pref mean ± SE = 0.3333 ± 0.1227, P = 0.013, n = 60; ST Pref mean ± SE = 0.2647 ± 0.1178, P = 0.0385, n = 68), and third (SR Pref mean ± SE = 0.3000 ± 0.1526, P = 0.0807, n = 40; ST Pref mean ± SE = 0.4737 ± 0.1177, P = 0.0005, n = 57) mating, and did not differ in the strength of their preference across these copulations (1st mating $F_{1,155} = 0.9107$, $P = 0.3414$; 2nd mating $F_{1,126} = 0.1623$, $P = 0.6878$; 3rd mating $F_{1,95} = 0.8226$, $P = 0.3667$). SR and ST males did not differ in the frequency of failing to mate at least once (SR: 23/104, ST: 13/89, $\chi^2_1 = 1.8069$, $P = 0.1789$, n = 193).

2.4.2 Eyespan and male preference

In the second experiment, larvae were exposed to variable amounts of food during development. Adult males showed considerable variation in eyespan (mean ± SD = 7.026 ± 1.495 mm, range 3.625 – 9.461 mm). Eyespan was strongly co-linear with body size (i.e. thorax length, $F_{1,191} = 788.5$, $P < 0.0001$), but did not differ with genotype ($F_{1,191} = 0.9322$, $P = 0.3355$), nor was there a difference in the allometric slope of eyespan on body size with genotype ($F_{1,191} = 0.0014$, $P = 0.9706$; Figure S1).

As before, when individuals from both genotypes were pooled, males showed a preference for large females overall (Pref mean ± SE = 0.2344 ± 0.0494, GLM: $t = 7.044$, $P < 0.0001$, n = 178), and in the first (Pref mean ± SE = 0.3371 ± 0.0707, $P < 0.0001$, n = 178), second (Pref mean ± SE = 0.2785 ± 0.0767, $P = 0.0005$, n = 158) and third matings (Pref mean ± SE =
0.2593 ± 0.083, \( P = 0.0033, n = 135 \)). Again, there was no male preference for large females in subsequent matings as sample size fell (fourth mating, \( \text{Pref mean} \pm SE = 0.1132 \pm 0.0970, P = 0.2853, n = 107 \); fifth mating, \( \text{Pref mean} \pm SE = 0.2500 \pm 0.1220, P = 0.0599, n = 64 \)).

Male eyespan had a strong positive effect on mating preference (\( F_{1,174} = 5.8333, P = 0.0168 \), Figure 3). When males were split into three groups based on eyespan (large >7.5mm, medium 6.0 – 7.5mm and small <6.0mm), male eyespan group affected preference (\( F_{2,173} = 6.8639, P = 0.0014, n = 197 \)), with larger males showing stronger preference than medium (\(|Z| = 2.754, P = 0.0159 \)) and small males (\(|Z| = 3.430, P = 0.0017 \)). Large males preferred to mate with large females (\( \text{Pref mean} \pm SE = 0.4110 \pm 0.0618, t = 3.840, P = 0.0003, n = 89 \)). Medium males (\( \text{Pref mean} \pm SE = 0.1919 \pm 0.0828, t = 1.910, P = 0.0611, n = 63 \)) and small males showed no preference (\( \text{Pref mean} \pm SE = -0.0702 \pm 0.1263, t = 0.4040, P = 0.6880, n = 50 \)).

As in the first experiment, there was no difference in the strength of preference according to genotype (\( F_{1,173} = 0.6657, P = 0.4159 \)). Both SR (\( \text{Pref mean} \pm SE = 0.2508 \pm 0.0887, t = 4.153, P = 0.0001, n = 69 \)) and ST males (\( \text{Pref mean} \pm SE = 0.2156 \pm 0.0600, t = 5.464, P < 0.0001, n = 128 \)) preferred large females. After controlling for the effect of eyespan group (large, medium, small eyespan), there was still no effect of genotype on the strength of preferences (all \( P > 0.4 \)), nor any interaction between eyespan
group and genotype \((F_{2,170} = 0.2449, P = 0.7830)\). Both SR and ST males preferred large females in the first (SR \(Pref\) mean ± SE = 0.3871 ± 0.1181, \(P = 0.0044, n = 61\); ST \(Pref\) mean ± SE = 0.2982 ± 0.0898, \(P = 0.0019, n = 114\)), second (SR \(Pref\) mean ± SE = 0.4286 ± 0.1218, \(P = 0.0018, n = 56\); ST \(Pref\) mean ± SE = 0.1800 ± 0.0988, \(P = 0.0066, n = 100\)), and third (SR \(Pref\) mean ± SE = 0.3191 ± SE 0.1397, \(P = 0.0011 n = 47\); ST \(Pref\) mean ± SE = 0.2093 ± 0.1061, \(P = 0.0007, n = 86\)) mating, and there was no difference in the strength of SR and ST preference across these matings (1\(^{st}\) mating \(F_{1,174} = 0.3541, P = 0.5525\); 2\(^{nd}\) mating \(F_{1,154} = 2.4044, P = 0.1230\); 3\(^{rd}\) mating \(F_{1,131} = 0.3874, P = 0.5437\)). The frequency of failing to mate at least once was unaffected by genotype (SR: 7/69, ST: 14/128, \(\chi^2_{1} = 0.0059, P = 0.9386, n = 197\)) or eyespan (large: 9/89, medium: 3/63, small: 10/38, \(\chi^2_{1} = 5.6826, P = 0.05835\)).

### 2.4.3 Mating frequency

SR males mated less often than ST males in the thirty-minute observation period (SR mean ± SE = 2.6127 ± 0.1445; ST mean ± SE = 3.2857 ± 0.1392, \(\chi^2_{1,330} = 5.5672, P = 0.0183\)). Genotype had a strong effect on mating frequency in large eyespan flies (\(\chi^2_{1,233} = 9.8030, P = 0.0017\)), but not in medium (\(\chi^2_{1,57} = 0.4153, P = 0.5193\)) or small eyespan flies (\(\chi^2_{1,36} = 0.0001, P = 0.9915\)). In the second experiment, males with large and medium eyespan mated more frequently than small eyespan males (large mean ± SE
$= 3.8876 \pm 0.2268$, medium mean $\pm$ SE $= 3.6031 \pm 0.2165$, small mean $\pm$ SE $= 2.1600 \pm 0.2414; \chi^2_{1,173} = 13.4863, P = 0.0005$).

### 2.5 Discussion

Male mate preferences have been observed across a range of species, even where initially unexpected, for example in polygynous species which lack paternal care or other forms of direct male investment in offspring or mating partners (Edward and Chapman 2011). In this study of stalk-eyed flies, I found that males show preference for large eyespan females. This mirrors previous laboratory and field studies in *T. dalmanni* (Cotton et al. 2015). As in other species, the likely benefit of this preference derives from mating with higher fecundity females (Olsson 1993; Dosen and Montgomerie 2004; Byrne and Rice 2006; Reading and Backwell 2007). Female eyespan reliably indicates fecundity among field caught stalk-eyed flies, where it explains a significant amount of variation in ovarian egg number, even after controlling for body size (Cotton et al. 2010, 2015).

There was no difference between drive and standard males in their strength of preference. In order to compare genotypes independent of differences in size, eyespan was restricted to a narrow range at the large end (7.5-8.5mm) of the distribution. Male eyespan is a highly condition-dependent trait, sensitive to both environmental (David et al. 2000; Cotton et al. 2004) and genetic stress (Wilkinson et al. 1998; Bellamy et al. 2013). By placing limits
on the eyespan of experimental males, I may have inadvertently picked out SR and ST males of equivalent high condition and thereby masked differences between the genotypes. This may be a problem as $X^{SR}$ is predicted to accumulate deleterious alleles due to a lack of recombination. Using large flies may even have selected SR males with higher condition than ST males. To address this concern, a second experiment used males that eclosed from eggs laid on variable quantities of food. This generated a much greater range in male eyespan among experimental males, with both smaller and larger eyespan (3.6 - 9.5mm). Again, there was no difference in the strength of mate preference between SR and ST males. Nor were there preference differences between SR and ST males that had small, medium or large eyespan. I conclude that meiotic drive does not directly affect male mate preference.

The two experiments are similar but not clones of each other. As well as the differences already mentioned in the eyespan range of experimental males, there were minor dietary differences for the tester females. In the first experiment, small females were fed a low value diet known to decrease egg production, and large females were fed a high value diet known to increase egg production (Cotton et al. 2015). In the second experiment, large and small eyespan females were fed the same diet, reducing their fecundity difference. Previous work shows that males independently prefer females with large eyespan and those with high fecundity (Cotton et al. 2015). There
was still male preference for the large eyespan females and no difference in preference between SR and ST males.

The experiments were deliberately designed to simulate the field behaviour of stalk-eyed flies. In the wild, leks form at dusk, attract a restricted number of females (mean 2, range 1-7) and are where most copulations take place at dawn the following day (Cotton et al. 2010). The experimental protocol tracked males for 30 minutes at dawn, allowing males to mate multiply and exert mate preference. The design used here presented males with a binary choice between large and small females and this is appropriate given the biology of stalk-eyed flies. Preference assessments based on choices made between two markedly different phenotypes have been criticised for a number of reasons, in particular that this approach fails to capture a “preference function” based on response to the full range of female phenotypes (Wagner 1998; Cotton et al. 2006). However, there is no particular reason to believe this would impact preferences differently in SR and ST males. In one respect, the experimental design is unrepresentative of natural behaviour, as females leave lek sites once they have mated and females do not mate multiple times with the same male (Cotton et al. 2015). The mating chamber’s design precluded female departure but this does not appear to prejudice the findings. In both experiments, there was no difference between SR and ST male preference for large females in the first, second and third matings. It seems unlikely that the design masked differences in male mate preference between the two genotypes.
My attempt to mimic wild conditions is complicated by the recent discovery of a cryptic *T. dalmanni* species (Paczolt et al. 2017). SR is carried by *T. dalmanni-1*, but has not been detected in the other species, *T. dalmanni-2*. The two species do not readily interbreed and can only be discriminated genetically or by close examination of male genitalia (GS Wilkinson, personal communication). Only *T. dalmanni-1* individuals were used in the experiments here. Previous field work (Cotton et al. 2010; Cotton et al. 2014; Cotton et al. 2015) was carried out in the Gombak valley in Malaysia where both species occur in sympatry (Andrew Pomiankowski, unpublished data). It is not yet known how the presence/absence of meiotic drive affects patterns of sexual selection in the two species.

Although there was no difference in the preference of SR and ST males, I found that large eyespan males showed strong preference and small eyespan males exhibited no preference. Vision is the dominant sensory mode for assessment of potential mates in stalk-eyed flies (Chapman et al. 2005; Chapman et al. 2017). Since stereoscopic vision and visual acuity improve as eyespan increases (Burkhardt and de la Motte, 1983; de la Motte and Burkhardt, 1983), males with larger eyespan will be better able to distinguish differences between females and express stronger preference, just as has been found for female mate preference in *T. dalmanni* (Hingle et al. 2001a). Mean eyespan is smaller in SR than ST males (Wilkinson et al. 1998; Cotton et al. 2014; Meade et al. 2020), and field samples show that
males with smaller eyespan attract fewer females to their lek sites (Cotton et al. 2010). On average SR males will attract fewer females to their leks, and have fewer opportunities for choice. However, the magnitude of this effect may be small as the eyespan difference between SR and ST laboratory-reared males is only ~5% (Meade et al. 2020).

I predicted that SR males would have weak preference if male choice is costly and condition-dependent, but this is not supported by the data. The absence of male-male competition at dawn when most mating takes place (Cotton et al. 2010) and the short amount of time before female lek departure do not point to obvious male preference costs associated with distinguishing between females that have already settled at a lek site. Smaller eyespan may mean that SR males may have fewer opportunities to choose between females and lose out to rival males in establishing ownership of favourable lek sites. But when SR males do attract multiple females, they will likely benefit from preferential mating with large females (leading to fecundity benefits), just like ST males.

A further observation was a lower mating frequency in large eyespan SR males, although this had no effect on their preference. Previous work in T. dalmanni has linked mating rate to accessory gland size, the organ that produces non-sperm components of the ejaculate (Baker et al. 2003; Rogers et al. 2005), and SR males have smaller accessory gland size (Meade et al. 2020). This deficit may arise due to a greater allocation of resources to testes
which are enlarged in large eyespan SR males, presumably to compensate for the destruction of sperm by meiotic drive (Meade et al. 2020). A lower mating frequency was also observed in males with small and medium eyespan, suggesting that SR males are constrained to behave in a similar way to these males. How this different aspect of male mating behaviour affects fitness needs further work.

This is the first study of how meiotic drive influences male mating preference. It has wider significance as drive is associated with lower genetic quality due to mutation accumulation in the XSR inversion. But there was no weakening in the strength of drive male preference. Our results suggest that the expression of male mate preference is not condition-dependent (Cotton et al 2006). Male (and female) mate preference may not incur significant costs when there are multiple females (males) to choose between. This contrasts with other aspects of male mating behaviour, like attracting females and warding off competitors, which are likely to be costly and condition-dependent. We observed a reduction in preference as male eyespan decreased and this is likely to affect drive males more, as their eyespan on average is reduced. To fully gauge the impact of these findings, further work on mate choice will focus on whether the expected reduced eyespan of drive males impacts their ability to dominate lek sites and attract females.
2.6 References


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2.7 Figure legends

Figure 1
Mating chambers used for male mate preference assay. A single male of unknown genotype was placed in the top compartment, with two tester females (one large, one small) in the bottom compartment. Males and females were kept separate by a removable partition until testing commenced. A string, resembling a rootlet, runs the length of the chamber, to provide a roosting site. Reproduced with permission from Cotton et al. (2015).

Figure 2
Frequency distribution of male preference values for SR (top) and ST (bottom) males from the first experiment. Preference is given by $Pref = (C_L - C_S)/(C_L + C_S)$, where $C_L$ and $C_S$ are the number of copulations with large and small females respectively. Positive values indicate preference for mating with large females, and negative values indicate preference for mating with small females.

Figure 3
Line graph showing the regression of male preference ($Pref$) on eyespan for ST and SR males from the second experiment. Shaded areas represent 95% confidence intervals.
2.8 Figures

Figure 1

- **Focal male**
- **Roosting string**
- **Removable partitions**
- **Tester females**
Figure 3

Preference vs. Male eyespan (mm) for different genotypes.
Meiotic drive reduces egg-to-adult viability in stalk-eyed flies
3.1 Abstract

A number of species are affected by *sex-ratio* meiotic drive (SR), a selfish genetic element located on the X chromosome that causes dysfunction of Y-bearing sperm. SR is transmitted to up to 100% of offspring, causing extreme sex ratio bias. SR in several species is found in a stable polymorphism at a moderate frequency, suggesting there must be strong frequency-dependent selection resisting its spread. I investigate the effect of SR on female and male egg-to-adult viability in the Malaysian stalk-eyed fly, *Teleopsis dalmanni*. SR meiotic drive in this species is old and appears to be broadly stable at a moderate (~20%) frequency. I use large-scale controlled crosses to estimate the strength of selection acting against SR in female and male carriers. I find that SR reduces the egg-to-adult viability of both sexes. In females, homozygous females experience greater reduction in viability ($s_f = 0.242$) and the deleterious effects of SR are additive ($h = 0.511$). The male deficit in viability ($s_m = 0.214$) is not different from that in homozygous females. The evidence does not support the expectation that deleterious side-effects of SR are recessive or sex-limited. We discuss how these reductions in egg-to-adult survival, as well as other forms of selection acting on SR, may maintain the SR polymorphism in this species.
3.2 Introduction

Meiotic drivers are selfish genetic elements that subvert the standard mechanisms of gametogenesis to promote their own transmission (Lindholm et al. 2016). During meiosis, a driver disables or prevents the maturation of gametes that contain the non-driving element (Burt and Trivers 2006; Lindholm et al. 2016). In extreme cases, drive can reach 100% transmission to the next generation. In male heterogametic species, drivers are most frequently found on the X-chromosome (Hurst and Pomiankowski 1991), commonly known as ‘Sex-Ratio’ or SR (Hurst and Werren 2001). These drivers target developing sperm carrying the Y chromosome, causing their dysfunction, which results in strongly female biased broods.

SR is predicted to spread rapidly due to its transmission advantage. When homozygous female fitness is not greatly reduced, SR could potentially spread to fixation and cause population collapse and extinction through massive sex ratio imbalance (Hamilton 1967; Hatcher et al. 1999). Empirical evidence for this is limited to laboratory environments where drive causes extinction in small populations (Lyttle 1977; Price et al. 2010; Galizi et al. 2014) and a single putative example under natural conditions (Pinzone and Dyer 2013). More typically, studies in wild populations find that drive exists as a low-frequency polymorphism (Pinzone and Dyer 2013; Price et al. 2014; Verspoor et al. 2018), with persistence that can span over a million years (Kovacevic and Schaeffer 2000; Paczolt et al. 2017). In order for SR to
persist as a polymorphism, there must be frequency-dependent selection, allowing spread when rare but retarding further increases in frequency as drive becomes more common. The selective counter-forces that fulfil this requirement may act in males or females but in general they are not well understood. I discuss potential causes of selection first in males and then females in the following sections.

Selection on male viability may be associated with the drive chromosome. It is likely to operate in a frequency-independent manner and as a consequence will not have a stabilizing effect on the frequency of drive (Edwards 1961; Carvalho and Vaz 1999). But it has been suggested that there will be negative frequency-dependent selection on male fertility (Jaenike 1996). This has intuitive appeal because the spread of SR causes the population sex ratio to become increasingly female biased. In such a population, the average male mating rate will increase. If SR male fertility increases at a lower rate than non-drive (ST) male fertility when males mate many times (for instance because SR males are sperm limited), then a polymorphism could be stabilised (Jaenike 1996). Decreased male fertility under multiple mating is a general feature observed in many drive systems (Jaenike 1996; Beckenback 1978; Atlan et al. 2004). However, for this effect alone to prevent SR fixation, SR male fertility must fall to less than half that of ST males as the mating rate increases (Jaenike 1996), a condition not met in a number of species that nonetheless are found with stable SR polymorphism (Carvalho and Vaz 1999). A related suggestion is that SR
males may be out-competed at higher mating rates, supported by some evidence that SR males are poor sperm competitors (Wu 1983a; Wilkinson and Fry 2001; Price et al. 2008). However, the strength of sperm competition weakens as SR spreads, as this reduces the number of competitor males in the population, which seems unlikely to exert a stabilizing effect on SR frequency. SR males may do poorly in other forms of male-male competition if SR is generally associated with poor performance. Such effects are likely to decrease as drive spreads and males become rare, again making it unlikely that this form of selection will stabilize drive. Models that combine the effects of decreased male fertility and reduced sperm competitive ability on SR frequency dynamics find they can lead to a stable polymorphism (Taylor and Jaenike 2002). But this equilibrium can be destabilised by perturbations in either the population sex ratio or the frequency of SR. In particular, given a meta-population of small demes, slight fluctuations in SR frequency are likely to cause drive to spread to fixation, resulting in population extinction (Taylor and Jaenike 2003).

Suppressors are another selective force operating in males that limit the spread of drive alleles. Most obviously, selection favours the evolution of suppression on chromosomes targeted by drivers for dysfunction. In an SR system with complete drive, if resistance is linked to the Y-chromosome, it restores transmission to Mendelian levels, while non-resistant Y-chromosomes are not transmitted at all (Thomson and Feldman 1975). Y-linked suppressors are therefore expected to spread quickly even if they
have deleterious side effects (Wu 1983b). Unlinked suppressors will also be favoured because drive in males causes gamete loss and is often associated with dysfunction amongst the surviving, drive-carrying sperm. Reduced sperm number is likely to reduce organismal fertility. Additionally, as SR spreads it causes the population sex ratio to become female-biased, providing a further advantage to suppressors as they increase the production of male offspring, which have higher reproductive value than female offspring (Fisher 1930; Carvalho et al. 1998). The spread of suppressors reduces the advantage of drive and could lead to its loss. But both types of suppressors are under negative frequency-dependent selection, because a lower frequency of drive reduces selection in their favour. Under some circumstances this could lead to a stable polymorphism at the drive locus. Y-linked and autosomal suppressors of SR drive have been detected in a number of species including *Drosophila simulans*, *D. affinis*, *D. subobscura*, *D. quinara*, *D. mediopunctata* and *Aedes aegypti* (Jaenike 2001). The evolution of suppressors can be remarkably rapid. For example, in the Paris SR system of *D. simulans*, the increase of SR from less than 10% to more than 60% in a mere five years has been matched by a similar increase in suppressor frequency over the same time period (Bastide et al. 2013). While suppressors are common, they are not universal and have not been detected in *D. pseudoobscura*, *D. recens* and *D. neotestacea* (Jaenike 2001). In these systems, other factors are therefore necessary to explain extant SR polymorphism.
Another force that may prevent SR fixation is reduced fitness of female carriers (Curtsinger and Feldman 1980). As male X-linked drive causes defects in spermatogenesis, there is no obvious mechanistic carry-over to female oogenesis. Likewise, examples of meiotic drive in female gametogenesis, which affect the biased segregation of chromosomes into the egg or polar bodies, show no carry-over to segregation bias in male gamete production (Burt and Trivers 2006). For selection to act against female carriers, the drive locus must either have direct pleiotropic fitness effects or be in linkage with alleles that impact fitness. Linkage is a plausible explanatory factor given that drive systems are often located in genomic regions with low recombination rates, such as in inversions (Beckenbach 1983; Silver 1993; Dyer et al. 2007; Reinhardt et al. 2014). If the inversion is at low frequency, it will rarely be homozygous and the recombination rate among SR chromosomes will be low. Inversions also severely limit the exchange of genes with the homologous region on the standard chromosome (as this requires a double cross-over within the inverted region; Navarro et al. 1997; Navarro and Ruiz 1997). The consequence is that low frequency inversions will be subject to weak selection and suffer the accumulation of a greater mutation load (Dyer et al. 2007; Kirkpatrick 2010). Recessive viability and sterility effects are expected as they will not be evident in females until the frequency of drive is high enough for homozygotes to be common. In contrast, hemizygosity in males means recessive and dominant effects are always expressed and will be more strongly selected against. In general, SR inversions are expected to be
enriched for sexually antagonistic alleles that benefit the sex in which drive occurs (Rydzewski et al. 2016). This means that we expect that loss of fitness will be greater in females and likely to be recessive. These effects produce relevant frequency dependence that restricts fixation of drive. Severe reductions in female viability and fertility in SR homozygotes, along with SR heterozygotes, have been reported in several Drosophila species (Wallce 1948; Curtsinger and Feldman 1980; Dyer et al. 2007). But it is surprising how rarely viability effects of drive in either sex have been studied, compared to fertility effects in males (Price and Wedell 2008). These deleterious consequences are likely to build up and lead to a reduction in SR frequency through time (Dyer et al. 2007).

Large-scale chromosomal inversions are not a universal feature of SR, however. Inversions are not present in the Paris SR system in D. simulans (Jaenike 2001). Despite this, SR must be weakly deleterious in this species as it is rapidly declining in frequency in populations that have recently become completely suppressed (Bastide et al. 2011). The deleterious effects of the Paris SR chromosome must arise due to the drive genes themselves or a tightly linked region. The genetically distinct Winters SR system in the same species also lacks association with an inversion (Kingan et al. 2010). It persists despite having been completely suppressed for thousands of years, suggesting it does not cause any pleiotropic fitness deficit (Kingan et al. 2010). These are the only well characterised examples of meiotic drive not being associated with inversions, so this feature may be a rarity.
Another aspect operating in females concerns behavioural resistance to the spread of SR. Laboratory experiments suggest that increased levels of polyandry can be selected as a defence mechanism against SR (Price et al. 2008). This benefit arises when drive male sperm are weak competitors against wildtype male sperm (Price and Wedell 2008). Recent modelling work shows that polyandry helps prevent invasion of SR but alone cannot prevent fixation of drive (Holman et al. 2015). As drive spreads, additional matings have a lower probability of involving wildtype males, so the disadvantage to drive sperm declines. There needs to be positive frequency-dependent costs to achieve a stable polymorphism (Holman et al. 2015), for instance, when homozygous females have lower viability than heterozygotes. If a stable polymorphism can evolve, the frequency of drive should decline with the rate of female remating. There is evidence in favour of this idea in D. neotestacea which exhibits a stable cline in SR frequency that correlates negatively with the frequency of polyandry (Pinzone and Dyer 2013), and a similar pattern has been reported in D. pseudoobscura (Price et al. 2014). Alternatively, females may simply avoid mating with SR males (Lande and Wilkinson 1999; Pomiankowski and Hurst 1999). In stalk-eyed flies, females prefer to mate with males with large eyespan (Wilkinson et al. 1998; Cotton et al. 2010), a trait that is reduced in SR males (Wilkinson et al. 1998; Johns et al. 2005; Cotton et al. 2014). Sexual selection may therefore be acting in this species to limit the spread of SR. However, this form of selection against drive is likely to be restricted to a sub-set of species with drive, as it requires
the linkage of SR with a conspicuous trait subject to mate choice (Pomiankowski and Hurst 1999). Another potential example is the autosomal \( t \)-locus system in mice which is proposed to be detectable in mate choice through olfaction (Coopersmith and Lenington 1990) but this preference has not been confirmed (Sutter and Lindholm 2015). A counter example is in \( D. \) pseudoobscura, where females do not avoid mating with SR males, though there would be considerable benefit to doing so (Price et al. 2012).

In this study, I determine the effect of SR meiotic drive on viability in the Malaysian stalk-eyed fly, \( Teleopsis dalmanni \). My objective was to assess whether there is a SR-linked deleterious mutation load leading to higher developmental mortality before adult eclosion. Populations of this species carry SR at a moderate level of \( \sim 20\% \) but with considerable variation among populations (Presgraves et al. 1997; Wilkinson et al. 2003; Paczolt et al. 2017). SR resides within a large paracentric inversion (or inversions) that covers most of the X chromosome (Johns et al. 2005). There is no recombination between SR and ST haplotypes (Paczolt et al. 2017) and the lower frequency of SR in the wild means SR homozygous recombination events are relatively rare (at 20\%, the recombination rate of SR is a quarter that of ST). SR is absent from a cryptic species of \( T. dalmanni \) estimated to have diverged \( \sim 1 \) Mya (Paczolt et al. 2017). X-linked meiotic drive is also present in the more distantly related species \( T. whitei \), which diverged 2-3.5 Mya (Swallow et al. 2005; Paczolt et al. 2017). But to what extent the mechanism or genetic basis is conserved remains to be established.
The ancient origin of the $X^{SR}$ chromosome and limited recombination across the $X^{SR}$ chromosome are predicted to have led to the accumulation of deleterious alleles. Consistent with a lack of recombination, there are 955 fixed sequence differences between transcripts linked to $X^{SR}$ and $X^{ST}$ (Reinhardt et al. 2014). The main evidence for a deleterious effect of $X^{SR}$ on fitness is the reduced eyespan of SR males (Wilkinson et al. 1998; Cotton et al. 2014). Male eyespan is an exaggerated, highly condition-dependent trait used in female mate choice (Wilkinson et al. 1998; Cotton et al. 2004), as well as signalling between males (Panhuys and Wilkinson 1999; Small et al. 2009), which reflects male genetic and phenotypic quality (David et al. 2000; Cotton et al. 2004; Howie et al. 2019). However, in a series of experiments Wilkinson et al. (2006) found little direct evidence that SR reduces fitness components. Although larval viability was not directly assessed, progeny production showed no difference between SR and ST homozygous females (Wilkinson et al. 2006). Another study compared offspring genotypes of heterozygous females mated to ST males, and reported little deviation from expected progeny genotype ratios assuming no viability selection differences (Johns et al. 2005). Adult survival did not vary with genotype in either males or females (Wilkinson et al. 2006). There was no evidence for a deleterious effect of $X^{SR}$ on female fecundity, rather heterozygotes were more productive, suggesting overdominance (Wilkinson et al. 2006). However, sample size in these experiments was small, and fecundity/fertility results were based on progeny counts which are confounded by genotype effects on
larval survival. The only significant detriment reported was in SR male fertility which was reduced when males were allowed to mate with large numbers of females (eight) for 24 hours (Wilkinson et al. 2006). However, a further experiment that measured male fertility through counts of fertile eggs (avoiding any confounding impact of larval survival), failed to show any difference between SR and ST male fertility (Meade et al. 2020).

To better understand these previous results, I was motivated to explicitly test for differences in larval survival. The experimental design was similar to that used in early investigations of *D. pseudoobscura* (Wallace 1948; Curtsinger and Feldman 1980). Controlled crosses were carried out to produce eggs with all possible SR and ST male and female genotypes. These were reared together to ensure exposure to similar environmental variation. The sample size was large to maximize power to detect genotypic survival differences. Offspring were genotyped at adult eclosion, yielding observed genotype ratios in order to estimate the selection coefficients operating against drive in both sexes. The principal aims were to test whether the SR-drive chromosome causes viability loss during egg-to-adult development, and whether fitness effects are recessive or sex-limited.

### 3.3 Methods

#### 3.3.1 Fly stocks and maintenance
A standard stock population was obtained from Ulu Gombak in Malaysia (3°19’N 101°45’E) in 2005 (by Sam Cotton and Andrew Pomiankowski). Stock flies are reared in high-density cage culture (cage size approx. 30 x 20 x 20cm) at 25°C on a 12:12 hour light:dark cycle, and fed puréed corn ad libitum. Fifteen minute artificial dawn and dusk phases are created by illumination from a single 60-W bulb at the start and end of each light phase. Meiotic drive is absent from the standard stock population.

A meiotic drive stock was created using flies collected from the same location in 2012 (Cotton et al. 2014). Meiotic drive is maintained in this stock by following a standard protocol (Presgraves et al. 1997; Meade et al. 2019). Females heterozygous for the drive chromosome are mated to males from the standard stock. It is expected that half their male offspring will inherit the drive chromosome. All male offspring are crossed to three females from the standard stock and the sex ratio of their progeny scored. Males that sire all-female broods of at least 15 individuals are considered to be carriers of meiotic drive. In the meiotic drive stock, drive strength is 100% percent, and no males are produced by X^{SR}/Y males carrying the drive chromosome (Meade et al. 2019). Progeny from drive males are female heterozygotes for the drive chromosome. They are subsequently mated to standard males, and the process is repeated.

3.3.2 Experimental crosses
To generate the five possible genotypes of both females ($X^{ST}/X^{ST}$, $X^{SR}/X^{ST}$, $X^{SR}/X^{SR}$) and males ($X^{ST}/Y$, $X^{SR}/Y$), two crosses were performed (Figure 1).

In Cross A, drive males ($X^{SR}/Y$) were mated to heterozygous females ($X^{SR}/X^{ST}$). This cross produces $X^{SR}/X^{SR}$ and $X^{SR}/X^{ST}$ female zygotes in equal proportions. In Cross B, standard males ($X^{ST}/Y$) were mated to heterozygous females ($X^{SR}/X^{ST}$). This cross produces $X^{ST}/Y$ and $X^{SR}/Y$ male, and $X^{ST}/X^{ST}$ and $X^{SR}/X^{ST}$ female zygotes in equal proportions. Experimental males were collected from the drive stock that were approximately 50:50 $X^{ST}/Y$ and $X^{SR}/Y$ males. They were crossed to standard stock females ($X^{ST}/X^{ST}$) and one larva per male was genotyped to define the paternal genotype. Experimental females heterozygous for drive ($X^{SR}/X^{ST}$) were collected from crosses between drive males and females from the standard stock.

Individual males were placed with three virgin females in 500ml pots. Females that died during the experiment were replaced, but males were not. 25 Cross A and 50 Cross B pots were set-up. The base of each pot was lined with moistened cotton wool covered with blue tissue paper to aid egg visualisation. The cotton bases were removed for egg collection and replaced three times per week. Fertilised eggs were identified under light microscopy as those that showed signs of development (e.g. segmental striations, development of mouthparts; Baker et al. 2001) and transferred to a 90mm petri dish containing a large cotton pad moistened with 15ml of water and 2.5ml of food. Three different food conditions were used that varied in their corn content: 25% corn, 50% corn, and 75% corn. In each mixture the
remainder was made up with a sucrose solution (25% sucrose/water w/w). To ensure the sucrose solution had a similar viscosity to puréed corn, an indigestible bulking agent was added (methylcellulose, 3% w/w; Rogers et al. 2008). 4 eggs from Cross A and 8 eggs from Cross B were transferred to each petri dish. This gives the five possible genotypes ($X^{ST}/X^{ST}$, $X^{SR}/X^{ST}$, $X^{SR}/X^{SR}$, $X^{ST}/Y$, $X^{SR}/Y$) in an expected 1:2:1:1:1 ratio (Table 1). Prior to the end of development, six Petri dishes were placed inside a large cage and all eclosing adult flies were collected. The cage was used as a level of analysis of the relative egg-to-adult viability of different genotypes in the subsequent analyses.

### 3.3.3 Genotyping

DNA was extracted in 96-well plates using a modification of a standard isopropanol precipitation protocol (Green and Sambrook 2017). Half a fly thorax was added to a well containing 4 μl Proteinase K (10 mg.ml$^{-1}$) and 100μl DIGSOL (25mM NaCl, 1mM EDTA, 10mM Tris–Cl pH 8.2), mechanically lysed, and incubated overnight at 55°C. The following day, 35μl of 4M ammonium acetate was added and plates were left on ice for 5 minutes before being centrifuged at 4500RPM at 4°C for 40 minutes. 80μl of supernatant was then aspirated into a new 96-well plate containing 80μl of isopropanol. The precipitate was discarded. Samples were then centrifuged again at 4500RPM and 4°C for 40 minutes to precipitate the DNA. The supernatant was then discarded, 100μl 70% ethanol was added, and
samples were spun again at 4500RPM and 4°C for 20 minutes. The supernatant was once again discarded and plates were left to air-dry for 45 minutes at room temperature. Finally, 30μl of Low TE (1mM Tris-HCL pH8, 0.1mM EDTA) was added to elute the DNA. DNA was PCR-amplified in 96-well plates, with each well containing 1μl of dried DNA, 1μl of primer mix (consisting of the forward and reverse primers of *comp162710* at a concentration of 0.2μM) and 1μl of QIAGEN Multiplex PCR Mastermix (Qiagen). The length of amplified fragments was determined by gel electrophoresis. A 3% agarose gel was made using 3g of molecular grade agarose, 100ml of 0.5x TBE buffer (45mM Tris (pH 7.6), 45mM boric acid, 1mM EDTA), and 4μl ethidium bromide. PCR products were diluted with 3μl ultrapure water and 2μl of gel loading dye was added. 4μl of this mixture was loaded into each well and assessed for size against a ladder made from the PCR-amplified DNA of multiple heterozygous drive females. *Comp162710* is an indel marker with small alleles (201bp) indicating the presence of the drive chromosome and large alleles (286bp) indicating the presence of the standard chromosome (GS Wilkinson 2017, personal communication; Meade et al. 2019).

### 3.3.4 Statistical analysis

I used two approaches to estimate the egg-to-adult viability costs of the X\(^{SR}\) chromosome. The first estimated the relative egg-to-adult viability cost of each genotype. The second estimated the strength of selection against drive
in males and females, as well as the dominance coefficient. Model outputs are given in details in the supplementary material, Table S1-S7.

3.3.5 Egg-to-adult viability of each genotype

In the first analysis, the number of eclosed adult flies of each genotype was compared to the number expected at the level of the cage. Each cage contained six petri dishes with 12 eggs, producing a maximum of 72 flies. Genotyping effort varied across cages and sexes. The expected number of each genotype was determined with respect to the genotyping effort of the relevant sex for a particular cage. For example, if 24 males were collected from a given cage, and 75% of these males were genotyped, then the expected number of $X_{SR}/Y$ individuals is $(24 \times 0.75) / 2 = 9$. Due to the nature of the experimental design, I expected twice as many $X_{SR}/X_{ST}$ females compared with $X_{SR}/X_{SR}$ and $X_{ST}/X_{ST}$ females. For example, in a cage with 36 genotyped females I expected 18 $X_{SR}/X_{ST}$ females and 9 each of the remaining two female homozygotes. I then divided the observed number of flies of a given genotype by the expectation for that genotype to obtain the cage estimate of egg-to-adult viability. I split the data by sex and analysed the relationship between egg-to-adult viability and genotype using linear mixed-effect modelling with lme4 (Bates et al. 2015) in R (R Core Team 2018). Genotype and food condition were modelled as fixed effects and cage ID and collection date as random effects. Significance of model terms was
determined using the lmerTest R package (Kuznetsova et al. 2017). Mean viability measures were estimated using model terms.

### 3.3.6 Estimating the strength of selection against drive

In the second analysis, I estimated the strength of selection against drive using Bayesian inference, separately for males and females. Cage survival frequencies for each genotype were pooled. The probability of drawing the male genotype distribution was calculated for values of the selection coefficient taken from a uniform prior distribution for $s_m = 0 - 1$, in 0.001 increments. I then used a binomial model to determine the likelihood of drawing the observed number of $X^{ST}/Y$ and $X^{SR}/Y$ males for each value of $s_m$. As I used a uniform prior, the posterior probability simplifies to the likelihood. The 95% and 99% credible intervals were determined from the probability density. The probability of observing the distribution of the three female genotypes was estimated under a multinomial where the values of $s_f$ and $h$ (Table 1) were taken from a uniform prior distribution for every combination of values of $s_f$ and $h$ ranging from 0 - 1, in 0.001 intervals. The 95% and 99% credible intervals were determined in the same way as in males, and displayed as a two-dimensional contour. Note that the probability of drawing $X^{SR}/X^{ST}$ females was multiplied by two because the experimental design was expected to generate twice as many heterozygote eggs compared to all of the other genotypes. To determine if $s_m$ and $s_f$ were of different strength, 1000 random samples each of $s_m$ and $s_f$ (taking $h$ equal to its mode) were
drawn from the posterior distributions with probability of drawing a value equal to its likelihood. A distribution of differences was obtained by subtracting the randomly drawn $s_r$ values from the randomly drawn $s_m$ values. A z-score was calculated to determine if this distribution is different from zero.

I also estimated the difference in the strength of selection between female genotypes. To compare egg-to-adult viability between wildtype ($X^{ST}/X^{ST}$) and heterozygous ($X^{SR}/X^{ST}$) females, the likelihood of observing the counts of these two genotypes was determined under a binomial as above but shrinking $h$ and $s_r$ to a single term with a uniform prior. The process was repeated to compare drive heterozygotes ($X^{SR}/X^{ST}$) and homozygotes ($X^{SR}/X^{SR}$).

### 3.4 Results

#### 3.4.1 Effect of food condition

Food condition had no overall effect on the egg-to-adult viability of males ($F_{2,72} = 0.1085, P = 0.8973$) or females ($F_{2,54} = 0.1552, P = 0.8566$), nor did it alter the genotype response (genotype-by-condition interaction, males $F_{2,79} = 0.8026, P = 0.4518$; females $F_{4,116} = 0.2044, P = 0.9355$). So, offspring counts were pooled across food conditions within sexes in the following analyses.
3.4.2 Egg-to-adult viability of each genotype

From a total of 96 cages, each containing 72 eggs, I collected a total of 1065 males and 2500 females, of which 798 and 1272 were genotyped respectively. Male genotype had a significant effect on egg-to-adult viability, with $X^{SR}/Y$ males showing significantly reduced viability ($F_{1,81} = 11.7296$, $P < 0.001$). $X^{ST}/Y$ males had a mean viability of 0.5412, and $X^{SR}/Y$ males had a mean viability of 0.4036 (Figure 2). Genotype also had a significant effect on egg-to-adult viability in females ($F_{2,120} = 4.7593$, $P = 0.0103$). Mean viability was 0.6294 in $X^{ST}/X^{ST}$ females, 0.5491 in $X^{SR}/X^{ST}$ females, and 0.4650 in $X^{SR}/X^{SR}$ individuals. A Tukey’s post-hoc comparison test revealed that the viability of $X^{ST}/X^{ST}$ females was greater than $X^{SR}/X^{SR}$ females ($P = 0.0104$), while $X^{SR}/X^{ST}$ females had intermediate viability, but not different from either homozygote ($X^{SR}/X^{ST} – X^{SR}/X^{SR}$ comparison: $P = 0.2949$; $X^{SR}/X^{ST} – X^{ST}/X^{ST}$ comparison: $P = 0.3293$; Figure 2).

3.4.3 Estimating the strength of selection against drive

The posterior probability of each value of the male selection parameter $s_m$ is given in Figure 3. The mode of $s_m = 0.214$ with a 95% credible interval 0.097 – 0.316 and a 99% credible interval 0.056 – 0.346. The probability of the modal value compared to the null hypothesis of no viability selection against drive males has a Bayes Factor $BF_{10} = 321.79$. 

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The posterior probability of each combination of the female selection parameters $s_f$ and $h$ values is shown in Figure 4. The modal values are $s_f = 0.242$ and $h = 0.511$, with the bivariate 95% and 99% credible interval displayed as a two-dimensional contour (Figure 4). The probability of the modal $s_f$ value compared to the null hypothesis of no viability selection against drive in females has a Bayes Factor $BF_{10} = 572.89$. The strength of selection against drive in males and females ($s_f$ and $s_m$; setting $h$ to its modal value), did not differ between the sexes ($|z| = 0.3785$, $\alpha = 0.01$ $P = 0.7047$).

In the pairwise comparison of individual female genotypes there was a difference between the egg-to-adult viability of $X^{ST}/X^{ST}$ and $X^{SR}/X^{ST}$ females, with a selection coefficient mode $= 0.126$ with a 95% credible interval $= 0.007 – 0.232$ and a 99% credible interval $= -0.017 – 0.261$. A similar difference was observed in the comparison of $X^{SR}/X^{ST}$ and $X^{SR}/X^{SR}$, with a selection coefficient mode $= 0.138$ with a 95% credible interval of $0.008 – 0.252$ and a 99% credible interval of $-0.038 – 0.287$.

### 3.5 Discussion

Due to their two-fold transmission advantage in males, X chromosomes that exhibit sex-ratio meiotic drive ($X^{SR}$) potentially can spread to fixation and cause population extinction (Hamilton 1967; Hatcher et al. 1999). Despite this, several meiotic drive systems exist in broadly stable polymorphisms
(Wilkinson et al. 2003; Pinzone and Dyer 2013; Price et al. 2014). This suggests that there are costs of carrying the $X^{SR}$ chromosome. In the stalk-eyed fly system, the $X^{SR}$ chromosome contains a large inversion (Johns et al. 2005), which is expected to accumulate deleterious mutations as they are less efficiently removed by recombination than those on the $X^{ST}$ chromosome. This mutation load is expected to lead to a decrease in fitness of the $X^{SR}$ chromosome. Here, controlled crosses were used to estimate one component of fitness, egg-to-adult viability, of meiotic drive genotypes. There was a reduction in viability linked to $X^{SR}$ in both males and females. In $X^{SR}$ hemizygous males this was $s_m = 21\%$ (Figure 3) and in $X^{SR}$ homozygous females $s_f = 24\%$ (Figure 4). The negative effect of $X^{SR}$ in females was largely additive ($h \approx 0.5$), with heterozygotes being intermediate in viability compared to homozygotes. The estimates of selection ($s_m$ and $s_f$) do not differ between the sexes. This probably reflects a lack of sexual dimorphism in fitness at the larval stage. In $D. melanogaster$, egg-to-adult viability measured for particular genotypes is strongly positively correlated across the sexes, whereas adult reproductive success is typically negatively correlated (Chippendale et al. 2001; Arnqvist and Tuda 2010).

In the experiment, individual males of known genotype, either SR or ST, were crossed with heterozygous females. Eggs were collected and combined in groups of 6 petri dishes each containing 12 eggs. The eggs were visually inspected for signs of development, so as to be able to exclude the possibility that differential fertility of the two paternal genotypes (i.e. SR or ST) affected
the subsequent output of adult flies. In addition, a pilot experiment showed equal levels of SR and ST male fertility in conditions similar to those used here (supplementary material, Table S8). The combination of eggs from the two crosses were expected to generate all five genotypes in an even ratio, except for heterozygous females which were expected at double the number of the other genotypes. The objective was to standardise competition between genotypes. It is hard to estimate whether this objective was attained, as only surviving adults were genotyped. The observed adult genotype frequencies were compared to infer genotype-specific survival in the egg-to-adult stage. The number of flies genotyped was sufficiently large ($N_m = 798, N_f = 1272$) to give reasonable assurance of the accuracy of the estimates. Even with this sizeable sample, the bounds on the estimates of $s_m, s_i$ and $h$ remain large (Figure 3-4) but I can be confident that drive is associated with loss of viability in both sexes. These results contrast with a prior study showing that adult lifespan is independent of SR genotype in males and females (Wilkinson et al. 2006), revealing a difference between larval and adult genotypic effects. This previous study also suggested that larval survival is independent of SR genotype (Wilkinson et al. 2006). The reasons for this difference are unclear; there could be differences that relate to food and housing, the mixture of genotypes undergoing larval competition or the SR haplotype used as those in Wilkinson et al. (2006) cause less than 100% transmission distortion. This suggests that further investigation is warranted in a number of directions.
This is the first study showing a reduction in SR viability in stalk-eyed flies. Similar methods have been applied previously in *D. pseudoobscura* (Wallace 1948; Curtsinger and Feldman 1980; Beckenbach 1983). Wallace observed strong selection against $X^{SR}$ in both sexes. In high density populations (Wallace 1948), Beckenbach found a reduction in $X^{SR}/Y$ viability but no homozygous $X^{SR}$ female viability effect (Beckenbach 1983). In contrast, Curtsinger and Feldman report stronger selection against homozygous $X^{SR}$ females (Curtsinger and Feldman 1980). Comparisons of these three studies provides strong evidence to suggest that viability selection is density-dependent, as the reduction in $X^{SR}$ viability was greatest under high density (Wallace 1948), and a lack of differential viability was observed in another experiment carried out at low density (Beckenbach 1983). In the present study, stalk-eyed fly larvae were cultured under low density and provided with excess food. Future work will need to determine whether varying levels of food stress enhance or restrict the deleterious effect of the $X^{SR}$ chromosome.

Strong viability selection against the $X^{SR}$ chromosome, as found here under laboratory conditions, may play a key role in determining the equilibrium level of the SR polymorphism in the wild. There are several other factors that could be involved in determining SR frequency, such as suppressors, polyandry and various forms of sexual behaviour which I discuss further here. First, in *D. simulans*, SR commonly co-occurs with suppressors which restrict the transmission advantage (Merçot et al. 1995; Kingan et al. 2010).
Although early work on the stalk-eyed fly drive system suggested that there were suppressors (Wilkinson et al. 1998), this has not been sustained by further work, either on the autosomes or Y chromosome (Paczolt et al. 2017). Second, polyandry may evolve to limit the spread of SR (Price et al. 2008). Polyandry is the norm in *T. dalmanni* (Baker et al. 2001; Wilksinson et al. 2003), and there is evidence that SR male sperm does less well under sperm competition (Wilkinson et al. 2006) and may suffer from interactions with non-sperm ejaculate components produced by standard males (though this has only been shown in the related species *T. whitei*; Wilkinson and Fry 2001). But it has not been shown whether variation in the degree of polyandry correlates with SR frequency in natural populations of stalk-eyed flies.

Third, it has long been suggested that mate choice may play a role in determining the frequency of drive (Coopersmith and Lenington 1990). This may be important in stalk-eyed flies as they are canonical examples of sexual selection driven by mate choice (Burkhardt and de la Motte 1985; 1988]. In *T. dalmanni*, drive males are expected to attract fewer females as they have reduced eyespan, and hence mate less often (Wilkinson et al. 1998; Cotton et al. 2014. However, there is as yet no evidence in stalk-eyed flies that the strength of female mate preference has been enhanced in populations subject to drive. Nor has there been investigation of whether females that carry SR show alterations in their mating behaviour. A related consideration is male mate preference (Bonduriansky 2001) which has been shown to be an important behavioural adaptation in *T. dalmanni* favouring
male matings with fecund females (Cotton et al. 2015). A recent study reported that SR had no direct effect on male mate choice (Finnegan et al. 2019). However, the strength of male mate preference positively covaries with male eyespan. As drive males have smaller eyespan (Cotton et al. 2014), we expect they will be less discriminating in their mate choice (Finnegan et al. 2019).

Finally, measurements of sperm number per mating report that SR males deliver as many sperm as ST males, and a single mating with a SR male results in the same female fertility as a mating with a ST male (Meade et al. 2019). Whether this pattern carries over to situations where a male can mate with multiple females is less clear. One experiment showed no difference between SR and ST males (Meade et al. 2020), whereas another experiment found lower fertility in SR males (Wilkinson et al. 2006) when multiple females were allowed to mate freely with a single male for a day. The cause of this difference is unclear, but drive males have been shown to have lower mating rates compared to standard males (Meade et al. 2020), and this could conceivably have contributed to lower fertility in females mated to SR males. As mentioned previously, SR males are poor sperm competitors compared with ST males, which must arise from reasons other than numerical sperm transfer from the male (Wilkinson et al. 2006).

The number of different factors set out above make it difficult to predict whether they are sufficient to explain the observed frequency of ~20%
(Wilkinson et al. 2003; Paczolt et al. 2017). Many could act as stabilizing forces which restrict the spread of drive in a frequency-dependent manner. Future work should aim to examine these factors, in combination with the intensity of egg-to-adult viability selection measured here, in a modelling framework in order to predict the evolutionary outcomes. This needs to be coupled to better estimation of ecological and demographic parameters across local populations of *T. dalmanni* in which SR frequency is known to be highly variable (Cotton et al. 2014).
3.6 References


Wu Cl. 1983b. The fate of autosomal modifiers of the sex-ratio trait in

3.7 Figure legends

**Figure 1**
Experimental protocol. Individual males of known genotype were crossed with three heterozygous females in 500ml pots. Cross A produces no males and $X^{SR}/X^{SR}$ and $X^{SR}/X^{ST}$ females, in equal proportions. Cross B produces $X^{SR}/Y$ and $X^{ST}/Y$ males and $X^{ST}/X^{ST}$ and $X^{SR}/X^{ST}$ females, in equal proportions. 4 eggs from Cross A and 8 eggs from Cross B were added to each egglay – a petri dish containing a moistened cotton pad and food. At pupation, 6 egglayes were placed into a population cage and their lids were removed so as to allow the adult flies to eclose.

**Figure 2**
Male and female genotype mean ± standard error egg-to-adult viability. Values were determined from the fraction of a given genotype observed in replicate cages.

**Figure 3**
The posterior probability density of the strength of selection against drive in males ($s_m$). The mode is shown as a dotted red line. The dashed black lines indicate the 95% credible interval. The dotted blue lines indicate the 99% credible interval.

**Figure 4**
The posterior probability density of the strength of selection against drive in females ($s_i$) and the dominance coefficient ($h$). Colour indicates probability density, with darker colours indicating higher likelihood. The black dashed contour shows the 95% credible interval and the blue dotted line shows the 99% credible interval.
3.8 Figures

Figure 1

Cross A
- $X^{SR}/Y$ male
- $X^{SR}/X^{ST}$ female
- 500ml pot
- 4 eggs

Cross B
- $X^{ST}/Y$ male
- $X^{SR}/X^{ST}$ female

Egglay

X 6

Cage
- Egglay
Figure 2

Egg-to-adult viability

$X^{SR}/Y$  $X^{ST}/Y$  $X^{SR}/X^{SR}$  $X^{SR}/X^{ST}$  $X^{ST}/X^{ST}$

Male Genotypes  Female Genotypes
Figure 3
Meiotic drive does not cause condition-dependent reduction of eyespan, the sexual ornament of *Teleopsis dalmanni* stalk-eyed flies.
4.1 Abstract

Like most meiotic drive systems, “sex-ratio” (SR) in *Teleopsis dalmanni* stalk-eyed flies is associated with a large inversion (or inversions) on the X chromosome. The SR X chromosome is expected to suffer the accumulation of deleterious mutations due to reduced recombination, with the standard X and between SR chromosomes, due to low effective population size. I test the prediction that the low genetic quality of SR haplotypes is reflected in heightened condition-dependent expression of eyespan. Male and female larvae were raised under benign and high larval food stress environments. SR males have reduced eyespan under the low stress treatment. There was no evidence of a condition-dependent decrease in eyespan under high stress. Similar but more complex patterns were observed for female eyespan, with evidence of additivity under low stress and heterosis under high stress. These results do not support the hypothesis that eyespan shows condition-dependent expression due to the increased mutation load of the SR haplotype. Instead, reduced eyespan in SR males likely reflects compensatory resource allocation to different traits during development in response to drive-mediated destruction of sperm.
4.2 Introduction

A key tenet of the handicap principle (Zahavi 1975; 1977) is that sexual ornaments show heightened condition-dependent expression (Pomiankowski and Møller 1995; Rowe and Houle, 1996; Cotton et al. 2004a). Historically, empirical support for this was limited as studies lacked comparison with non-sexual control traits (Cotton et al. 2004a). But there are now a number of studies showing that heightened condition dependence is a feature of many sexual traits used in mate preference (Bonduriansky and Rowe, 2005; Johns et al. 2014; Izzo and Tibbetts, 2015). A canonical example is eyespan in the Malaysian stalk-eyed fly, *Teleopsis dalmanni* (Cotton et al. 2004b). Stalk-eyed flies are characterised by lateral elongation of the head capsule into eyestalks (Wilkinson and Dodson, 1997). Many species of stalk-eyed fly, including *Teleopsis dalmanni*, are highly sexually dimorphic for this trait, with males possessing much larger eyespan than females (Burkhardt and de la Motte, 1985). Eyespan is used as a signal in male-male interactions (Panhuis and Wilkinson, 1999; Small et al. 2009) and female choice (Wilkinson and Reillo, 1994; Hingle et al. 2001). In the wild, females prefer to roost and mate with males with larger eyespan, both in absolute terms, and relative to body size (Wilkinson and Reillo, 1994; Cotton et al. 2010). Male eyespan is highly sensitive to both environmental (David et al. 1998; Cotton et al. 2004a) and genetic stress (David et al. 2000; Bellamy et al. 2013; Howie et al. 2019).
Male eyespan in *T. dalmanni* is also associated with *sex-ratio* meiotic drive (SR), a type of selfish genetic element located on the X-chromosome that causes selective destruction of Y-bearing sperm and the production of female-biased broods (Jaenike 2001; Lindholm et al. 2016). The X\textsuperscript{SR} chromosome exists at moderate frequencies (~20%) in wild populations (Wilkinson et al. 2003; Cotton et al. 2014; Paczolt et al. 2017). Male carriers of X\textsuperscript{SR} have reduced eyespan both under laboratory conditions (Wilkinson et al. 1998; Johns et al. 2005; Meade et al. 2019) and in the wild (Cotton et al. 2014). The drive and standard (X\textsuperscript{ST}) chromosomes are differentiated by a large paracentric inversion (or inversions; Johns et al. 2005), spanning at least one third of the chromosome (Paczolt et al. 2017). Inversions are a common feature of many meiotic drive systems that restrict recombination (Hoffmann and Rieseberg, 2008; Kirkpatrick 2010) and are presumed to have been selected to maintain linkage on X\textsuperscript{SR} between genes contributing to meiotic drive (Charlesworth and Hartl, 1978; Jaenike, 2001). The lack of recombination has led to high divergence of X\textsuperscript{SR} and X\textsuperscript{ST}, with almost 1,000 fixed differences (Reinhardt et al. 2014). Long term recombination suppression not only between X\textsuperscript{SR} and X\textsuperscript{ST} but also within X\textsuperscript{SR} itself (due to its moderate population frequency) is expected to lead to a weaker response to selection and an increase in the accumulation of deleterious mutations (Gordo and Charlesworth, 2001), as observed *in extremis* on Y-chromosomes (Orr and Kim, 1998). Several meiotic drive inversions are associated with mutations that severely impact fitness (Jaenike 2001). For example, in the *t*-haplotype autosomal drive system in the house mouse,
**Mus musculus**, many drive haplotypes carry factors that cause embryonic lethality when homozygous (Silver, 1985). In *Drosophila recens*, the entire X chromosome is locked up in a series of overlapping inversions and is fixed for a recessive mutation causing female sterility (Dyer et al. 2007). In the stalk-eyed fly, the large number of fixed differences between $X^{SR}$ and $X^{ST}$ is consistent with this mutation accumulation (Reinhardt et al. 2014), and carriers of the $X^{SR}$ chromosome in both sexes have reduced egg-to-adult viability (Finnegan et al. 2019). These findings lead to the hypothesis that the $X^{SR}$ haplotype carries an increased mutation load, leading to an overall reduction in genetic quality, and this is reflected in the condition-dependent reduction of eyespan.

There is some evidence that the X chromosome in *T. dalmanni* is associated with additive genetic variance for eyespan. First, in a quantitative trait locus (QTL) study of eyespan, Johns et al. (2005) found a major X-linked QTL accounting for 36% of the variation, located just 1.3cM from the putative drive locus and associated with small eyespan in the $X^{SR}$ haplotype. Second, Reinhardt et al. (2014) used RNAseq to identify transcripts that are differentially expressed between $X^{SR}$ and $X^{ST}$. While many of these transcripts were associated with testes development, as might be expected, a group of transcripts were associated with eye development, including two genes – *chiffon* and *CG4598* – that had previously been observed among genes that are differentially expressed in stalk-eyed flies artificially selected for long and short eyespan (Baker et al. 2009). However, none of these
studies considered whether these putative markers have condition-dependent effects on male eyespan.

While understanding the evolution and maintenance of male sexual ornaments has been the central focus of a wide body of work, the homologous trait in females has received comparatively little attention (Amundsen, 2000). The evolution of the female trait may simply represent selection on male ornaments against the background of a shared genetic architecture (Darwin 1871; Lande 1980). Alternatively, female ornamentation may have evolved or have been maintained by male mate preferences (Amundsen 2000) or female-female competition (LeBas 2006). In stalk-eyed flies, female eyespan is a condition-dependent trait, although to a lesser extent than in males (Cotton et al. 2004b). It does, however, reliably indicate female fecundity, even after controlling for variation in body size (Cotton et al. 2010), and males prefer to mate with females that have larger eyespan (Cotton et al. 2015; Finnegan et al. 2020). To date, there is mixed evidence that the X^{SR} affects female eyespan. The X-linked QTL that explains over a third of variation in male eyespan explains just 9% of variation in female eyespan (Johns et al. 2005). In wild flies, no association was found between female eyespan and ms395 allele size, a marker that is strongly associated with relative male eyespan (Cotton et al. 2014). As female eyespan acts as a sexual signal of quality, the relationship between X^{SR} and female eyespan warrants further study.
Here, I used manipulations of larval dietary stress to determine how drive alters eyespan in males and females, and whether trait expression is condition-dependent. I reared larvae of all possible male and female $X^{SR}$ and $X^{ST}$ genotypes under two food treatments – low food stress and high food stress – and examined the resulting variation in eyespan. My aims were to determine first, whether there is little or no difference between SR and ST males reared under low stress and second, whether an increase in stress leads to an greater difference between SR and ST males, as expected if the $X^{SR}$ chromosome is associated with reduced genetic quality. I also predicted that $X^{SR}$ would have a similar, but weaker, condition-dependent effect on the expression of female eyespan. By comparing standard, heterozygous and homozygous drive females, we also sought to establish the dominance relationship for eyespan. Since mildly deleterious effects are generally recessive (Fry and Nuzhdin 2003; Charlesworth and Willis 2009), my expectation was that homozygous drive females would show a disproportionate reduction in eyespan.
4.3 Methods

4.3.1 Stocks

A standard (ST) stock was obtained from Ulu Gombak in Malaysia (3°190 N 101°450 E) in 2005 by Andrew Pomiankowski and Sam Cotton. Meiotic drive (SR) is absent from the standard stock. Flies are maintained in high density cage culture (cage size approx. 30 × 20 × 20 cm) at 25°C with a 12 hour light:dark cycle that includes 15-minute artificial dawn and dusk periods. Stock flies are fed 100% corn ad libitum.

A meiotic drive stock was obtained in 2012 by Sam Cotton from the same Ulu Gombak location. Meiotic drive is maintained following a standard protocol (Presgraves et al. 1997; Meade et al. 2020). Briefly, females heterozygous for SR (X<sup>SR</sup>X<sup>ST</sup>) are crossed to ST (X<sup>ST</sup>Y) males and the female offspring are discarded. The male offspring from this cross, half of which are expected to have inherited SR, are crossed individually to ST (X<sup>ST</sup>X<sup>ST</sup>) females and their offspring sex ratio is recorded. Males that produce all-female broods of 15 or more are considered SR (X<sup>SRY</sup>). Drive strength is 100% in our SR stock so SR males do not sire any male offspring. All offspring of SR males are heterozygous females that are then mated to ST males, and the process is repeated.

4.3.2 Focal flies
Focal females used in experimental crosses were heterozygous for SR, taken from the SR stock. To obtain males with known genotypes, males were collected from the SR stock and crossed individually to ST females. Their larvae were genotyped for SR to determine the paternal genotype (for genotyping details see below). Larvae from SR males were heterozygous for SR whereas larvae from ST males were either females homozygous for ST or males hemizygous for ST.

### 4.3.3 Experimental crosses and egg collection

In order to produce all five genotypes of fly ($X^{ST}X^{ST}$, $X^{SR}X^{ST}$, $X^{SR}X^{SR}$, $X^{ST}Y$, and $X^{SR}Y$), two crosses were employed following a standard design (Finnegan et al. 2019). In Cross A, $X^{SR}X^{ST}$ females were crossed to $X^{SR}Y$ males (5 of each per cage), generating $X^{SR}X^{ST}$ and $X^{SR}X^{SR}$. In Cross B, $X^{SR}X^{ST}$ females were crossed to $X^{ST}Y$ males (5 of each per cage), generating $X^{ST}X^{ST}$ and $X^{SR}X^{ST}$ females, and $X^{ST}Y$ and $X^{SR}Y$ males. Four replicates were set up for Cross A and eight for Cross B. Eggs from experimental crosses were collected daily (i.e. when $\leq$ 24 hours old) and groups of 12 were allocated per petri dish, each dish containing a damp cotton wool pad and food. Two larval food treatments were used, based on earlier work (Cotton et al. 2004b). High stress allocated 0.12g of pureed sweetcorn per egg, and low stress allocated 0.3g per egg. Adults were frozen and later measured for eyespan (the distance between the distal tips of the eye-bulbs, Cotton et al.)
2004b) and thorax length (the distance between the anterior-most point of the prothorax and the posterior-most edge of the thorax; Cotton et al. 2004b), using ImageJ (v.1.46). Measured flies were then stored in 100% ethanol for subsequent genotyping.

A second experiment was carried out using identical food treatments and rearing conditions. However eggs from Cross A and Cross B were no longer reared separately but instead mixed together in each petri dish. Four eggs from Cross A were mixed with eight eggs from Cross B, generating all genotypes ($X^STX^ST$, $X^SRX^ST$, $X^SRX^SR$, $X^STY$, and $X^SRY$) in a 1:2:1:1:1 ratio on average. This design was used previously for measuring egg-to-adult survival in $T. dalmanni$ (Finnegan et al. 2019). Morphology measures of eclosed adults were obtained in the same way as in the first experiment.

**4.3.4 Genotyping**

To extract DNA, the abdomen of each fly was removed and placed in a 96-well plate containing 50µl of squish buffer (5µl 10x Taq Buffer with KCl and 15mM MgCl$_2$ (Thermo Scientific), 3µl proteinase K, and 42µl UltraPure H$_2$O). Abdomens were mechanically lysed, and wells were topped up with a further 100µl squish buffer. The 96-well plates were then transferred to a 2720 Thermal Cycler (Applied Biosystems) and incubated at 37°C for 30 minutes, before being heated to 95°C for 3 minutes to denature the proteinase K. Extracted DNA was stored at 4°C.
DNA was PCR amplified on a 2720 Thermal Cycler (Applied Biosystems) in 96-well plates containing 1ul of DNA, 0.1ul of 5x Phusion Taq polymerase (New England BioLabs), 0.2ul of dNTPs, 6.2ul UltraPure water, and 0.5ul each of the 10uM forward and reverse primers for comp162710. Comp162710 is an indel marker developed in the laboratory of Jerry Wilkinson (personal communication) to identify $X^{SR}$ chromosomes which carry a small allele (201bp) and $X^{ST}$ chromosomes which carry a large allele (286bp). This marker has been successfully used previously (Meade et al. 2020). Comp167210 fragment lengths were assayed by gel electrophoresis on a 3% agarose gel with a 0.5x TBE buffer.

**4.3.5 Statistical analysis**

I first analysed the effect of food treatment, genotype, and the food treatment by genotype interaction on absolute male eyespan in a linear model. Then, I analysed the effect of residual eyespan after body size has been accounted for by including thorax in the model. Female eyespan was analysed using similar models that also included a factor representing which parental cross females came from. As parental cross had a strong effect on female eyespan, I then split the data by cross and reanalysed. In a further analysis, I controlled for the effect of cross. I adjusted Cross B female eyespan values by the percentage difference between heterozygous $X^{SR}X^{ST}$ eyespan in Cross A and Cross B and repeated the analyses using the adjusted values.
Pairwise comparisons of female genotypes were made using Tukey post-hoc comparison tests.

### 4.4 Results

#### 4.4.1 Male eyespan

A total of 468 males were collected, of which 423 were successfully genotyped. Food treatment had a strong effect on absolute male eyespan which was smaller under high stress (low stress mean ± se = 7.869 ± 0.065mm; high stress mean ± se = 4.6893 ± 0.0609, $F_{1,416} = 1222.9622$, $P < 0.0001$; Figure 1). SR males had smaller eyespan than ST males under both food treatments (low stress SR mean ± se = 7.7853 ± 0.0958; low stress ST mean ± se = 7.9638 ± 0.0981; high stress SR mean ± se = 4.5749 ± 0.0907; high stress ST mean ± se = 4.7824 ± 0.0933, $F_{1,416} = 5.1820$, $P = 0.023$; Figure 1). The magnitude of this difference did not differ between low and high stress (food treatment by genotype interaction term $F_{1,413} = 0.1229$, $P = 0.7261$). After controlling for body size, residual male eyespan was still strongly affected by food treatment ($F_{1,413} = 90.07440$, $P < 0.0001$). SR males had reduced residual eyespan compared to ST males ($F_{1,413} = 8.5065$, $P = 0.0037$), and there was no interaction with food treatment ($F_{1,413} = 0.2786$, $P = 0.5979$).

#### 4.4.2 Female eyespan
A total of 1159 females were collected, of which 1086 were successfully
genotyped. As in males, the high stress food treatment had a strong negative
effect on absolute female eyespan (low stress mean ± se = 5.6557 ± 0.0188;
high stress mean ± se = 4.1473 ± 0.0208, F_{1,1063} = 2824.0524, P < 0.0001).
There was a significant effect of cross on female eyespan (F_{1,1063} = 5.7000, P
= 0.0171), so genotypes were compared separately for cross A and cross B
(Figure 2). \(X^{SR}\) homozygotes had smaller absolute eyespan than
heterozygous females (\(F_{1,586} = 6.1437, P = 0.0135\)). There was a significant
food treatment by genotype interaction (\(F_{1,586} = 4.0692, P = 0.0434\)) as \(X^{SR}\)
homozygotes were smaller than heterozygotes under high stress (\(X^{SR}X^{ST}\)
mean ± se = 4.2421 ± 0.0409, \(X^{SR}X^{SR}\) mean ± se = 4.0958 ± 0.0399, \(F_{1,331} =
7.9483, P = 0.0051\)) but not under low stress (\(X^{SR}X^{ST}\) mean ± se = 5.7118 ±
0.0401, \(X^{SR}X^{SR}\) mean ± se = 5.7096 ± 0.0347, \(F_{1,254} = 0.0274, P = 0.8687\)).
Genotype did not affect cross B absolute female eyespan (\(F_{1,475} = 1.2887, P
= 0.2569\)) nor was there a food treatment by genotype interaction (\(F_{1,475} =
2.8579, P = 0.0916\)). After controlling for body size, genotype no longer
explained variation in residual female eyespan in cross A (\(F_{1,585} = 0.0703, P
= 0.7910\)) or cross B (\(F_{1,474} = 0.1824, P = 0.6695\)), and there was no food
treatment by genotype interaction in either cross (cross A \(F_{1,585} = 0.2084, P =
0.6482\); cross B \(F_{1,474} = 0.2221, P = 0.6377\)).

To compare the three female genotypes (Figure 3), I controlled for cross by
equalising measurements of female heterozygotes which were common to
cross A and B (see Methods). Absolute eyespan depended on genotype
\( (F_{2,1064} = 4.6997, P = 0.0093) \) and the effect of genotype on absolute
eyespan varied across food treatment (food treatment by genotype
interaction term \( F_{2,1064} = 3.4041, P = 0.0336 \)). Under low stress, \( X^{ST} \)
homozygous females had the biggest absolute eyespan (mean ± se = 
5.74013 ± 0.0425), that was larger than \( X^{SR} \) homozygous females (mean ± 
se = 5.7096 ± 0.0347; Tukey \( P = 0.0025 \)). Heterozygous females had
intermediate absolute eyespan (mean ± se = 5.7118 ± 0.0285), not
significantly different from either homozygote (Tukey post-hoc comparison
test, \( X^{ST}X^{ST} - X^{ST}X^{SR} \) comparison \( P = 0.0507 \); \( X^{SR}X^{ST} - X^{SR}X^{SR} \) comparison \( P \\
= 0.3581 \)). Under high stress, females heterozygous for SR had the biggest
absolute eyespan (mean ± se = 4.2422 ± 0.0315), larger than \( X^{ST} \)
homozygotes (mean ± se = 4.1328 ± 0.0420, Tukey \( P = 0.0130 \)) and \( X^{SR} \)
homozygotes (mean ± se = 4.0958 ± 0.0399, Tukey \( P = 0.0038 \)). As before,
when controlling for body size genotype did not affect residual female
eyespan (\( F_{2,1063} = 0.5412, P = 0.5822 \)), and there was no food treatment by
genotype interaction (\( F_{2,1063} = 0.5656, P = 0.5682 \)).

In a second experiment, eggs from cross A and cross B were mixed together,
so that the three female genotypes emerged from each petri dish. This
eliminated specific differences associated with cross, and so avoids the need
to equalise them statistically. Female absolute eyespan again depended on
food treatment (\( F_{1,446} = 1678.7142, P < 0.0001 \)) and genotype (\( F_{2,446} \\
= 5.6035, P = 0.0039 \)). There was no food treatment by genotype interaction
(\(F_{2,446} = 2.0007, P = 0.1364\)). However, the largest genotype is different across the treatments. In low stress, \(X^{ST}X^{ST}\) eyespan (mean ± se = 6.0766 ± 0.02522) is larger than \(X^{SR}X^{SR}\) (mean ± se = 5.9246 ± 0.0352; Tukey post-hoc comparison test \(P = 0.0020\)) and \(X^{SR}X^{ST}\) is intermediate (mean ± se = 5.9888 ± 0.02659; Tukey post-hoc comparison test, \(X^{SR}X^{ST} - X^{ST}X^{ST}\) comparison \(P = 0.0872\), \(X^{SR}X^{ST} - X^{SR}X^{SR}\) comparison \(P = 0.2529\)). In high stress, heterozygous \(X^{SR}X^{ST}\) eyespan (mean ± se = 4.4860 ± 0.0577) is larger than \(X^{SR}X^{SR}\) (mean ± se = 4.2596 ± 0.0691; Tukey post-hoc comparison test \(P = 0.0424\)) and \(X^{ST}X^{ST}\) is intermediate (mean ± se = 4.4257 ± 0.0676, Tukey post-hoc comparison test \(X^{SR}X^{ST} - X^{ST}X^{ST}\) comparison \(P = 0.7784\), \(X^{SR}X^{ST} - X^{SR}X^{SR}\) comparison \(P = 0.2498\)). After controlling for body size, genotype affected residual eyespan in low stress (\(F_{2,246} = 4.7519, P = 0.0094\)), but not in high stress (\(F_{2,198} = 1.5412, P = 0.2167\)). The results from experiment 2 are therefore in broad agreement with those of experiment 1.

4.5 Discussion

Male eyespan in stalk-eyed flies is a canonical example of an exaggerated sexual character that is highly condition-dependent, in response both to environmental (David et al. 1998; Cotton et al. 2004b) and genetic stress (David et al. 2000; Bellamy et al. 2013). *T. dalmanni* stalk-eyed flies show reduced eyespan in males carrying SR meiotic drive. Here I tested the hypothesis that this reduction is due to a condition-dependent response to the low genetic quality of the \(X^{SR}\) chromosome. As reported previously
(Wilkinson et al. 1998; Cotton et al. 2010), eyespan was reduced in SR males and this effect persisted after control for body size. The reduction in SR male eyespan was evident under low as well as high environmental stress. But there is no evidence that the difference in eyespan between males carrying the $X_{SR}$ and $X_{ST}$ chromosomes was amplified under high environmental stress.

The food treatments used in the experiment follow previous work in $T.\ dalmanni$ (Cotton et al. 2004b). The “low” stress treatment constituted a plentiful amount of the standard food of stalk-eyed flies in the laboratory. The “high” stress treatment level was chosen as that at which, in previous work, trait size declined but before any substantial increase in mortality (Cotton et al. 2004b). Previous work has shown that genetic differences in the male sexual ornament are constrained under low stress but amplified as environmental stress increases (David et al. 2000; Bellamy et al. 2013; Howie et al. 2019). This is not the pattern I observe with the $X_{SR}$ haplotype, where the smaller eyespan of SR males is consistent across environmental stress treatments. The lack of a condition-dependent response is further supported by experimental work using dietary stress based on varying protein:carbohydrate ratios (rather than varying the amount of food per larva). This additionally showed reduced SR eyespan across diets but no amplification of the difference between SR and ST males (Cotton, 2016).
My findings relate to meiotic drive because the SR selfish genetic element is thought to be associated with low genetic quality. Meiotic drive genes are typically located in or close to chromosomal inversions or other areas of low recombination and are at low population frequency (Silver 1993; Johns et al. 2005; Dyer et al. 2007; Larracuente and Presgraves 2012). This means that they are subject to weak selection and predicted to suffer the accumulation of deleterious alleles (Kirkpatrick 2010). This is borne out by many examples in which the transmission advantage of drive is matched by viability and fertility deficits in males and females (Curtsinger and Feldman 1980; Jaenike 1996; Dyer et al. 2007; Finnegan et al. 2019). In *T. dalmanni*, a large inversion(s) covers at least a third and maybe substantially more of the XSR chromosome (Johns et al. 2005; Reinhardt et al. 2014) and is estimated to be at least half a million years old (Paczolt et al. 2017). Why then is there an absence of evidence for XSR causing a condition-dependent deficit in male eyespan? One possibility arises from the relatively high frequency of XSR, around 20%, in natural populations (Presgraves et al. 1997; Wilkinson et al. 2003; Paczolt et al. 2017). At this frequency, the rate of recombination of XSR is only a quarter of that of the standard XST chromosome, and this may be sufficient to allow the removal of a substantial fraction of deleterious mutations occurring on the XSR chromosome. There may also have been the opportunity for beneficial mutants to spread, allowing a degree of compensatory adaptation to meiotic drive (Meade et al. 2020). Overall, there must be some countervailing fitness deficit to constrain the spread of drive. In *T. dalmanni* previous studies have documented reduced egg-to-adult viability in males.
and females carrying $X^{SR}$ (Finnegan et al. 2019) and evidence that SR males do worse than ST males under conditions of sperm competition (Wilkinson et al. 2006). However, it appears that these and other shortfalls in fitness are not sufficient to exert a condition-dependent effect on male eyespan.

An alternative hypothesis is that the $X^{SR}$ chromosome may simply be fixed for alleles that directly reduce eyespan. If drive arises on, or becomes associated with, a haplotype that contains genetic variation for small eyespan, this haplotype could spread due to the transmission advantage of drive, despite producing males with less attractive (smaller) secondary sexual traits. Theoretical analysis shows that this is possible but only when there is a complete lack of recombination between drive and standard chromosomes (Lande and Wilkinson 1999). Such a situation is unlikely to be stable, however. It requires the fortuitous association of drive with small eyespan variation, which is then maintained in the long term. Events such as double crossovers, gene conversions and back mutations, while rare, would generate a haplotype with the transmission advantage of drive but without a reduction of male attractiveness. There seems no obvious reason why this non-equilibrium association would not break down.

My results can also be interpreted in terms of changes in resource investment in SR males. In SR males, fertility is comparable to that of ST males despite sperm destruction (Meade et al. 2019; Meade et al. 2020). This is accomplished by SR males having greatly enlarged testes which
allows them to deliver the same number of sperm per ejaculate as ST males (Meade et al. 2019) and to maintain their fertility even under conditions of multiple mating (Meade et al. 2020). But the devotion of increased resources to testes must mean SR males have less to invest in other traits. This may explain the reduced accessory gland size of SR males, as both reproductive organs develop over a period of several weeks post-eclosion (Baker et al. 2003; Rogers et al. 2008; Meade et al. 2020). It is less obvious why increased investment in testes constrains eyespan development. However, Fry (2006) found that topical application of a juvenile hormone analogue to final instar larvae led to the development of males with larger testes and smaller eyespan than untreated controls. These observations suggest that reduced accessory gland size and smaller eyespan in SR males are side-effects of their greater investment in sperm production. These changes could be adaptive as modelling work shows that males with fewer resources are expected to produce ejaculates similar to those males who are resource-rich, but at the expense of investment in traits that contribute to the mating rate (Tazzyman et al. 2009). In stalk-eyed flies there is prior evidence that increased testis size can maintain fertility, with consequent smaller accessory glands whose size is associated with mating rate (Rogers et al. 2005), and reduced eyespan causing males to attract and mate with fewer females.

Female eyespan also shows high condition dependence in *T. dalmannii*, but to a lesser extent than in the homologous male trait (Cotton et al. 2004b). In wild samples, there is no evidence of reduced eyespan in females carrying
the XSR chromosome (Cotton et al. 2014). Here I examined female eyespan condition dependence and found it was more complex than in males. Under low and high environmental stress, XSR homozygotes had smaller eyespan than XST homozygotes (though this difference was not significant under high stress). As with males, there was no evidence for a condition-dependent amplification of genetic differences; the eyespan difference between XSR and XST homozygotes was not exaggerated by high environmental stress, like hemizygous male differences. The pattern in heterozygous females was different. Under low stress, heterozygotes were intermediate between the homozygotes. But under high stress there was evidence for heterosis as heterozygous females had the largest eyespan, greater than either homozygote. This heterosis likely reflects the masking of deleterious alleles (Wilton and Sved 1979) when the non-recombinant and hence highly diverged XSR and XST chromosomes are brought together (Reinhardt et al. 2014). Our results suggest heterosis is dependent on environmental conditions as under benign stress additive differences between haplotypes dominate, whereas under elevated stress, low fitness recessive mutations are exposed and homozygotes size declines, but heterozygotes mask this reduction.

An unforeseen complication in this study arose from the experimental design. In order to collect the full range of male and female genotypes, two experimental crosses were carried out, cross A (XSRXST mated to XSRY) and cross B (XSRXST mated to XSTY). Larvae from the two crosses were kept
separately throughout egg-adult development. While the rearing conditions of the two crosses were identical (larval density, food type, all other environmental variables), there was a clear effect of cross on female eyespan as heterozygous female eyespan was larger in cross A than cross B samples. These heterozygous offspring have the same nuclear genotype, they share the same maternal genotype (all heterozygotes), while their parents are drawn from the same stock cages and do not differ in maternally inherited cytotype. These offspring did differ in paternal genotype ($X^{SR}Y$ in cross A and $X^{ST}Y$ in cross B) but there is no obvious paternal effect to explain the difference in eyespan. A possible cause is that in cross A only female offspring are produced, whereas in cross B the offspring sex ratio is approximately 1:1, suggesting that male larvae have a negative competitive effect on female eyespan. This was despite efforts to limit the amount of competition between larvae by plating a small number of eggs (12) onto each petri dish. It is not immediately clear why this would be the case. Differences in male and female larval competitive ability has been reported previously in fruitflies and mosquitoes (Nunney 1983; Steinwascher 2018). In *D. melanogaster*, Nunney (1983) reported that male larvae of some strains were better at exploiting a limited food supply than females. This was true even for a strain where females eclosed earlier than males, as is the case in stalk-eyed flies (S. Finnegan, unpublished data). In our analysis I dealt with this inconsistency by statistically controlling for the effect of cross on female eyespan. Furthermore an additional experiment was carried out in which eggs were mixed together from cross A (4 eggs) and cross B (8 eggs), as in
a previous study (Finnegan et al. 2019). The pair of experiments gave qualitatively similar results, implying that the statistical adjustment for the effect of cross was appropriate. It further implies that adult size may be influenced by the sex and genotype of conspecifics that share the larval environment. This hypothesis is no under investigation.

In summary, meiotic drive causes a reduction in male eyespan, the sexual ornament in stalk-eyed flies. This occurs under low and high food stress, in a manner that is not strongly condition-dependent. A similar reduction is observed in female eyespan, again across environmental stress levels, but the pattern is complicated by heterosis in heterozygotes which is dependent on environmental stress. It seems likely that the reduced eyespan in SR males reflects contrasting resource allocation to different traits during development in order to compensate for the destruction of sperm caused by meiotic drive.
4.6 References


rhinoceros beetle, *Trypoxylus dichotomos* (Coleoptera: Scarabaeidae).

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4.7 Figure legends

Figure 1
Absolute male $X^{SR}Y$ (red) and $X^{ST}Y$ (blue) eyespan in low and high stress larval food treatments. Points denote mean absolute eyespan ± one standard error.

Figure 2
Absolute female eyespan in low stress and high stress larval food treatments, where females from each cross category were reared separately. Cross A produces $X^{SR}X^{SR}$ (orange) and $X^{SR}X^{ST}$ (green) genotypes. Cross B produces $X^{ST}X^{SR}$ (green) and $X^{ST}X^{ST}$ (purple) genotypes. Points denote mean absolute eyespan ± one standard error.

Figure 3
Relative eyespan of $X^{SR}X^{SR}$ (orange), $X^{SR}X^{ST}$ (green), and $X^{ST}X^{ST}$ (purple) females (after controlling for thorax length), in low and high stress food treatments, after adjusting for cross. Points show mean relative eyespan ± one standard error.

Figure 4
Absolute female eyespan in low and high stress larval food treatments in the second experiment when eggs from cross A and cross B were reared together to generate all three female genotypes: $X^{SR}X^{SR}$ (orange), $X^{SR}X^{ST}$
(green), and $X^{ST}X^{ST}$ (purple). Points show mean absolute eyespan ± one standard error.
4.8 Figures

Figure 1

Eyespan (mm)

Low stress  High stress

Food treatment

Genotype

$X^{SR_Y}$  $X^{ST_Y}$
Figure 2

The graph shows the effect of different genotypes and stress levels on Eyespan (mm). The x-axis represents different food treatments: Low stress, High stress, Low stress, and High stress. The y-axis represents the Eyespan in millimeters, ranging from 3.0 to 7.0.

- Genotype $X^{SR}X^{SR}$
- Genotype $X^{SR}X^{ST}$
- Genotype $X^{ST}X^{ST}$

Cross A and Cross B are compared in the graph.
Figure 3

Eyespan (mm)

Food treatment

Genotype
- $X^{SR}X^{SR}$
- $X^{SR}X^{ST}$
- $X^{ST}X^{ST}$
Figure 4

![Graph showing the relationship between genotype and eyespan (mm) under low and high stress conditions. Different genotypes are represented by different colors: $X^{SR}X^{SR}$ (orange), $X^{SR}X^{ST}$ (green), and $X^{ST}X^{ST}$ (purple). The x-axis represents food treatment levels (low stress and high stress), and the y-axis represents eyespan (mm).]
Experimental population genetics of meiotic drive in the stalk-eyed fly, *Teleopsis dalmanni*
5.1 Abstract

Populations of many species carry sex-ratio meiotic drive (SR), a selfish genetic element located on the X chromosome that biases transmission to ensure it is transmitted to more than 50% of progeny. Due to this transmission advantage, SR is often predicted to rapidly spread through populations that carry it, potentially even causing population extinction due to a lack of males. Despite this, SR is often found at low to moderate frequency in the wild. SR frequency is stable over time in *Teleopsis dalmanni* stalk-eyed fly populations in the Gombak valley, but varies considerately between populations and is negatively correlated with population size. In order to better understand these findings, I established 24 cage populations of stalk-eyed flies with meiotic drive at a moderate initial frequency. Larvae in these populations were reared on high quality or low quality food. The frequency of SR generally declined in cage populations, being lost altogether from some after just six generations, but SR was maintained at a higher frequency in low food lines, where productivity and population size was lower. I discuss several possible explanations for these findings. I also found evidence that one cage population was driven extinct by meiotic drive, suggesting that small populations are vulnerable to perturbations in population sex ratio and SR frequency.
5.2 Introduction

It has long been hypothesized that sex-chromosome meiotic drive – a phenomenon whereby a selfish genetic element on a sex chromosome manipulates sperm development to ensure it is transmitted to more than 50% of progeny – has large impacts on populations carrying it (Novitski 1947). Hamilton was the first to model how a driving X or Y chromosome could lead to the extinction via sex ratio bias at the level of the population as X drive leads to a deficit in males and hence a lack of egg fertility and Y drive leads to a deficit in females and hence low population fecundity (Hamilton 1967). The potential for Y-drive to cause extinction was subsequently demonstrated experimentally (Lyttle 1977). Using X-ray irradiation, Lyttle was able to link the *Drosophila melanogaster* Y chromosome to the naturally occurring autosomal segregation distorer (SD) found on chromosome 2, creating a pseudo-Y drive. This pseudo-Y drive spread rapidly through small laboratory populations, causing extinction in as few as seven generations. Y-chromosome drive is expected to spread much more quickly than X-chromosome drive. This is because Y-chromosome drives are passed from male to male, and so are expressed in every generation, while X chromosomes spend, on average, twice as much time in females as in males. Nonetheless, X-chromosome drives still have the potential to spread extremely rapidly (Hamilton 1967; Bastide et al. 2013). X-chromosome drives are also considerably more common (Jaenike 2001).
Only a few studies have explicitly tracked the frequency of X chromosome meiotic drive, all in laboratory populations of *D. pseudoobscura*. Wallace (1948) initiated several populations with X-chromosome drive (referred to in this and many other species as *sex-ratio* or SR) at either 50% or 75%. Despite these high initial frequencies, considerably higher than is naturally observed in the wild (Price et al. 2014), drive did not fix. Instead, the frequency of SR quickly fell. In cages maintained at 25°C, SR had been completely replaced by the wildtype X chromosome (ST) in just seven generations. In cages maintained at 16.5°C, drive still persisted after 12 generations, albeit at a very low frequency (~5%). In this study, Wallace (1948) also reported reduced male fertility, female fecundity, and egg-to-adult viability of drive carriers; all selective forces that must contribute to the decline in SR frequency. Later, Beckenbach (1983) tracked SR frequency in a number of populations of *D. pseudoobscura* kept under varying conditions. In low density populations that were only allowed a single mating, drive did not change much from its initial 50% frequency over 9 generations. When flies were kept at either high density with a single mating opportunity, or low density and allowed to mate freely, drive declined to 10-15% after 9 generations. When populations were kept at high density and allowed to mate freely, conditions most similar to Wallace (1948), SR rapidly fell in frequency and was essentially lost by the end of the experiment. This experiment highlighted that the deficits to male fertility and larval viability are particularly pronounced under conditions of increased competition.
More recently, Price et al. (2008a) established cage populations of *D. pseudoobscura* with drive at 30%, the maximum frequency observed across the species’ natural range (Price et al. 2014), and allowed them to mate freely. After 10 generations, these populations evolved increased female remating rates, while the mating rates in control populations without drive did not change. Higher female remating rates are likely to particularly disadvantage SR males as they do poorly under conditions of increased sperm competition (Price et al. 2008b). The authors also report that the frequency of drive fell from an initial 30% to less than 5% over the course of the experiment. In subsequent work, the authors once again started populations with drive at 30% frequency. In several populations, males and females were mixed only once for four hours, allowing only a single opportunity for mating, as females do not mate more than once within this time period (Price et al. 2010). In several other populations, males and females were mixed for four hours up to six times. They found that the frequency of drive declined in the multiply-mating populations over 9 generations, while it remained unchanged in the single-mating populations. These findings replicate those of earlier work (Beckenbach 1983). Where this work goes further is that it shows that single-mating populations are associated with an increased extinction risk. By generation 15, 5 of 12 single-mating populations had been driven extinct. In contrast, none of the 36 multiple-mating populations went extinct. These extinctions were clearly caused by meiotic drive because populations were large and productive but
had extremely female-biased population sex ratios in the generations preceding extinction (Price et al. 2010).

These laboratory experimental evolution studies are generally in broad agreement, and yet give results that somewhat disagree with both theory and collections of wild flies. Early theoretical work suggested that drive should spread rapidly through populations (Hamilton 1967). More recent work has suggested that meiotic drive can reach a stable polymorphism if it is also associated with severe fitness costs to carriers (Holman et al. 2015; Larner et al. 2019). In the wild, SR is indeed found in a stable polymorphism in *D. pseudoobscura*, where it exists in a stable north-south cline ranging from 0 to 30% along its range (Price et al. 2014). However, the frequency of drive has consistently fallen in laboratory populations (Wallace 1948; Beckenbach 1983; Price et al. 2008a), even being lost altogether (Wallace 1948; Beckenbach 1983), except in populations kept under unnatural conditions of forced monandry (Price et al. 2010). Nevertheless, these laboratory studies have provided insights into the selective forces that affect the frequency of sex chromosome meiotic drive.

How has the frequency of drive changed in wild populations of other species? In the cosmopolitan *D. simulans*, the frequency of drive has increased dramatically as it has spread throughout Africa and the Middle East. For example, SR in this species rose from <5% to more than 70% in just six years in Egypt (Bastide et al. 2013). However, this huge increase in
SR frequency was closely followed by an increase in the frequency of other genes that suppress the effect of drive (Bastide et al. 2013). In *D. pseudoobscura*, no suppressors have so far been detected (Price et al. 2019). In *D. neotestacea*, another species without suppression, the pattern of meiotic drive in the wild mirrors that of *D. pseudoobscura*, with SR existing along a stable north-south cline (Pinzone and Dyer 2013).

In the stalk-eyed fly, *Teleopsis dalmanni*, previous work has shown that the frequency of *sex-ratio* drive varies slightly between highly geographically separated populations on the Sunda shelf (Wilkinson et al. 2003), but the frequency of drive is broadly stable over time, at approximately 20% (Wilkinson et al. 2003; Paczolt et al. 2017). More recently, we intensively sampled from the Gombak valley (Meade et al. unpublished data), one of the sites sampled in Wilkinson et al. (2003). Flies of this species are found roosting and mating on exposed root hairs along the banks of rainforest streams (de la Motte and Burkhardt 1983). Flies in this valley are found in a number of small, distinct populations with little migration between them (Cotton et al. 2014). Population size varies among these populations, and flies from different sites also differ in size, suggesting there are general differences in environmental quality (Cotton 2016). Over several years, we sampled from multiple stream sites along a 2km stretch of the Gombak valley. We found that the frequency of drive across the whole valley did not change over approximately 10 years, but that the frequency within individual streams is highly variable, ranging from 0-50%. The frequency of drive in
these streams is negatively correlated with population size, with small populations having a higher frequency of drive.

Motivated by the findings above, I set out to determine how the frequency of drive changes in laboratory populations over time. I established a total of 24 cage populations with SR at a moderate frequency. 12 populations were assigned to “high” lines and were fed high-quality food, and 12 populations were assigned to “low” lines and fed low-quality food. This work is ongoing, but here I report preliminary findings.

5.3 Methods

5.3.1 Stocks

A stock population was obtained in 2005 from the Gombak valley in peninsula Malaysia (3°19’N 101°45’E) by Sam Cotton and Andrew Pomiankowski. All flies in this population only carry the standard X chromosome. This is designated the ST stock. A second stock population was made from male flies collected from the same location in 2012 by Alison Cotton and Sam Cotton. This stock includes a mixture of drive and standard chromosomes and is referred to as the SR stock. Drive is maintained in this stock following a standard protocol (Presgraves et al. 1997; Meade et al. 2019). Individual SR stock males are crossed with ST stock females and their offspring sex ratio is scored. Males siring female-biased broods (100%
females and >15 total offspring) are designated SR (X^{SR}Y). Their female progeny are therefore heterozygous for drive (X^{SR}X^{ST}). Progeny from males not designated as SR are discarded. Heterozygous females are then mated to males from the ST stock (X^{ST}Y), which is expected to produce SR (X^{SR}Y) and ST (X^{ST}Y) males in equal proportions (assuming no viability cost). These male offspring, referred to as SR stock males, are crossed to ST stock females and the process is repeated. Flies from both stocks are maintained in a 12h:12h light:dark cycle at 25°C, and fed 100% puréed corn *ad libitum*.

5.3.2 Setup of experimental populations

The experiment was commenced with populations containing flies with all possible genotypes. A preliminary cross was carried out between 26 SR stock males (1:1 X^{SR}Y : X^{ST}Y) and 26 heterozygous females (X^{SR}X^{ST}). This is expected to produce X^{ST}Y, X^{SR}Y, X^{ST}X^{ST}, X^{SR}X^{ST} and X^{SR}X^{SR} genotypes in a 1: 1 : 1: 3: 2 ratio. Progeny were collected for three weeks, and then adults were left for a further 4 weeks to ensure they were sexually mature. They were then sexed and placed in 24 population cages, 12 populations each were assigned to the “high” and “low” treatment, each containing 26 males and 26 females. Adult flies were fed 100% pureed corn *ad libitum*. These populations are referred to as the F0 generation.

The experimental protocol is given in Figure 1. Eggs were collected from the adult flies on “egg-lays”, petri dishes containing a large cotton pad moistened
with 15ml of water. On each egg-lay, 2.5ml of the appropriate food was added for larvae to consume during development. In the high lines, this food consisted of 100% pureed sweetcorn. In the low lines, larval food was 25% pureed corn and 75% sugar solution (25% sugar w/v). To ensure that low food had the same viscosity as high food, an indigestible bulking agent (3% carboxymethylcellulose w/v) was added (Rogers et al. 2008; Cotton et al. 2015; Finnegan et al. 2019). Previous work found that larvae reared on this low food were smaller at eclosion than flies reared on high quality food (Finnegan, unpublished data). Six egg-lays were placed into each population cage twice a week for three weeks. Following egg collection, the surviving adults were collected and frozen for future genotyping.

Larvae were allowed to develop and pupate in egg-lays for two weeks. The egg-lays were then placed in a new population cage and a further three weeks was allowed to ensure that most flies had eclosed. Once all egg-lays for a particular line had reached this point, all flies were removed and anaesthetised on ice. A random sample of ~100 flies was taken from each population and the sex ratio of this sample recorded. The remaining flies were frozen for future genotyping. If the population consisted of fewer than 100 individuals then the sex ratio was recorded and all flies were taken to form the next generation. The maximum population size was chosen as ~100 to prevent overcrowding in population cages. Flies for the next generation were placed in new population cages and allowed to develop for a further 4 weeks to ensure that adults were sexually mature. The process was then
repeated: 3 weeks for egg-lay collection, 2 weeks larval development, 3 weeks for emergence, 4 weeks to ensure sexual maturity (i.e. 12 weeks per generation).

5.3.3 Genotyping

Adult flies at the end of a generation (i.e. after completion of egg collections) were genotyped. If there were less than 47 individuals per sex per population then additional flies were genotyped when there was an excess of 100 when the populations were sexed. It was not always possible to reach this maximum of 47. The founding population was not always sufficiently large, or had a biased sex ratio, or because the population size declined by the end of the generation and there was an insufficient excess to bolster numbers. In addition, there were occasional genotyping errors. The average number of genotyped flies per population per generation (combined across sex) was \( \text{mean} \pm \text{se} \) 41.4576 ± 2.3292.

DNA was extracted by placing the thorax of each fly into a well of a PCR plate containing \( 3\mu l \) proteinase K, \( 15\mu l \) of 10x Taq buffer (Thermo Scientific) and \( 132\mu l \) UltraPure water. PCR plates were incubated at 37°C for 30 minutes and then heated to 95°C for 3 minutes to denature the proteinase. To amplify DNA, \( 1\mu l \) of DNA, \( 0.1\mu l \) of Phusion Taq polymerase (New England BioLabs), \( 0.2\mu l \) of dNTPs, \( 6.2\mu l \) of UltraPure water, and \( 0.5\mu l \) each of the forward and reverse primers for the marker Comp162710 were added.
to each well of a PCR plate. Comp162710 is an indel marker developed by GS Wilkinson (personal communication). The $X^{SR}$ chromosome is associated with a small allele (201bp) and the $X^{ST}$ is associated with a large allele (286bp; Meade et al. 2019). Fragment lengths were assayed on gel electrophoresis with a 3% agarose gel with a 0.5x TBE buffer.

5.3.4 Statistical analysis

All statistical analysis was carried out in R (R Core Team 2019). The frequency of drive was modelled using a linear mixed-effects model with generation, population sex ratio, and treatment as fixed effects, and line as a random effect. Significance of model terms was determined using Type III with the lmerTest package (Kuznetsova et al. 2017). A Welch’s t-test was used to determine if the frequency of drive was significantly different at the beginning and the end of the experiment. Differences between the frequency of drive at F6 in the high and low lines were also assessed using a Welch’s t-test. The effect of generation on population size was analysed using a linear mixed effect model with line as a random effect and generation as a fixed effect. The F0 was excluded from this analysis as the population size was artificially set at 52 individuals for this generation. The effects of generation, drive frequency and treatment on population sex ratio were modelled in a linear mixed effects model with line as a random effect and significance of model terms was determined as above.
5.4 Results

Over six generations SR frequency generally declined in experimental populations \((F_{1,4} = 21.2306, \ P < 0.0001)\). Across all cages, the frequency of SR was lower at F6 than it was at F0 \((t = -4.8988, \ df = 23, \ P < 0.0001)\). By F6, SR was maintained at a lower frequency in the high lines than in the low lines \((\text{high mean ± se frequency} = 0.0344 ± 0.0094; \text{low mean ± se frequency} = 0.1375 ± 0.0281; t = 3.4753, \ df = 11.0240, \ P = 0.0052; \text{Figure 2})\).

By F6, 4 of 12 high lines had lost drive compared with 2 of 10 low lines (or at least SR is at such a low frequency it cannot be detected). The frequency of SR is not estimated for one low line at F6 because only 3 individuals survived until the end of the generation and could not be successfully genotyped. Another low line did not survive to F6. This population appears to have been driven extinct by SR. In the F4, the frequency of drive was 65% in a population of 82 individuals, of which only one individual was male. This male was genotyped as SR. Accordingly, the F5 generation was 100% female.

Throughout the experiment population size declined in low lines \((F_{5,66} = 6.6737, \ P < 0.0001)\) but not in high lines \((F_{5,55} = 0.5366, \ P = 0.7476)\), where it stayed at or close to the population cap of 100 individuals (Figure 3). Population sex ratio, estimated as the ratio of the number of females to males, was female-biased throughout the experiment and did not change from F1-F6 \((\text{F1 mean ± se sex ratio} = 0.6546 ± 0.0198; \text{F6 mean ± se sex ratio} = 0.6555 ± 0.0141)\).
ratio = 0.6354 ± 0.0276; $F_{4,75} = 0.6956, P = 0.5973$), nor did it differ between high and low lines (high mean ± se sex ratio = 0.6089 ± 0.0106; low mean ± se sex ratio = 0.6333 ± 0.0134; $F_{1,23} = 0.1768, P = 0.6780$), but did depend on drive frequency ($F_{1,66} = 6.7004, P = 0.0119$).

### 5.5 Discussion

While still ongoing, the results presented here allow some preliminary conclusions to be drawn from this experiment. First, the frequency of SR meiotic drive generally declines in freely-mating cage populations of stalk-eyed flies. Second, while still declining, SR persists at higher frequencies in lines where larvae are reared on low-quality food. Apart from one cage, the population trajectories recorded here are contrary to early predictions that X-linked meiotic drive should quickly lead to population extinction due to the loss of males (Hamilton 1967), and yet coincide with previous work in $D. pseudoobcura$ (Wallace 1948; Beckenbach 1983; Price et al. 2008a), that finds a reduction in SR frequency.

What factors might explain the reduction in SR frequency, and its persistence at higher frequency in low lines? Previous work (Finnegan et al. 2019) has shown that drive carriers have reduced egg-to-adult viability. One possible explanation is that larval mortality is increased in low lines, and this occurs in a genotype-dependent manner, with drive carriers being particularly disadvantaged. In previous work I found no difference in mortality across
condition, and no interaction between genotype and larval food condition. However, while larval food in the low lines here is the same as the low food condition used previously, the high lines in this experiment are provided with 100% corn, compared with a corn-sugar mix in previous work, which may have led to a different response. For differences in viability to explain the results here, the relative viability of drive carriers would have to be higher in low food quality lines, which is counterintuitive. One possibility is that differences in density between high and low lines caused differences in genotype-specific viability. In this experiment, high lines were more productive than low lines, implying that larvae were reared at higher densities in high lines (Figure 3). In *D. pseudoobscura*, the negative effect of drive on larval viability is greater at high densities (Beckenbach 1983). If, as in *D. pseudoobscura*, mortality of drive-bearing larvae is greater at higher densities, this could explain why high lines in this experiment have a lower frequency of drive. Further work investigating the egg-to-adult viability of stalk-eyed flies under a range of larval densities would be needed to support this idea.

Alternatively, differences in food quality may have led to differences in adult morphology, which may have had an impact on the mating behaviour of adult flies. While I did not measure the morphology of flies in this experiment, previous work employing these larval food conditions has shown that larvae reared on the food used in the low lines of this experiment eclose as smaller adults with smaller eyespan compared with larvae reared on 100% corn food,
as in the high lines (S. Finneghan, unpublished data). Eyespan is an important condition-dependent trait in stalk-eyed flies (Cotton et al. 2004) and females prefer to mate with males with large eyespan (Wilkinson and Reillo 1994; Cotton et al. 2010). However, while drive males have reduced eyespan compared to standard males (Wilkinson et al. 1998; Cotton et al. 2014), the magnitude of this difference does not increase under worsening larval conditions (Chapter 4). Differences in adult morphology caused by the different larval food environments used in high and low lines are therefore not expected to have affected mate choice dynamics in this experiment. However, it might pay to assess eyespan in these lines to gauge the importance of differences between drive and standard males.

One clear difference between high and low lines is that the population size declined in low lines but remained close to the cap of ~100 individuals in high lines. The reduced population size in low lines may reduce the opportunities for females to express their mate preferences. Though given the relatively small size of population cages, the costs of assessing potential mates is likely small, and so it is unlikely that females are unable to select preferred males. Another way that population size is likely to affect mating behaviour is via the mating rate. As the population size increases, the mating rate is likely to increase. Increased mating rates are expected to disproportionately affect drive males in a number of ways. First, SR males are unable to match the mating frequency of standard males (Wilkinson et al. 2003; Finneghan et al. 2020; Meade et al. 2020), likely because they have reduced accessory
glands (Meade et al. 2020), the organs associated with mating rate (Rogers et al. 2005). Second, while SR and ST males have similar fertility when the mating rate is low, Wilkinson et al. (2006) reported reduced SR male fertility at high mating rates, though this result has not been replicated (Meade et al. 2020). Finally, *T. dalmanni* are highly promiscuous with females mating multiple times each morning (Wilkinson et al. 1998; Wilkinson et al. 2003). SR males in many species have low paternity when females mate multiply and their sperm is in competition with that of ST males (Price and Wedell 2008). Wilkinson et al. (2006) found that the SR *T. dalmanni* males sired on average only 25% of the offspring of multiply-mated females. In the closely related *T. whitei*, the transfer of seminal fluid from an ST male was found to incapacitate SR male sperm in the female reproductive tract (Fry and Wilkinson 2004), although this result has not been replicated in this or any other species. As *T. dalmanni* SR males do not transfer fewer sperm per mating than ST males (Meade et al. 2019), further work is needed to clarify why SR males do poorly in sperm competitive conditions. Nonetheless, this deficit is expected to be particularly pronounced as the mating rate increases in high density populations. The rate of female mating has been shown to be an important factor determining SR frequency in cage populations of *D. pseudoobscura* (Beckenbach 1983; Price et al. 2008; Price et al. 2010). Populations kept under conditions of monandry maintain drive at high frequencies, while SR generally declines in cages where females mate multiply (Beckenback 1983; Price et al. 2010). Remarkably, Price et al. (2008a) also found that cages with moderate initial SR frequencies evolved
higher female remating rates than those without drive. It would be interesting to assay the remating rate of females in high and low lines in this experiment to determine whether they differ from one another, or from the baseline remating rate of stock flies. In the wild, the Gombak valley may also represent an interesting natural laboratory to explore the relationship between female remating rate and SR frequency, as the small populations in this valley vary in their population size and SR frequency.

After six generations of experimental evolution, SR has been lost from a total of 6/24 populations: 4 in the high lines, and 2 in the low lines. Given a general trend of declining SR frequency, it is likely that SR will be lost from further populations as the experiment continues. Despite this, one population cage in this experiment was driven extinct after only five generations. This extinction was clearly caused by the spread of meiotic drive, because the population was fairly large (>80 individuals) but entirely female. In the preceding generation, the population was of a similar size and contained only a single male, who was genotyped as SR. There is no obvious reason why this population increased in frequency while all others declined. In models designed to assess the relationship between SR frequency and the level of sperm competitive disadvantage experienced by SR sperm, Taylor and Jaenike (2002) found that sperm competition may allow a stable SR polymorphism but that small perturbations in SR frequency due to genetic drift may be enough to drive SR to be lost or to fix (Taylor and Jaenike 2003). This is because as SR increases in frequency, the population becomes
increasingly female-biased and the frequency of female remating decreases and the reduction of SR sperm competitive ability becomes less important. It is possible that such stochastic perturbations in the population sex ratio played a role in the extinction of one cage population in this experiment. It has been suggested in small, structured meta-populations, SR polymorphism may be maintained at the global level by the repeated extinction and recolonization of subpopulations (Taylor and Jaenike 2002; Price et al. 2019). Given the results presented here, and the finding that T. dalmanni in the Gombak valley exist in small, structured populations that vary in SR frequency (L. Meade unpublished data), this hypothesis warrants further study.

In conclusion, preliminary results from this experiment show that SR is declining in frequency in nearly all cage populations of stalk-eyed flies, and has already been lost in a quarter of them after only six generations. SR has persisted at higher frequency in cages fed on a low quality larval diet. This diet primarily appears to affect the productivity of cage populations, and so it is likely that differences in SR frequency in high and low cages are mediated by density-dependent effects. Future work should assay female remating rates in these populations to determine what effect this has on SR frequency.
5.6 References


5.7 Figure legends

Figure 1
Diagrammatic representation of the scheme used to maintain experimental populations. Egg-lays are collected for 3 weeks and the adults from the previous generation are then frozen for genotyping. Larvae are allowed 2 weeks to develop and pupate before egg-lays are placed into a population cage to eclose. Cages are left for 3 weeks to allow the majority of flies to eclose. Flies are then sexed and the population is capped at approximately 100 individuals, and the excess are frozen. Flies are then allowed 4 weeks to ensure sexual maturity before egg-lays are collected again, forming the next generation.

Figure 2
The frequency of SR meiotic drive over six generations. Each semi-opaque line represents one population cage. The solid line shows the regression of generation on SR frequency for High and Low lines. The shaded areas represent 95% confidence intervals. Note that F3 and F5 are not included as flies from these generations have not been genotyped.

Figure 3
Mean ± standard error population size of cage populations over six generations. Note that the population size is capped at approximately 100 individuals.
Figures

Figure 1

Egg-lays are collected. Flies are given 4 weeks to ensure sexual maturity. Then flies are sexed and the population is capped at approximately 100 individuals. Flies in excess of population cap frozen for possible genotyping.

After collection of egg-lays, adult flies are frozen for genotyping.

Flies are given 4 weeks to ensure sexual maturity. Then flies are sexed and the population is capped at approximately 100 individuals. Flies in excess of population cap frozen for possible genotyping.

3 weeks are allowed for emergence. Then flies are sexed and the population is capped at approximately 100 individuals. Flies in excess of population cap frozen for possible genotyping.

Larvae allowed 2 weeks to develop and pupate. Then flies are sexed and the population is capped at approximately 100 individuals. Flies in excess of population cap frozen for possible genotyping.

Egg-lays forming the next generation are collected for 3 weeks.

Egg-lays allowed for 3 weeks. Egg-lays collected for 3 weeks.
Figure 2

![Graph showing SR frequency across generations for High and Low groups. The x-axis represents generation (F0 to F6), and the y-axis represents SR frequency ranging from 0.00 to 1.00.]
Figure 3

![Population size vs. Generation graph](image)

- **Y-axis**: Population size
- **X-axis**: Generation

- High and Low population size trends across generations F1 to F6.
Chapter 6

General discussion
6.1 General overview

Meiotic drive genes are a type of selfish genetic element that gain a transmission advantage by causing dysfunction of non-carrier sperm (Burt and Trivers 2006; Lindholm et al. 2016). Due to this strong transmission advantage, meiotic drive genes are predicted to rapidly spread through populations that carry them. In the case of sex-linked drives, this could potentially even cause population extinction due to the lack of one of the sexes (Hamilton 1967). While there is evidence for large increases in drive frequency in just a few years in one species (Bastide et al. 2011), drive in many other species is found at low to moderate frequencies that are stable across long timescales (Wilkinson et al. 2003; Pinzone and Dyer 2013; Price et al. 2014). This suggests there must be strong selection acting against drive genes in order to counteract their transmission advantage and prevent further spread. Such selective counter-forces could come about as a by-product of the action of the drive genes themselves. For example, the loss of up to half of all sperm may reduce drive male fertility (Hartl et al. 1976; Wu 1983a,b; Jaenike 1996; Atlan et al. 2004; Angelard et al. 2008) or sperm competitive ability (Atlan et al. 2004; Wilkinson et al. 2006; Price et al. 2008a; Manser et al. 2011). Alternatively, drive chromosomes may suffer reductions in fitness due to the accumulation of deleterious mutations in drive-associated inversions. Many drive chromosomes are associated with inversions, where they maintain linkage between multiple genes necessary
for the drive phenotype (Jaenike 2001; Lindholm et al. 2016). Such
inversions may cover as much as the entire length of a driving chromosome
(Dyer et al. 2007). This represents a large mutational target. As
recombination is weak on the inversion, because homozygotes are rare and
inverted and non-inverted homologous chromosomes do not typically pair,
the removal of deleterious mutations by recombination is prevented (Navarro
et al. 1997). Mutations within inverted regions could feasibly have a negative
impact on a wide range of important traits, which may contribute to reducing
the fitness of drive chromosomes and counter-acting their spread. The
search for negative fitness consequences of the sex-ratio meiotic drive
chromosome (X<sup>SR</sup>) in the stalk-eyed fly, *Teleopsis dalmanni*, has been a
central theme of this thesis.

6.2 Summary of findings

6.2.1 Chapter 2 – Does meiotic drive alter male mate preference?

There are several conditions necessary for the evolution of male mate
preference (Bonduriansky 2001). Males must be limited in their mating
capacity, there needs to be variation in female quality, and the cost of
assessing potential mates must be low enough to not outweigh the benefits
of choice (Bonduriansky 2001). In my study species, the Malaysian stalk-
eyed fly *Teleopsis dalmanni*, these conditions are met. The production of
sperm is generally costly (Dewsbury 1982). In stalk-eyed flies, males further
are limited in their daily mating capacity by the time taken to recover accessory gland organ size (Rogers et al. 2005) and females vary substantially in fecundity (Cotton et al. 2010). The direct costs of assessing mates are likely to be small due to the lek-style mating system exhibited by this species (Cotton et al. 2010). Previous work has indeed shown that males prefer to mate with females with large eyespan, indicative of high fecundity, in both the laboratory and the field. In this chapter I took this work further by investigating if and how sex-ratio meiotic drive (SR) affected male mate preference. As SR in this species is associated with a large, low frequency, inversion that restricts recombination (Johns et al. 2005; Paczolt et al. 2017), the X^{SR} chromosome is expected to have accumulated deleterious mutations. I therefore predicted that low-quality SR males would be less able to bear the costs of mating and would express weaker mate preference. Using a series of binary choice trials, I found that both SR and ST males preferred to mate with large females, and that they do not differ in the strength of this male mate preference. However, SR males were unable to mate at the same frequency as ST males, likely as a result of their reduced accessory gland size (Meade et al. 2020). These results suggest that the expected reduced quality of SR males does not lead to a severe weakening of male preference. This may be because the cost of assessing females is low once multiple females have already settled with a male. I also found that mate preference depended on eyespan, with large eyespan males preferring to mate with large eyespan females, while small eyespan males showed no preference. As visual acuity improves with increased eyespan (Burkhardt and de la Motte
1983; de la Motte and Burkhardt 1983), larger eyespan males may be better at distinguishing differences in female quality, as has been shown in female preference (Hingle et al. 2001a). To a lesser extent this may impact on the mate preferences of SR males as SR is associated with reduced eyespan in this species (Wilkinson et al. 1998; Cotton et al. 2014), although this difference is relatively small (~5%; Meade et al. 2020). Future work is needed in order to determine how the reduced eyespan of SR males affects their ability to attract and mate with multiple females in the wild.

6.2.2 Chapter 3 – Meiotic drive reduces egg-to-adult viability in stalk-eyed flies

Meiotic drive is often predicted to spread rapidly in a population. Despite this, drive in many species is found at low to moderate frequencies that are stable across decades (Wilkinson et al. 2003; Pinzone and Dyer 2013; Price et al. 2014; Paczolt et al. 2017; Verspoor et al. 2018). In the stalk-eyed fly *Teleopsis dalmanni*, SR is approximately 500,000 years old and appears to be broadly stable at a moderate (~20%) frequency (Paczolt et al. 2017). In order for SR to exist at a stable polymorphism there must be frequency-dependent selection allowing SR to spread when rare but preventing further increases in frequency when common. Recessive fitness costs such as reduced female viability represent one possible selective counter-force that may fulfil this requirement. This is because such costs will not be evident until the frequency of SR is high enough for homozygotes to be common.
The X\textsuperscript{SR} chromosome in \textit{T. dalmanni} is associated with at least one large inversion (Johns et al. 2005; Paczolt et al. 2017) as in many other species (Stalker 1961; Beckenbach 1983, Dyer et al. 2007; Pieper and Dyer 2016; Verspoor et al. 2018). Low frequency inversions restrict recombination and so are expected to accumulate deleterious alleles (Kirkpatrick 2010), which may impact viability. In this chapter, I used large-scale controlled crosses to assess whether the expected increased mutation load of the X\textsuperscript{SR} has led to an increase in mortality at pre-adult life stages. I found that both male and female SR carriers had reduced egg-to-adult viability compared to standard (ie wildtype) flies. I estimated the strength of selection against homozygous SR females as \( S_r = 0.242 \), and the effect of SR on viability was broadly additive \( (h = 0.511) \), with heterozygote females having viability intermediate of that of either homozygote. The viability deficit suffered by SR males was not significantly different from that of homozygous females \( (S_m = 0.214) \). I conclude that strong viability selection against the X\textsuperscript{SR} chromosome, as measured in this chapter, must play a key role in maintaining SR polymorphism in the wild.

\textbf{6.2.3 Chapter 4 – Meiotic drive does not cause condition-dependent reduction of eyespan, the sexual ornament of \textit{Teleopsis dalmanni} stalk-eyed flies}

A central thrust of the handicap hypothesis for the evolution of sexual ornaments, such as the large eyespan of stalk-eyed flies, is that these
ornaments show heightened condition-dependent expression (Zahavi 1975). Male eyespan is used as a signal of high quality in male-male interactions (Panhuis and Wilkinson 1999; Small et al. 2009), and females prefer to roost and mate with males with large eyespan, both for absolute eyespan and eyespan relative to body size (Wilkinson and Reillo 1994; Hingle et al. 2001b; Cotton et al. 2010). Male eyespan is highly sensitive to both environmental (David et al. 1998; Cotton et al. 2004) and genetic stress (David et al. 2000; Bellamy et al. 2013; Howie et al. 2019), and so it therefore constitutes an honest signal of underlying genetic quality (Zahavi 1975). In *T. dalmanni*, eyespan is also associated with sex-ratio meiotic drive, with SR males having reduced eyespan compared to standard males (Wilkinson et al. 1998; Cotton et al. 2014). These eyespan differences could be the result of fixed genetic differences between the X_{SR} and X_{ST} chromosomes (Johns et al. 2005; Reinhardt et al. 2014). Alternatively, reduced eyespan may be a reflection of an increased mutation load due to the accumulation of deleterious mutations within X_{SR}-associated inversions (Kirkpatrick 2010), which would be expected to have condition-dependent effects. In this chapter I tested this hypothesis by rearing male and female larvae of all possible genotypes (X_{ST}/Y, X_{SR}/Y, X_{ST}/X_{ST}, X_{SR}/X_{ST}, X_{SR}/X_{SR}) under low and high stress food treatments and examining the resulting variation in eyespan. In females, the effect of SR on eyespan is additive under low stress and heterotic under high stress. In males, I found that SR males had smaller eyespan than ST males in both treatments, and this difference is not amplified when reared at high stress. This suggests that expression of eyespan in meiotic drive males is not
strongly condition-dependent in this species. These results may be interpreted in terms of strategic resource allocation. Despite the expected destruction of half of all sperm, SR males transfer similar numbers of sperm per ejaculate as standard males (Meade et al. 2019). This is apparently achieved due to increased investment in testes development, which are larger in SR males (Meade et al. 2020). However, resources are not limitless, and so this must be traded off against reduced investment elsewhere. This likely explains why SR males have reduced accessory glands (Meade et al. 2020). Less obviously, increased investment in testes may incur trade-offs with lifespan (Fry 2006). These trade-offs may be adaptive as they allow SR males to maintain high fertility albeit at the cost of attracting fewer females and mating at a reduced rate (Meade et al. 2020).

6.2.4 Chapter 5 – Experimental population genetics of meiotic drive in the Malaysian stalk-eyed fly, *Teleopsis dalmanni*.

It has been predicted that *sex-ratio* meiotic drive (SR) should rapidly spread through populations that carry it, potentially even causing extinction due to a lack of males (Hamilton 1967). Despite this, SR is often found at a stable polymorphism in many species in the wild (Wilkinson et al. 2003; Pinzone and Dyer 2013; Price et al. 2014). When the frequency of drive has been tracked in laboratory cage populations of the fruitfly *Drosophila pseudoobscura*, SR did not spread or stay at stable polymorphism. Instead SR generally declined in frequency when males and females are kept
together and allowed to mate freely (Wallace 1948; Beckenbach 1983; Price et al. 2008b; Price et al. 2010). In wild populations of the stalk-eyed fly *Teleopsis dalmanni* in the Gombak valley, drive frequency has not changed over ~10 years. However, within small populations the frequency of drive is highly variable, ranging from 0-50% (L. Meade, unpublished data). Drive frequency appears to be negatively correlated with population size, with small populations having a higher frequency of SR. In order to better understand these findings, I established freely-mating cage populations of stalk-eyed flies with drive at a moderately high initial frequency (mean ≈ 40%) and tracked the frequency of drive over successive generations. Cage populations were kept on either high quality larval food or low quality larval food. In this chapter I present preliminary results from this work. I found that drive generally declined in frequency in cage populations, as in previous work in *D. pseudoosbcura* (Wallace 1948; Beckenbach 1983; Price et al. 2008b; Price et al. 2010). The primary effect of the low food treatment was to reduce the productivity of these cages. Interestingly, this led to drive being maintained at a higher frequency in these lines, mimicking the finding in the wild that small populations carry SR at higher frequencies. I also found evidence that one population had been driven extinct by meiotic drive, suggesting that small populations are vulnerable to stochastic fluctuations in SR frequency and population sex ratio. One possible explanation for the decline in SR frequency in this experiment is that high levels of multiple mating in cage populations may have particularly disadvantaged SR males, as these males are expected to be poor sperm competitors (Price and
Wedell 2008). This may explain the difference in SR frequency in high and low lines, as the female mating rate is expected to be correlated with population size. Future work should endeavour to understand how polyandry and sperm competition vary between wild populations of stalk-eyed flies that are known to differ in SR frequency.

6.3 Future directions

This thesis has explored the effect of meiotic drive on mate preferences, viability and the expression of a sexual ornament. I have additionally tracked the frequency of drive in experimental cage populations. Through this work I have identified further areas that warrant study in this species.

6.3.1 The effect of meiotic drive on fitness under a range of environmental conditions.

Previous work assessing the impact of meiotic drive on a range of traits has typically reared flies under benign, low-density conditions (Wilkinson et al. 2006; Finnegan et al. 2019). Wilkinson et al. (2006) showed that the fecundity of female carriers of meiotic drive is not reduced. In fact, they find some evidence that females heterozygous for drive have higher fecundity. In chapter 3 of this thesis, I show meiotic drive reduces the viability of male and female larvae. In the fruitfly, *D. pseudoobscura*, the effect of sex-ratio meiotic
Wallace (1948) found evidence that female SR heterozygotes had the highest fecundity and viability, and the viability of SR males and SR homozygotes was reduced. Curtsinger and Feldman (1980) also found heterosis for fecundity but found that the effect of SR on viability was additive in females. On the other hand, Beckenbach (1983) found no difference in egg production between the three female genotypes and found that SR reduced viability in males, but not in females. Recently, Larner et al. (2019) found that SR homozygous females produced fewer offspring than other female genotypes, and found no evidence for heterosis. The most parsimonious explanation for the differences between these experiments is that the effect of SR on these traits is density-dependent, with costs to SR carriers being greatest at high density (Beckenbach 1983; Finnegan et al. 2019; Larner et al. 2019).

In the Gombak valley, *T. dalmanni* stalk-eyed flies are distributed in a number of small populations with little migration between them (Cotton et al. 2014). Population density is highly variable between these populations, and flies sampled show high variability in relative eyespan and fecundity (Cotton et al. 2014; Meade et al. 2017). These traits are strongly influenced by food quality and the density under which larvae are reared (David et al. 1998; Hingle et al. 2001a; Cotton et al. 2004), suggesting these populations vary considerably in their typical larval environment. Nonetheless, it is clear that
the natural environment is considerably more variable and likely harsher than
the laboratory environment. As such, the impact of meiotic drive on fecundity
and viability in stalk-eyed flies should be assessed under a broader range of
ecologically relevant conditions. For example, flies should be reared under a
range of larval densities and the effect of meiotic drive on viability, fecundity,
and trait assessed. Other conditions could include reduced food quality or a
range of temperatures.

6.3.2 Sperm competition and polyandry

Meiotic drive in many species is associated with a reduction in fertility (Hartl
et al. 1976; Wu 1983a,b; Jaenike 1996; Atlan et al. 2004; Angelard et al.
2008) and sperm competitive ability (Atlan et al. 2004; Wilkinson et al. 2006;
Price et al. 2008a; Manser et al. 2011). The reduced sperm competitive
ability of drive males may come about due to the transfer of fewer and/or
lower quality sperm (Price and Wedell 2008; Manser et al. 2011: Bravo
Nuñez et al. 2018). In the stalk-eyed fly, T. dalmanni, Wilkinson et al. (2006)
showed that when females mate to both SR and ST males, the paternity
share of SR males is ~0.25. They attributed this deficit to the transfer of
fewer sperm that are potentially also of lower quality (Wilkinson et al. 2006).
However, Meade et al. (2019) found that SR and ST males actually transfer
similar numbers of sperm per mating. In the related species, T. whitei,
females store the same number of sperm after mating with an SR or an ST
male, but SR sperm do not survive well in the seminal fluid of ST males (Fry
and Wilkinson 2004). This suggests that the poor sperm competitive ability of *T. whitei* SR males (Wilkinson and Fry 2001) is mediated by differences in seminal fluid, not the number of sperm transferred. Given that the accessory glands – the organs that produce seminal fluid – are reduced in *T. dalmanni* SR males (Meade et al. 2020), this idea warrants further study in this species. It would be interesting to examine how drive affects protein expression in these organs.

A plausible explanation for the results obtained in chapter 5 is that a high rate of multiple mating in females fostered sperm competition which particularly disadvantaged SR males, as in other species (Price et al. 2010; Pinzone and Dyer 2013; Price et al. 2014). There is therefore now a real need to test the impact of sperm competition and polyandry in the wild as well as under controlled laboratory conditions. While the mating rates of wild males have been investigated (Cotton et al. 2015), little is known about the rate of multiple mating females in the wild. In the laboratory, females are highly promiscuous, mating as much as 5 times an hour (Reguera et al. 2004). However, mating rates are expected to be much lower than this in the wild as the laboratory measures corralled males and females into mating chambers, so they could not disperse. In the wild, males and females gather at dusk where they form leks on exposed root hairs (Burkhardt and de la Motte 1983). Leks typically consist of one male and several females (Cotton et al. 2010), and so females are not expected to mate with multiple males per day. However, while males return to the same lek every night, females on average
move ∼1 m between leks, suggesting that females mate with different males across mating sessions (Wilkinson et al. 1998). The impact of sperm competition on paternity in the laboratory, where a female is typically mated to SR and ST males sequentially (Wilkinson et al. 2006), is likely to be different from that of the wild, where females may not mate with another male for 24 hours. Future work should examine how sperm competition affects SR male paternity over these short timescales. On a related note, mating in *T. dalmanni* males is associated with a reduction in the size of accessory glands, which take 8-48 hours to recover in ST males (Rogers et al. 2005). As the seminal fluid produced by these organs may play an important role in sperm competition (Fry and Wilkinson 2004; Wilkinson et al. 2006), and SR males have smaller accessory glands (Meade et al. 2020), it would be interesting to establish whether SR and ST males differ in the time that it takes for these organs to recover to their maximum size after mating. Wilkinson et al. (2003) found no difference in mating rates between four geographically distinct populations of *T. dalmanni* but these populations also did not differ by very much in their frequency of meiotic drive. However, within one of these populations, the Gombak valley, we find that flies are split into several small subpopulations, with little migration between them (Cotton et al. 2014). The frequency of drive in these subpopulations differs quite dramatically (0-50%) and is negatively correlated with population size (L. Meade, unpublished data). In *D. pseudoobscura* and *D. neotestacea* the frequency of drive across populations is correlated with the rate of female multiple mating (Pinzone and Dyer 2013; Price et al. 2014). The Gombak
valley is an interesting natural laboratory and presents an opportunity to determine if drive frequency and female multiple mating are also positively correlated in *T. dalmani*.

### 6.3.3 Genomics of stalk-eyed fly species

Meiotic drive in *T. dalmani* is associated with at least one large inversion that restricts recombination between drive (X<sup>SR</sup>) and standard (X<sup>ST</sup>) chromosomes (Johns et al. 2005; Paczolt et al. 2017). This, combined with the age of the X<sup>SR</sup> chromosome (500,000ya; Paczolt et al. 2017) means that it is expected to be highly diverged from the X<sup>ST</sup> chromosome. As the X chromosome is gene-rich, this divergence is expected to lead to considerable variation at a range of traits. Reinhard et al. (2014) analysed expression differences between X<sup>SR</sup> and X<sup>ST</sup> using RNAseq on replicate pools of testes. They found over 500 differentially expressed transcripts, hundreds of which carried fixed differences between X<sup>SR</sup> and X<sup>ST</sup>. However, while testes-specific expression is clearly important for a selfish gene that affects sperm development, the X<sup>SR</sup> chromosome also has significant effects on other traits in *T. dalmani* including viability (Finnegan et al. 2019), eye development (Wilkinson et al. 1998; Cotton et al. 2014), and accessory gland development (Meade et al. 2020). Recently, Lindholm et al. (2019) examined expression differences in the liver, brain, and gonads of both sexes of mice with and without the autosomal driver t-haplotype. They showed that, while the majority of differentially expressed transcripts were associated with the
testes, the brain also showed strong upregulation of t-haplotype genes that were not differentially expressed in testes. This suggests that further work should look for fixed differences in other genes along the inverted region of the X<sup>SR</sup> in stalk-eyed flies.

Historically, genomic examination of meiotic drive chromosomes has been hampered by the difficulties associated with sequencing inverted regions. However, recent advances in long-read sequencing may ameliorate this issue (Amarasinghe et al. 2020). Long-read sequencing of X<sup>SR</sup> and X<sup>ST</sup> chromosomes has the potential to provide considerable insight into the evolution of the X<sup>SR</sup> chromosome and may enable detection of the causative drive genes themselves. Given the relatively ancient divergence of X<sup>SR</sup> and X<sup>ST</sup> chromosomes, it has been suggested that selection may have had time to allow the genome to adapt to the deficits in sperm production caused by drive (Meade et al. 2020). Examination of dN/dS rates with genes in the inverted region may allow determination of which genes are under selection on this chromosome.

Long-read sequencing may also enable us to better understand the evolutionary history of meiotic drive in three closely related species of stalk-eyed fly. In the Gombak valley, there are three species of stalk-eyed fly that live in sympatry. We now know that was previously thought of as *T. dalmanni* is likely actually two cryptic species, informally termed *T. dalmanni-1* and *T. dalmanni-2* (Paczolt et al. 2017). These species are indistinguishable by eye
but are reproductively isolated (Christianson et al. 2005; Rose et al. 2014). Some \textit{T. dalmanni}-1 individuals carry \textit{sex-ratio} meiotic drive, but extremely female-biased brood sex ratios have not been detected in \textit{T. dalmanni}-2, suggesting that meiotic drive is either absent or completely suppressed in this species (Paczolt et al. 2017). Meiotic drive has also been detected in the closely-related but phenotypically distinct \textit{T. whitei} (Paczolt et al. 2017). In order to better understand the evolution of drive within this group, Paczolt et al. (2017) sequenced three \textit{X}-linked genes, an \textit{autosomal} gene, and the 16S \textit{rRNA mitochondrial} gene in these species. They found considerable divergence between \textit{X}^{SR} and \textit{X}^{ST} \textit{genes} in \textit{T. dalmanni}-1 but very little divergence between these \textit{chromosomes} in \textit{T. whitei}. This suggests that either meiotic drive evolved so recently in \textit{T. whitei} that there is insufficient differentiation in the markers used to detect it, that drive in \textit{T. whitei} is not associated with a large inversion, or that it is determined by a small region that is distant from markers. As sperm development of SR \textit{T. whitei} \textit{males} differs from that of SR \textit{T. dalmanni}-1 \textit{males} (Wilkinson and Sanchez 2001; Wilkinson et al. 2014), this suggests that meiotic drive has evolved independently in these species (Paczolt et al. 2017). Alternatively, these three species are derived from a common ancestor with drive, which has been lost from one species (\textit{T. dalmanni}-2), and lost/gained an association with an inversion that distinguishes the other two species. Sequencing of the \textit{T. whitei} \textit{X}^{SR} and \textit{X}^{ST} \textit{chromosomes} may therefore provide further insight into the evolution of two distinct \textit{segregation distorder} systems in two species with similar life histories. It would also be interesting to establish if there are
shared phenotypic differences between SR and ST individuals in these species, such as reduced eyespan of SR males. The absence of meiotic drive in *T. dalmanni-2* is also worthy of further study, particularly given the suggestion that meiotic drive could be involved in hybrid incompatibilities (Frank 1991; Hurst and Pomiankowski 1991; Wilkinson et al. 2014; Verspoor et al. 2018).

### 6.4 Conclusion

In this thesis I have investigated a number of ways in which meiotic drive impacts upon the fitness of its carriers. My work represents a significant contribution towards understanding why many populations remain polymorphic for meiotic drive. Future researchers will now need to extend this research to analyses of populations of wild flies to fully understand the ecological and evolutionary dynamics that determine how meiotic drive is maintained.
6.5 References


Appendices
Appendix A

Chapter 2: Does meiotic drive alter male mate preference?

A.1 Model Outputs

A.1.1 Experiment 1

A.1.1.1 Supplementary Table S1

Pooled across genotypes, males show preference for large females.

```r
ml <- glm(cbind(L.matings, S.matings) ~ 1, data=subset(MC1, !is.na(Pref)),
          family=quasibinomial, weights=Total.matings)

             Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.6589939 0.1048181 6.287024 0
```

Males preferred large females in the first, second, and third matings. In the fourth and fifth matings, there was no preference for large females, in large part reflecting reduced sample size. Below are model output tables for these analyses.

First:

Number of matings with large female = 116.
Number of matings with small female = 46.
Mean preference ± SE in the first mating = 0.4321 ± 0.0711.

```r
## Exact binomial test
## data: c(116, 46)
## number of successes = 116, number of trials = 162, p-value = 3.67e-08
```
## alternative hypothesis: true probability of success is not equal to 0.5
## 95 percent confidence interval: 0.6399905 0.7840189
## sample estimates:
## probability of success
## 0.7160494

Second:
Number of matings with large female = 86.
Number of matings with small female = 46.
Mean preference ± SE in the second mating = 0.3030 ± 0.0833.

## Exact binomial test
## data: c(86, 46)
## number of successes = 86, number of trials = 132, p-value = 0.0006313
## alternative hypothesis: true probability of success is not equal to 0.5
## 95 percent confidence interval: 0.5637418 0.7323227
## sample estimates:
## probability of success
## 0.6515152

Third:
Number of matings with large female = 72.
Number of matings with small female = 29.
Mean preference ± SE in the second mating = 0.4257 ± 0.0905.

## Exact binomial test
## data: c(72, 29)
## number of successes = 72, number of trials = 101, p-value = 2.237e-05
## alternative hypothesis: true probability of success is not equal to 0.5
## 95 percent confidence interval: 0.6143106 0.7985450
## sample estimates:
## probability of success
## 0.7128713

Fourth: Number of matings with large female = 36.
Number of matings with small female = 25.
Mean preference ± SE in the second mating = 0.1803 ± 0.1270.

## Exact binomial test
## data: c(36, 25)
## number of successes = 36, number of trials = 61, p-value = 0.2
## alternative hypothesis: true probability of success is not equal to 0.5
## 95 percent confidence interval: 0.4567764 0.7144956
## sample estimates:
## probability of success
## 0.5901639

Fifth: Number of matings with large female = 17.
Number of matings with small female = 10.
Mean preference ± SE in the second mating = 0.2593 ± 0.1894.
# Exact binomial test

## data: c(17, 10)
## number of successes = 17, number of trials = 27, p-value = 0.2478
## alternative hypothesis: true probability of success is not equal to 0.5
## 95 percent confidence interval:
## 0.4236796 0.8059928
## sample estimates:
## probability of success
## 0.6296296

A.1.1.2 Supplementary Table S2

SR and ST males do not differ in the strength of their preference. SR preference mean ± SE = 0.3970 ± 0.0800. ST preference mean ± SE = 0.3367 ± 0.0806.

```r
ml <- glm(cbind(L.matings, S.matings) ~ 1 + Genotype, data=MC1,
family=quasibinomial, weights=Total.matings)
Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.6307416 0.1666489 3.784854 0.0002194
GenotypeST 0.0327068 0.2177318 0.150216 0.8807895
```

A.1.1.3 Supplementary Table S3

The preference of SR males is significantly different from zero.

```r
ml <- glm(cbind(L.matings, S.matings) ~ 1, data=subset(SR_total,
!is.na(Pref)), family=quasibinomial, weights=Total.matings)
Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.6307416 0.138061 4.56857 1.76e-05
```

A.1.1.4 Supplementary Table S4

The preference of ST males is significantly different from zero.

```r
ml <- glm(cbind(L.matings, S.matings) ~ 1, data=subset(ST_total,
!is.na(Pref)), family=quasibinomial, weights=Total.matings)
Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.6634484 0.1618788 4.098428 0.0001043
```

A.1.1.5 Supplementary Tables S5-S7

SR and ST males do not differ in the strength of their preference across the first, second, or third matings.

First:

SR mean preference ± SE = 0.5062 ± 0.0964 ST mean preference ± SE = 0.3864 ± 0.1073

```r
ml <- glm(family = quasibinomial, data = Pref1, formula = `1st` ~ Genotype)
Df Deviance Resid. Df Resid. Dev F Pr(>F)
NULL NA NA 156 186.2628 NA
Genotype 1 0.9224502 155 185.3404 0.9106982 0.3414151
```

Second:
SR mean preference ± SE = 0.3333 ± 0.1227 ST mean preference ± SE = 0.2647 ± 0.1178

\[
\text{ml} \left< \text{glm}(\text{family = quasibinomial, data = Pref1, formula = `2nd` ~ Genotype)}\right>
\]

\[\begin{array}{llllll}
\text{Df} & \text{Deviance} & \text{Resid. Df} & \text{Resid. Dev} & F & \text{Pr}(F) \\
\text{NULL} & NA & NA & 127 & 165.9926 & NA \\
\text{Genotype} & 1 & 0.1648357 & 126 & 165.8277 & 0.1622601 & 0.6877668 \\
\end{array}\]

Third:

SR mean preference ± SE = 0.3000 ± 0.1528 ST mean preference ± SE = 0.4737 ± 0.1177

\[
\text{ml} \left< \text{glm}(\text{family = quasibinomial, data = Pref1, formula = `3rd` ~ Genotype)}\right>
\]

\[\begin{array}{llllll}
\text{Df} & \text{Deviance} & \text{Resid. Df} & \text{Resid. Dev} & F & \text{Pr}(F) \\
\text{NULL} & NA & NA & 96 & 118.3377 & NA \\
\text{Genotype} & 1 & 0.8399021 & 95 & 117.4978 & 0.1788765 \\
\end{array}\]

A.1.1.6 Supplementary Table S8

SR and ST males do not differ in the rate at which they failed to mate at least once. MC1$Mated is a new column of the of the MC1 dataset that displays a 1 if Total.matings > 0 and a 0 otherwise.

Failure rate of SR males = 23/104. Failure rate of ST males = 13/89.

\[
\text{ml} \left< \text{glm(data=MC1, formula = MC1$Mated ~ Genotype, family = binomial)}\right>
\]

\[\begin{array}{llllll}
\text{Df} & \text{Deviance} & \text{Resid. Df} & \text{Resid. Dev} & \text{Pr}(>Chi) \\
\text{NULL} & NA & NA & 192 & 185.7239 & NA \\
\text{Genotype} & 1 & 1.806934 & 191 & 183.9169 & 0.1788765 \\
\end{array}\]

A.1.2 Experiment 2

A.1.2.1 Supplementary Table S9

Eyespan depends on thorax length but not genotype.

\[
\text{ml} \left< \text{lm(data = MC2, Eyespan ~ Thorax*Genotype)}\right>
\]

\[\begin{array}{llllll}
\text{Df} & \text{Sum Sq} & \text{Mean Sq} & \text{F value} & \text{Pr}(>F) \\
\text{Thorax} & 1 & 343.0963353 & 343.0963353 & 788.5067497 & 0.0000000 \\
\text{Genotype} & 1 & 0.4056111 & 0.4056111 & 0.9321786 & 0.3355183 \\
\text{Thorax:Genotype} & 1 & 0.0005910 & 0.0005910 & 0.0013582 & 0.9706402 \\
\text{Residuals} & 191 & 83.1082297 & 0.4351216 & NA & NA \\
\end{array}\]

A.1.2.2 Supplementary Table S10

Pooled across genotypes, males prefer large females.

\[
\text{ml} \left< \text{glm(data=MC2, formula = cbind(L.matings, S.matings) ~ 1, family=quasibinomial, weights=Total.matings)}\right>
\]

\[\begin{array}{lllll}
\text{Estimate} & \text{Std. Error} & \text{t value} & \text{Pr}(>|t|) \\
\text{(Intercept)} & 0.6074548 & 0.086241 & 7.043688 & 0 \\
\end{array}\]
A.1.2.3 Supplementary Table S11

Eyespan has a strong effect on mate preference

\[ \text{m1} \left< \text{glm} (\text{data} = \text{MC2}, \text{formula} = \text{cbind}(L.\text{matings}, S.\text{matings}) \sim \text{Eyespan}\ast\text{Genotype}, \text{family} = \text{quasibinomial}, \text{weights} = \text{Total.\text{matings}}) \right. \]

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<th>Resid. Dev</th>
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<td>172</td>
<td>1087.322</td>
<td>1.1991764</td>
</tr>
</tbody>
</table>

A.1.2.4 Supplementary Table S12

When male are split into groups on the basis of their eyespan, eyespan group (M_Size) has a strong effect on mate preference.

Large male preference mean ± SE = 0.4110 ± 0.0618.
Medium male preference mean ± SE = 0.1919 ± 0.0828. Small male preference mean ± SE = -0.0702 ± 0.1263.

\[ \text{m1} \left< \text{glm} (\text{data} = \text{subset}(\text{MC2}, !\text{is.na}(\text{Genotype})), \text{formula} = \text{cbind}(L.\text{matings}, S.\text{matings}) \sim \text{M_Size}\ast\text{Genotype}, \text{family} = \text{quasibinomial}, \text{weights} = \text{Total.\text{matings}}) \right. \]

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A.1.2.5 Supplementary Table S13

There was no difference in the strength of preference according to genotype.

SR preference mean ± SE = 0.2508 ± 0.0887. ST preference mean ± SE = 0.2156 ± 0.0600.

\[ \text{m1} \left< \text{glm} (\text{data} = \text{MC2}, \text{formula} = \text{cbind}(L.\text{matings}, S.\text{matings}) \sim \text{Eyespan}\ast\text{Genotype}, \text{family} = \text{quasibinomial}, \text{weights} = \text{Total.\text{matings}}) \right. \]

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</table>

A.1.2.6 Supplementary Table S14

The preference of SR males is significantly different from zero. SR preference mean ± SE = 0.2508 ± 0.0887.

\[ \text{m1} \left< \text{glm} (\text{data} = \text{MC2}_\text{SR}, \text{formula} = \text{cbind}(L.\text{matings}, S.\text{matings}) \sim \text{1}, \text{family} = \text{quasibinomial}, \text{weights} = \text{Total.\text{matings}}) \right. \]
**A.1.2.7 Supplementary Table S15**

The preference of ST males is significantly different from zero. ST preference mean ± SE = 0.2156 ± 0.0600.

```
m1 <- glm(data=MC2_ST, formula = cbind(L.matings, S.matings) ~ 1, family=quasibinomial, weights = Total.matings)
```

**A.1.2.8 Supplementary Tables S16-18**

SR and ST males do not differ in the strength of their preference across the first, second, or third matings.

**First:**

SR preference mean ± SE = 0.3871 ± 0.1181. ST preference mean ± SE = 0.2982 ± 0.0898.

```
m1 <- glm(data = Pref1, formula = `1st`~ Genotype, family = quasibinomial)
```

**Second:**

SR preference mean ± SE = 0.4286 ± 0.1218. ST preference mean ± SE = 0.1800 ± 0.0989.

```
m1 <- glm(data = Pref1, formula = `2nd`~ Genotype, family = quasibinomial)
```

**Third:**

SR preference mean ± SE = 0.3191 ± 0.1397. ST preference mean ± SE = 0.2093 ± 0.1061.

```
m1 <- glm(data = Pref1, formula = `3rd`~ Genotype, family = quasibinomial)
```

**A.1.2.9 Supplementary Table S19**

SR and ST males do not differ in the rate at which they failed to mate at least once. Failure rate also does not depend on eyespan. MC1$Mated is a new column of the of the MC1 dataset that displays a 1 if Total.matings > 0 and a 0 otherwise.

Failure rate of SR males = 7/69.

Failure rate of ST males = 14/128.
Failure rate of large males = 9/89.
Failure rate of medium males = 3/63. Failure rate of small males = 10/38.

\[ ml \leftarrow \text{glm}\left(\text{data=MC2, formula = MC2}$\text{Mated} \sim \text{M}_\text{Size}\text{+Genotype, family = binomial}\right) \]

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Deviance</th>
<th>Resid. Df</th>
<th>Resid. Dev</th>
<th>Pr(&gt;Chi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NULL</td>
<td>NA</td>
<td>NA</td>
<td>196</td>
<td>133.7020</td>
<td>NA</td>
</tr>
<tr>
<td>M_Size</td>
<td>2</td>
<td>5.6826286</td>
<td>194</td>
<td>128.0193</td>
<td>0.0583499</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>0.0059434</td>
<td>193</td>
<td>128.0134</td>
<td>0.9385490</td>
</tr>
</tbody>
</table>

### A.1.3 Analysis of mating frequency

#### A.1.3.1 Supplementary Table S20

In the combined experiment 1 and experiment 2 dataset, genotype has a strong effect on mating frequency, as does experiment.

SR mean total matings ± SE = 2.6127 ± 0.1445.
SR mean total matings ± SE = 3.2857 ± 0.1392.

Experiment 1 mean total matings ± SE = 2.4975 ± 2.4975.
Experiment 2 mean total matings ± SE = 3.3713 ± 3.3713.

\[ ml \leftarrow \text{glm}\left(\text{data=subset(MC_Comb, !is.na(Pref)), formula = Total.matings} \sim \text{Experiment}\text{*Genotype, family=poisson}\right) \]

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Deviance</th>
<th>Resid. Df</th>
<th>Resid. Dev</th>
<th>Pr(&gt;Chi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NULL</td>
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<td>NA</td>
<td>332</td>
<td>290.8451</td>
<td>NA</td>
</tr>
<tr>
<td>Experiment</td>
<td>1</td>
<td>10.962471</td>
<td>331</td>
<td>279.8826</td>
<td>0.0009298</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>5.567241</td>
<td>330</td>
<td>274.3154</td>
<td>0.0182996</td>
</tr>
<tr>
<td>Experiment:Genotype</td>
<td>1</td>
<td>2.806618</td>
<td>329</td>
<td>271.5088</td>
<td>0.0938761</td>
</tr>
</tbody>
</table>

#### A.1.3.2 Supplementary Table S21

Genotype had a strong effect on mating frequency in large eyespan flies. Here large eyespan includes all flies from experiment 1 and "large" flies from experiment 2, which have eyespan>7.5mm. Flies in experiment 1 mated fewer times overall. As experiment 1 only used large flies, this pulls the mean total number of copulations carried out by large males down considerably. This makes the combined dataset inappropriate for use in the analysis of the effect of eyespan on mating frequency, but it may still be used to estimate the effect of genotype on mating frequency.

SR large male preference mean ± SE = 2.4519 ± 0.1631. ST large male preference mean ± SE = 3.4452 ± 0.1774.

\[ ml \leftarrow \text{glm}\left(\text{data=subset(MC_Comb, !is.na(Pref) & !is.na(Genotype) & M_Size=="Large"), formula = Total.matings} \sim \text{Experiment}\text{*Genotype, family=poisson}\right) \]

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Deviance</th>
<th>Resid. Df</th>
<th>Resid. Dev</th>
<th>Pr(&gt;Chi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NULL</td>
<td>NA</td>
<td>NA</td>
<td>235</td>
<td>218.9937</td>
<td>NA</td>
</tr>
<tr>
<td>Experiment</td>
<td>1</td>
<td>19.9382313</td>
<td>234</td>
<td>199.0554</td>
<td>0.0000080</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>9.8029762</td>
<td>233</td>
<td>189.2525</td>
<td>0.0017423</td>
</tr>
<tr>
<td>Experiment:Genotype</td>
<td>1</td>
<td>0.3364147</td>
<td>232</td>
<td>188.9161</td>
<td>0.5619061</td>
</tr>
</tbody>
</table>

#### A.1.3.3 Supplementary Table S22

Genotype does not affect the mating frequency of medium flies.
SR medium male preference mean ± SE = 3.6522 ± 0.3957. ST medium male preference mean ± SE = 3.5641 ± 0.2644.

```
m1 <- glm(data=subset(MC_Comb, !is.na(Pref) & !is.na(Genotype) & M_Size=="Medium"),
               formula = Total.matings ~ Genotype, family=poisson)
```

<table>
<thead>
<tr>
<th>Df</th>
<th>Deviance</th>
<th>Resid. Df</th>
<th>Resid. Dev</th>
<th>Pr(&gt;Chi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NULL</td>
<td>58</td>
<td>40.52450</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>0.4153436</td>
<td>57</td>
<td>40.10916</td>
</tr>
</tbody>
</table>

**A.1.3.4 Supplementary Table S23**

Genotype does not affect the mating frequency of small flies.
SR small male preference mean ± SE = 2.4667 ± 0.3887. ST small male preference mean ± SE = 2.2188 ± 0.3102.

```
m1 <- glm(data=subset(MC_Comb, !is.na(Pref) & !is.na(Genotype) & M_Size=="Small"),
               formula = Total.matings ~ Genotype, family=poisson)
```

<table>
<thead>
<tr>
<th>Df</th>
<th>Deviance</th>
<th>Resid. Df</th>
<th>Resid. Dev</th>
<th>Pr(&gt;Chi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Genotype</td>
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<td>0.0001139</td>
<td>36</td>
<td>24.94022</td>
</tr>
</tbody>
</table>

**A.1.3.5 Supplementary Table S24**

To determine the effect of eyespan on mating frequency we look just in experiment 2, as experiment 1 used only one size class of males.
Large mean total matings ± SE = 3.8876 ± 0.2268.
Medium mean total matings ± SE = 3.6032 ± 0.2165.
Small mean total matings ± SE = 2.1600 ± 0.2414.

```
m1 <- glm(data=subset(MC2, !is.na(Pref) & !is.na(Genotype)), formula = Total.matings ~ M_Size, family=poisson)
```

<table>
<thead>
<tr>
<th>Df</th>
<th>Deviance</th>
<th>Resid. Df</th>
<th>Resid. Dev</th>
<th>Pr(&gt;Chi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NULL</td>
<td>175</td>
<td>145.6481</td>
</tr>
<tr>
<td>M_Size</td>
<td>2</td>
<td>15.36234</td>
<td>173</td>
<td>130.2857</td>
</tr>
</tbody>
</table>
A.2 Supplementary Figures

A.2.1 Supplementary Figure S1

Experiment 2 regression of body size on eyespan for SR and ST males.
Appendix B

Chapter 3: Meiotic drive reduces egg-to-adult viability in stalk-eyed flies

B.1 Model outputs

B.1.1 Supplementary table S1

The effect of food condition on egg-to-adult viability in males:

```
ml <- lmer(data=Male_Survival, formula = W ~ Genotype*Condition + (1|Cage_ID) + (1|Collection_Date))
```

|                | Estimate  | Std. Error | df  | t value | Pr(>|t|) |
|----------------|-----------|------------|-----|---------|----------|
| (Intercept)    | 0.3828775 | 0.0545171  | 55.57003 | 7.0230708 | 0.0000000 |
| GenotypeXY     | 0.1790490 | 0.0654798  | 79.00000 | 2.734174 | 0.0077113 |
| ConditionL     | 0.0769641 | 0.0720155  | 147.04295 | 1.0687149 | 0.2869495 |
| ConditionM     | 0.0308253 | 0.0730934  | 148.96913 | 0.421754 | 0.6738334 |
| GenotypeXY:ConditionL | -0.1157585 | 0.0969522 | 79.00000 | -1.1939743 | 0.2360609 |
| GenotypeXY:ConditionM | -0.0157272 | 0.0980011 | 79.00000 | -0.1604799 | 0.8729127 |

<table>
<thead>
<tr>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>NumDF</th>
<th>DenDF</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>0.7431435</td>
<td>0.7431435</td>
<td>1</td>
<td>127.3557</td>
<td>4.643296</td>
</tr>
<tr>
<td>Condition</td>
<td>0.0144249</td>
<td>0.0072124</td>
<td>2</td>
<td>72.97766</td>
<td>0.1085266</td>
</tr>
<tr>
<td>Genotype:Condition</td>
<td>0.1066840</td>
<td>0.0533420</td>
<td>2</td>
<td>116.0000</td>
<td>0.8026450</td>
</tr>
</tbody>
</table>

B.1.2 Supplementary table S2

The effect of food condition on egg-to-adult viability in females:

```
ml <- lmer(data=Female_Survival, formula = W ~ Genotype*Condition + (1|Cage_ID) + (1|Collection_Date))
```

|                | Estimate  | Std. Error | df  | t value | Pr(>|t|) |
|----------------|-----------|------------|-----|---------|----------|
| (Intercept)    | 0.4577565 | 0.0710439  | 127.3557 | 6.443296 | 0.0000000 |
| GenotypeSRX    | 0.0785903 | 0.0942983  | 116.0000 | 0.8334220 | 0.4063195 |
| GenotypeXX     | 0.2052136 | 0.0942983  | 116.0000 | 2.1762178 | 0.0315662 |
| ConditionL     | 0.0185047 | 0.0972369  | 165.6508 | 0.1903051 | 0.8493031 |
| ConditionM     | 0.0041773 | 0.0984081  | 165.5148 | 0.0424482 | 0.9661925 |
| GenotypeSRX:ConditionL | -0.0260082 | 0.1317608 | 116.0000 | -0.1973899 | 0.8438679 |
| GenotypeXX:ConditionL | -0.0958206 | 0.1317608 | 116.0000 | -0.7272316 | 0.4685493 |
| GenotypeSRX:ConditionM | 0.0442427 | 0.1333579 | 116.0000 | 0.3317589 | 0.7406700 |
| GenotypeXX:ConditionM | -0.0240328 | 0.1333579 | 116.0000 | -0.1802124 | 0.8573003 |

<table>
<thead>
<tr>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>NumDF</th>
<th>DenDF</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>0.8327350</td>
<td>0.4163675</td>
<td>2</td>
<td>116.0000</td>
<td>4.6824068</td>
</tr>
<tr>
<td>Condition</td>
<td>0.0275940</td>
<td>0.0137970</td>
<td>2</td>
<td>53.53907</td>
<td>0.1551592</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>NumDF</th>
<th>DenDF</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype:Condition</td>
<td>0.0727005</td>
<td>0.0181751</td>
<td>4</td>
<td>116.0000</td>
<td>0.2043948</td>
</tr>
</tbody>
</table>
### B.1.3 Supplementary table S3

As food condition did not affect egg-to-adult viability, condition was removed from subsequent analysis. Below are the full model results from linear mixed effect models examining the effect of genotype on egg-to-adult viability.

The effect of genotype on egg-to-adult viability in males:

```r
ml <- lmer(data=Male_Survival, formula = W ~ Genotype + (1|Cage_ID) + (1|Collection_Date))
```

| Estimate   | Std. Error | df       | t value   | Pr(>|t|)   |
|------------|------------|----------|-----------|------------|
| (Intercept)| 0.4167260  | 0.0390008| 16.94126  | 0.0000000  |
| GenotypeXY | 0.1375502  | 0.0401625| 81.00000  | 0.0009681  |

<table>
<thead>
<tr>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>NumDF</th>
<th>DenDF</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>0.7757225</td>
<td>0.7757225</td>
<td>1</td>
<td>81</td>
<td>11.72957</td>
</tr>
</tbody>
</table>

### B.1.4 Supplementary table S4

The effect of genotype of egg-to-adult viability in females:

```r
ml <- lmer(data=Female_Survival, formula = W ~ Genotype + (1|Cage_ID) + (1|Collection_Date))
```

| Estimate   | Std. Error | df       | t value   | Pr(>|t|)   |
|------------|------------|----------|-----------|------------|
| (Intercept)| 0.4654582  | 0.0424106| 29.18295  | 0.0000000  |
| GenotypeSRX| 0.0841424  | 0.0532743| 120.00000 | 0.1168722  |
| GenotypeXX | 0.1643466  | 0.0532743| 120.00000 | 0.0025278  |

<table>
<thead>
<tr>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>NumDF</th>
<th>DenDF</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>0.8239569</td>
<td>0.4119784</td>
<td>2</td>
<td>120</td>
<td>4.759265</td>
</tr>
</tbody>
</table>

### B.1.5 Supplementary table S5

The viability of both male genotypes was estimated directly from the model output of the more simplified linear model below.

```r
ml <- lm(data=Male_Survival, formula = W ~ Genotype)
```

| Estimate   | Std. Error | t value   | Pr(>|t|)   |
|------------|------------|-----------|------------|
| (Intercept)| 0.4063265  | 0.0307031 | 13.234068  | 0.0000000  |
| GenotypeXY | 0.1375502  | 0.0434207 | 3.167849   | 0.0018358  |

Here the X$^{SR}$/Y genotype is used as the comparison, so its egg-to-adult viability is the model intercept term, 0.40633.

The viability of X$^{ST}$/Y (labelled as simply GenotypeXY in the model), is calculated by adding the intercept term and the effect term together: 0.40633 + 0.13755 = 0.54388.

### B.1.6 Supplementary table S6

The viability of each female genotype was estimated in the same way as above:

```r
ml <- lm(data=Female_Survival, formula = W ~ Genotype)
```

| Estimate   | Std. Error | t value   | Pr(>|t|)   |
|------------|------------|-----------|------------|
| (Intercept)| 0.4649979  | 0.0395727 | 11.750485  | 0.0000000  |
| GenotypeSRX| 0.0841424  | 0.0559642 | 1.503505   | 0.1344614  |
| GenotypeXX | 0.1643466  | 0.0559642 | 2.936639   | 0.0037515  |
B.1.7 Supplementary table S7

To determine if the three female genotypes had significantly different viabilities, we used a Tukey’s post-hoc comparison test:

<table>
<thead>
<tr>
<th></th>
<th>diff</th>
<th>lwr</th>
<th>upr</th>
<th>p adj</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRX-SRSR</td>
<td>0.0841424</td>
<td>-0.0481157</td>
<td>0.2164006</td>
<td>0.2916928</td>
</tr>
<tr>
<td>XX-SRSR</td>
<td>0.1643466</td>
<td>0.0320885</td>
<td>0.2966048</td>
<td>0.0104345</td>
</tr>
<tr>
<td>XX-SRX</td>
<td>0.0802042</td>
<td>-0.0520539</td>
<td>0.2124623</td>
<td>0.3260922</td>
</tr>
</tbody>
</table>
B.2 Fertility trial - Supplementary table S8

Below are the results of a trial designed to test the fertility of eggs laid by X^{SR}/X^{ST} females crossed to X^{SR}/Y (Cross A) and X^{ST}/Y (Cross B) males. One day old eggs were collected and counted, then allowed to develop for a further five days. After five days of development, the vast majority of fertilised eggs have hatched, and the remainder show clear signs of development (eg segmental striations, darker colouration, development of mouthparts, etc.). At this time, the number of hatched/fertilised eggs were counted, along with the number of unfertilised eggs. In this trial, eggs were not inspected for signs of development before they were collected, and yet fertility remains high. There is no obvious difference in the fertility of Cross A and Cross B.

<table>
<thead>
<tr>
<th>Date</th>
<th>Cross</th>
<th>Pot.ID</th>
<th>Total.eggs</th>
<th>Unfert</th>
<th>Fert</th>
<th>Percent.Fert</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-Nov</td>
<td>A</td>
<td>A1</td>
<td>12</td>
<td>3</td>
<td>9</td>
<td>0.7500000</td>
</tr>
<tr>
<td>15-Nov</td>
<td>A</td>
<td>A2</td>
<td>131</td>
<td>12</td>
<td>119</td>
<td>0.9083969</td>
</tr>
<tr>
<td>15-Nov</td>
<td>A</td>
<td>A3</td>
<td>76</td>
<td>6</td>
<td>70</td>
<td>0.9210526</td>
</tr>
<tr>
<td>15-Nov</td>
<td>B</td>
<td>B1</td>
<td>81</td>
<td>8</td>
<td>73</td>
<td>0.9012346</td>
</tr>
<tr>
<td>15-Nov</td>
<td>B</td>
<td>B2</td>
<td>67</td>
<td>6</td>
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<td>15-Nov</td>
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<td>B3</td>
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</tr>
<tr>
<td>21-Nov</td>
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<td>A1</td>
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<tr>
<td>21-Nov</td>
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<td>21-Nov</td>
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<td>A3</td>
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<td>3</td>
<td>73</td>
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</tr>
<tr>
<td>21-Nov</td>
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<tr>
<td>21-Nov</td>
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<td>B3</td>
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<td>3</td>
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</tr>
<tr>
<td>23-Nov</td>
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<td>0</td>
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<td>1.0000000</td>
</tr>
<tr>
<td>23-Nov</td>
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<td>A2</td>
<td>69</td>
<td>3</td>
<td>66</td>
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</tr>
<tr>
<td>23-Nov</td>
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<td>A3</td>
<td>43</td>
<td>3</td>
<td>40</td>
<td>0.9302326</td>
</tr>
<tr>
<td>23-Nov</td>
<td>B</td>
<td>B1</td>
<td>57</td>
<td>4</td>
<td>53</td>
<td>0.9298246</td>
</tr>
<tr>
<td>23-Nov</td>
<td>B</td>
<td>B2</td>
<td>49</td>
<td>0</td>
<td>49</td>
<td>1.0000000</td>
</tr>
<tr>
<td>23-Nov</td>
<td>B</td>
<td>B3</td>
<td>42</td>
<td>0</td>
<td>42</td>
<td>1.0000000</td>
</tr>
<tr>
<td>17-Dec</td>
<td>A</td>
<td>A1</td>
<td>59</td>
<td>2</td>
<td>57</td>
<td>0.9661017</td>
</tr>
<tr>
<td>17-Dec</td>
<td>A</td>
<td>A2</td>
<td>69</td>
<td>2</td>
<td>67</td>
<td>0.9710145</td>
</tr>
<tr>
<td>17-Dec</td>
<td>A</td>
<td>A3</td>
<td>35</td>
<td>0</td>
<td>35</td>
<td>1.0000000</td>
</tr>
<tr>
<td>17-Dec</td>
<td>B</td>
<td>B1</td>
<td>84</td>
<td>0</td>
<td>84</td>
<td>1.0000000</td>
</tr>
<tr>
<td>17-Dec</td>
<td>B</td>
<td>B2</td>
<td>58</td>
<td>1</td>
<td>57</td>
<td>0.9827586</td>
</tr>
<tr>
<td>17-Dec</td>
<td>B</td>
<td>B3</td>
<td>52</td>
<td>3</td>
<td>49</td>
<td>0.9423077</td>
</tr>
<tr>
<td>19-Dec</td>
<td>A</td>
<td>A1</td>
<td>47</td>
<td>0</td>
<td>47</td>
<td>1.0000000</td>
</tr>
<tr>
<td>19-Dec</td>
<td>A</td>
<td>A2</td>
<td>134</td>
<td>4</td>
<td>130</td>
<td>0.9701493</td>
</tr>
<tr>
<td>19-Dec</td>
<td>A</td>
<td>A3</td>
<td>13</td>
<td>2</td>
<td>11</td>
<td>0.8461538</td>
</tr>
<tr>
<td>19-Dec</td>
<td>B</td>
<td>B1</td>
<td>99</td>
<td>8</td>
<td>91</td>
<td>0.9191919</td>
</tr>
<tr>
<td>19-Dec</td>
<td>B</td>
<td>B2</td>
<td>29</td>
<td>3</td>
<td>26</td>
<td>0.8965517</td>
</tr>
<tr>
<td>19-Dec</td>
<td>B</td>
<td>B3</td>
<td>34</td>
<td>0</td>
<td>34</td>
<td>1.0000000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cross</th>
<th>Total.eggs</th>
<th>Total.Unfertilised</th>
<th>Fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>986</td>
<td>48</td>
<td>0.9513185</td>
</tr>
<tr>
<td>B</td>
<td>916</td>
<td>56</td>
<td>0.9388646</td>
</tr>
</tbody>
</table>
Appendix C

Chapter 4: Meiotic drive does not cause condition-dependent reduction of eyespan, the sexual ornament of Teleopsis dalmanni stalk-eyed flies

C.1 Model outputs

C.1.1 Supplementary table S1

Male absolute eyespan depends on food treatment, development time, and genotype, but there is no genotype:treatment interaction.

```
ml <- lm(data=subset(N4_M, !is.na(Genotype) & !is.na(Eyespan)), formula = Eyespan ~ Treatment + Dev.time + Genotype + Treatment:Genotype)
```

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>1066.9738137</td>
<td>1066.9738137</td>
<td>1222.9621572</td>
<td>0.0000000</td>
</tr>
<tr>
<td>Dev.time</td>
<td>1</td>
<td>32.2698643</td>
<td>32.2698643</td>
<td>36.976208</td>
<td>0.0000000</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>4.5210319</td>
<td>4.5210319</td>
<td>5.189930</td>
<td>0.0233295</td>
</tr>
<tr>
<td>Treatment:Genotype</td>
<td>1</td>
<td>0.1072219</td>
<td>0.1072219</td>
<td>0.1228975</td>
<td>0.7260904</td>
</tr>
<tr>
<td>Residuals</td>
<td>416</td>
<td>362.9393632</td>
<td>0.8724504</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
```

C.1.2 Supplementary table S2

Summary table of the above model, showing the direction of effects. Low stress flies are bigger than high stress flies. Development time has a negative effect on male absolute eyespan, such that slow-developing flies are smaller than fast-developing flies. X^{ST}/Y males have bigger absolute eyespan than X^{SR}/Y flies.

```
Estimate   Std. Error  t value  Pr(>|t|)
(Intercept)   5.9273710  0.2387544 24.8262249  0.0000000
TreatmentLow stress  3.1281694  0.1265994 24.7092021  0.0000000
Dev.time       -0.0542727  0.0088248 -6.1500049  0.0000000
GenotypeXY    0.1747462  0.1315471  1.3283919  0.1847769
TreatmentLow stress:GenotypeXY  0.0642786  0.1833559  0.3505673  0.7260904
```

C.1.3 Supplementary table S3

Male eyespan strongly covaries with body size (thorax length). After controlling for body size, there are still strong effects of treatment, development time, and genotype on residual male eyespan.

```
ml <- lm(data=subset(N4_M, !is.na(Genotype) & !is.na(Eyespan) & !is.na(Thorax) & !is.na(Dev.time)), formula = Eyespan ~ Thorax + Treatment + Dev.time + Genotype + Treatment:Genotype)
```

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorax</td>
<td>1</td>
<td>1324.5174339</td>
<td>1324.5174339</td>
<td>5170.0692436</td>
<td>0.0000000</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>23.0761111</td>
<td>23.0761111</td>
<td>90.0743842</td>
<td>0.0000000</td>
</tr>
<tr>
<td>Dev.time</td>
<td>1</td>
<td>5.4639089</td>
<td>5.4639089</td>
<td>21.3276071</td>
<td>0.0000052</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>2.1792722</td>
<td>2.1792722</td>
<td>8.5064856</td>
<td>0.0037318</td>
</tr>
<tr>
<td>Treatment:Genotype</td>
<td>1</td>
<td>0.0713882</td>
<td>0.0713882</td>
<td>0.2786462</td>
<td>0.5978734</td>
</tr>
<tr>
<td>Residuals</td>
<td>413</td>
<td>105.8062619</td>
<td>0.2561895</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
```
### C.1.4 Supplementary table S4

Summary table of the above model, showing that the direction of all effects remains the same as in the model of absolute male eyespan.

|                | Estimate   | Std. Error | t value  | Pr(>|t|)  |
|----------------|------------|------------|----------|-----------|
| (Intercept)    | -0.9324120 | 0.2529445  | -3.6862323 | 0.0002580 |
| Thorax         | 3.4007454  | 0.1073957  | 31.6657800 | 0.0000000 |
| Treatment Low  | 0.9092178  | 0.0980957  | 9.2686826  | 0.0000000 |
| Dev. time      | -0.0227566 | 0.0048960  | -4.6479617 | 0.0000045 |
| Genotype XY    | 0.1720795  | 0.0716443  | 2.4018574  | 0.0167529 |
| Treatment Low  | -0.0526286 | 0.0997000  | -0.5278695 | 0.5978734 |

### C.1.5 Supplementary table S5

Female absolute eyespan depends on treatment, cross, development time, genotype, and the interaction between genotype and treatment.

```r
ml <- lm(data = subset(N4_F, !is.na(Genotype) & !is.na(Eyespan) & !is.na(Thorax) & !is.na(Dev.time)),
          formula = Eyespan ~ Treatment + Cross + Dev.time + Genotype + Treatment:Genotype)
```

<table>
<thead>
<tr>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>599.057090</td>
<td>599.0570898</td>
<td>2824.052352</td>
</tr>
<tr>
<td>Cross</td>
<td>1</td>
<td>1.2091147</td>
<td>1.2091147</td>
<td>5.699963</td>
</tr>
<tr>
<td>Dev. time</td>
<td>1</td>
<td>18.784745</td>
<td>18.7847452</td>
<td>88.554338</td>
</tr>
<tr>
<td>Genotype</td>
<td>2</td>
<td>1.304755</td>
<td>0.6523774</td>
<td>3.075413</td>
</tr>
<tr>
<td>Treatment:Genotype</td>
<td>2</td>
<td>1.884786</td>
<td>0.9423929</td>
<td>4.442593</td>
</tr>
<tr>
<td>Residuals</td>
<td>1063</td>
<td>225.490751</td>
<td>0.2121268</td>
<td>NA</td>
</tr>
</tbody>
</table>

### C.1.6 Supplementary table S6

Summary table of the above model, showing the direction of effects. Low stress flies have larger eyespan than high stress flies. Cross A flies have larger eyespan than cross B flies. Development time has a negative effect on female absolute eyespan. X<sup>ST</sup>X<sup>ST</sup> and X<sup>SR</sup>X<sup>ST</sup> females both have larger absolute eyespan than X<sup>SR</sup>X<sup>SR</sup>, the reference genotype in this model.

|                | Estimate   | Std. Error | t value  | Pr(>|t|)  |
|----------------|------------|------------|----------|-----------|
| (Intercept)    | 4.6335873  | 0.0667789  | 69.387005 | 0.0000000 |
| Treatment Low  | 1.5609067  | 0.0514984  | 30.309838 | 0.0000000 |
| Cross          | -0.0443774 | 0.0420177  | -1.056159 | 0.2911356 |
| Dev. time      | -0.0229205 | 0.0024096  | -9.512104 | 0.0000000 |
| Genotype SRX   | 0.1713990  | 0.0480885  | 3.564240  | 0.0038111 |
| Genotype XX    | 0.0784939  | 0.0663349  | 1.183296  | 0.2369564 |
| Treatment Low  | -0.1964182 | 0.0997000  | -2.960955 | 0.0031350 |
| Cross          | -0.0939316 | 0.0783023  | -1.199601 | 0.2305615 |

To highlight the effect of cross on absolute female eyespan, we can compare the size of X<sup>ST</sup>X<sup>ST</sup> females, as these are the only genotype produced in both crosses. For example, under low stress, cross A X<sup>ST</sup>X<sup>ST</sup> females mean ± se eyespan = 5.7118 ± 0.0401mm, while low stress cross B X<sup>SR</sup>X<sup>ST</sup> females have mean ± se absolute eyespan of 5.5876 ± 0.03975mm. Under high stress, cross A X<sup>ST</sup>X<sup>ST</sup> females have mean ± se absolute eyespan of 4.2422 ± 0.04086mm, while cross B mean ± se absolute eyespan = 4.1947 ± 0.0489. In the following models, absolute and residual female eyespan is therefore analysed separately for cross A and cross B.
C.1.7 Supplementary table S7

In cross A, absolute female eyespan depends on food treatment, development time, genotype, and the interaction between food treatment and genotype.

```r
ml <- lm(data = subset(N4_F, !is.na(Genotype) & !is.na(Eyespan) & !is.na(Thorax) & Cross == "A" & !is.na(Dev.time)),
          formula = Eyespan ~ Treatment + Dev.time + Genotype + Treatment:Genotype)
```

<table>
<thead>
<tr>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>345.0079063</td>
<td>345.0079063</td>
<td>1725.025422</td>
</tr>
<tr>
<td>Dev.time</td>
<td>1</td>
<td>18.6022096</td>
<td>18.6022096</td>
<td>93.010287</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>1.2287535</td>
<td>1.2287535</td>
<td>6.143717</td>
</tr>
<tr>
<td>Treatment:Genotype</td>
<td>1</td>
<td>0.8192455</td>
<td>0.8192455</td>
<td>4.096194</td>
</tr>
<tr>
<td>Residuals</td>
<td>586</td>
<td>117.2009586</td>
<td>0.2000016</td>
<td>NA</td>
</tr>
</tbody>
</table>

C.1.8 Supplementary table S8

Summary table of the above model, showing the direction of effects. Low stress females have larger eyespan than high stress females. Development time has a negative effect on female absolute eyespan, such that slow-developing flies are smaller than fast-developing flies. Overall, heterozygous X^SRX^ST females have bigger eyespan than homozygous X^SRX^SR females, though there is a strong treatment:genotype interaction. This is because the two genotypes have similar eyespan under low stress (X^SRX^ST mean ± se = 5.7118 ± 0.0401, X^SRX^SR mean ± se = 5.7096 ± 0.0347) but heterozygotes are have larger eyespan under high stress (X^SRX^ST mean ± se = 4.2421 ± 0.0409, X^SRX^SR mean ± se = 4.0958 ± 0.0399).

```r
summary(ml)
```

| Estimate | Std. Error | t value | Pr(>|t|) |
|----------|------------|---------|----------|
| (Intercept) | 4.8471681  | 0.0847214 | 57.213023 | 0.0000000 |
| TreatmentLow stress | 1.5403430  | 0.0502797   | 30.635479  | 0.0000000 |
| Dev.time | -0.0320229  | 0.0032977  | -9.710797  | 0.0000000 |
| GenotypeSRX | 0.1566053  | 0.0489532   | 3.199078   | 0.0014531 |
| TreatmentLow stress:GenotypeSRX | -0.1522440  | 0.0752229  | -2.023906  | 0.0434331 |

C.1.9 Supplementary table S9

In cross B, absolute female eyespan depends on food treatment and development time, but not genotype or the treatment:genotype interaction.

```r
ml <- lm(data = subset(N4_F, !is.na(Genotype) & !is.na(Eyespan) & !is.na(Thorax) & Cross == "B" & !is.na(Dev.time)),
          formula = Eyespan ~ Treatment + Dev.time + Genotype + Treatment:Genotype)
```

<table>
<thead>
<tr>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>254.6454353</td>
<td>254.6454353</td>
<td>1152.180915</td>
</tr>
<tr>
<td>Dev.time</td>
<td>1</td>
<td>3.3622641</td>
<td>3.3622641</td>
<td>15.213060</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>0.2848235</td>
<td>0.2848235</td>
<td>1.288726</td>
</tr>
<tr>
<td>Residuals</td>
<td>475</td>
<td>104.9805537</td>
<td>0.2210117</td>
<td>NA</td>
</tr>
</tbody>
</table>

C.1.10 Supplementary table S10

Summary table of the above model, showing the direction of effects. As in cross A, low stress flies have larger absolute eyespan than high stress flies, and development time has a negative effect on absolute female eyespan. Here, X^STX^ST homozygous females are smaller than X^SRX^SR females (the reference genotype in this model), though this difference does
not explain a significant amount of variation in female absolute eyespan, according to the anova of the full model (table S10 above).

|                  | Estimate | Std. Error | t value | Pr(>|t|) |
|------------------|----------|------------|---------|----------|
| (Intercept)      | 4.5515503| 0.1001089  | 45.465998| 0.0000000|
| TreatmentLow stress | 1.344670 | 0.0636040  | 21.138105| 0.0000000|
| Dev.time         | -0.0138335| 0.0034929  | -3.960508| 0.0000863|
| GenotypeXX       | -0.1142291| 0.0578978  | -1.972942| 0.0490815|
| TreatmentLow stress:GenotypeXX | 0.146979 | 0.0869418  | 1.690545 | 0.0915799|

C.1.11 Supplementary table S11

In cross A, female eyespan strongly covaries with body size (thorax length). After controlling for thorax length, residual female eyespan is still affected by food treatment and development time, but genotype no longer explains variation in female (residual) eyespan. There is also no longer a significant food treatment:genotype interaction.

```
ml <- lm(data=subset(N4_F, !is.na(Genotype) & !is.na(Eyespan) & !is.na(Thorax) & Cross=="A" & !is.na(Dev.time)), formula = Eyespan ~ Thorax + Treatment + Dev.time + Genotype + Treatment:Genotype)
```

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorax</td>
<td>1</td>
<td>426.1842901</td>
<td>426.1842901</td>
<td>5920.2816975</td>
<td>0.0000000</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>11.9836627</td>
<td>11.9836627</td>
<td>166.4694366</td>
<td>0.0000000</td>
</tr>
<tr>
<td>Dev.time</td>
<td>1</td>
<td>2.5585687</td>
<td>2.5585687</td>
<td>35.5420131</td>
<td>0.0000000</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>0.0050591</td>
<td>0.0050591</td>
<td>0.0702776</td>
<td>0.7910263</td>
</tr>
<tr>
<td>Treatment:Genotype</td>
<td>1</td>
<td>0.0150018</td>
<td>0.0150018</td>
<td>0.2083961</td>
<td>0.6481968</td>
</tr>
<tr>
<td>Residuals</td>
<td>585</td>
<td>42.1124910</td>
<td>0.0719872</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

C.1.12 Supplementary table S12

Summary table of the above model, showing the direction of effects. Eyespan is strongly colinear with thorax length. Low stress flies have larger eyespan than high stress flies. Development time has a negative effect on eyespan.

```
ml <- lm(data=subset(N4_F, !is.na(Genotype) & !is.na(Eyespan) & !is.na(Thorax) & Cross=="B" & !is.na(Dev.time)), formula = Eyespan ~ Thorax + Treatment + Dev.time + Genotype + Treatment:Genotype)
```

|                  | Estimate | Std. Error | t value | Pr(>|t|) |
|------------------|----------|------------|---------|----------|
| (Intercept)      | 1.1037894| 0.1265607  | 8.7214201| 0.0000000|
| Thorax           | 1.9009512| 0.0588589  | 32.2967698| 0.0000000|
| TreatmentLow stress | 0.5095728| 0.0439151  | 11.6036012| 0.0000000|
| Dev.time         | -0.0123200| 0.0020703  | -5.9507326| 0.0000000|
| GenotypeSRX      | 0.0029189| 0.0297522  | 0.0981072| 0.9218808|
| TreatmentLow stress:GenotypeSRX | -0.0206856| 0.0453130| -0.4565043| 0.6481968|

C.1.13 Supplementary table S13

In cross B, female eyespan strongly covaries with body size (thorax length). After controlling for thorax length, residual female eyespan is still affected by food treatment and development time.

```
ml <- lm(data=subset(N4_F, !is.na(Genotype) & !is.na(Eyespan) & !is.na(Thorax) & Cross=="B" & !is.na(Dev.time)), formula = Eyespan ~ Thorax + Treatment + Dev.time + Genotype + Treatment:Genotype)
```

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorax</td>
<td>1</td>
<td>319.1789661</td>
<td>319.1789661</td>
<td>4033.7781540</td>
<td>0.0000000</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>6.0016204</td>
<td>6.0016204</td>
<td>75.843728</td>
<td>0.0000000</td>
</tr>
<tr>
<td>Dev.time</td>
<td>1</td>
<td>1.1861547</td>
<td>1.1861547</td>
<td>14.990628</td>
<td>0.0001232</td>
</tr>
</tbody>
</table>
Supplementary table S14

Summary table of the above model, showing the direction of effects. As in all other analyses, low stress flies are larger than high stress flies, and development time has a negative effect on absolute female eyespan.

| Estimate | Std. Error | t value | Pr(>|t|) |
|----------|------------|---------|---------|
| (Intercept) | 0.9450355 | 0.1372628 | 6.8848619 | 0.0000000 |
| Thorax | 1.9362649 | 0.0663065 | 29.2017532 | 0.0000000 |
| TreatmentLow stress | 0.3857034 | 0.0502625 | 7.6737759 | 0.0000000 |
| Dev.time | -0.0080653 | 0.0020993 | -3.8419779 | 0.0001387 |
| GenotypeXX | 0.0221236 | 0.0349563 | 0.6328933 | 0.5271085 |
| TreatmentLow stress:GenotypeXX | -0.0246702 | 0.0523524 | -0.4712328 | 0.6376913 |

Supplementary table S15

The finding that genotype explained variation in absolute but not residual female eyespan suggests that genotype affects female body size overall, with which eyespan scales allometrically, not eyespan per se. To test this we analysed thorax length, a more direct measurement of overall body size, as the dependent variable for cross A and cross B separately.

In cross A, female thorax length depends on treatment, development time, genotype, and the treatment:genotype interaction, mirroring the finding for absolute eyespan.

```
ml <- lm(data=subset(N4_F, !is.na(Genotype) & !is.na(Eyespan) & !is.na(Thorax) & Cross=="A" & !is.na(Dev.time)), formula = Thorax ~ Treatment + Dev.time + Genotype + Treatment:Genotype)
```

<table>
<thead>
<tr>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>41.0386920</td>
<td>41.0386920</td>
<td>1157.336953</td>
</tr>
<tr>
<td>Dev.time</td>
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<td>1.9318655</td>
<td>54.480765</td>
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<tr>
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<td>0.3845181</td>
<td>10.843841</td>
</tr>
<tr>
<td>Treatment:Genotype</td>
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<td>0.1692891</td>
<td>4.774143</td>
</tr>
<tr>
<td>Residuals</td>
<td>586</td>
<td>20.7793188</td>
<td>0.0354596</td>
<td>NA</td>
</tr>
</tbody>
</table>

Supplementary tables S16-18

Summary table for the above model, showing the direction of effects. As for eyespan, thorax length is reduced in high stress compared to low stress, and development time has a negative effect on thorax length. X^SRX^ST heterozygous females have larger thoraces than X^SRX^SR homozygotes (the reference genotype in this model). As with absolute female eyespan there is a treatment:genotype interaction.

```
ml <- lm(data=subset(N4_F, !is.na(Genotype) & !is.na(Eyespan) & !is.na(Thorax) & Cross=="B" & !is.na(Dev.time)), formula = Thorax ~ Treatment + Dev.time + Genotype + Treatment:Genotype)
```

| Estimate | Std. Error | t value | Pr(>|t|) |
|----------|------------|---------|---------|
| (Intercept) | 1.9692135 | 0.0356733 | 55.201330 | 0.0000000 |
| TreatmentLow stress | 0.5422391 | 0.0211711 | 25.612268 | 0.0000000 |
| Dev.time | -0.0103648 | 0.0013885 | -7.464544 | 0.0000000 |
| GenotypeSRX | 0.0808471 | 0.0206125 | 3.922227 | 0.0000981 |
| TreatmentLow stress:GenotypeSRX | -0.0692066 | 0.0316738 | -2.184981 | 0.0292856 |

To dissect this interaction term, we compared female thorax length in low stress and high stress separately. In low stress, X^SRX^ST and X^SRX^SR have similar thorax length (X^SRX^ST mean ± se thorax length = 2.3030 ± 0.0181mm, X^SRX^SR mean ± se thorax length = 2.2909 ± 0.0139mm).
In high stress, X^{SR}X^{ST} females are larger than X^{SR}X^{ST} (X^{SR}X^{ST} mean ± se = 1.8036 ± 0.0169mm, X^{SR}X^{ST} mean ± se = 1.7219 ± 0.0158mm).

In high stress, X^{SR}X^{ST} females are larger than X^{SR}X^{ST} (X^{SR}X^{ST} mean ± se = 1.8036 ± 0.0169mm, X^{SR}X^{ST} mean ± se = 1.7219 ± 0.0158mm).

To dissect the interaction term further, we analysed the effect of genotype on thorax length for each treatment separately. In low stress, X^{SR}X^{ST} and X^{SR}X^{ST} females have similar thorax lengths (X^{SR}X^{ST} mean ± se = 2.3085 ± 0.0181mm, X^{SR}X^{ST} mean ± se = 2.2914 ± 0.0167mm).
In low stress, heterozygous females $X^{Sr}X^{ST}$ are larger than females homozygous for the standard allele $X^{ST}X^{ST}$ (mean ± se = 1.7856 ± 0.0203mm, $X^{ST}X^{ST}$ mean ± se = 1.7150 ± 0.0161mm).

C.1.18 Supplementary tables S23-S25

We controlled for differences between cross A and cross B by first analysing the percentage difference in thorax and eyespan between heterzygous females in cross A and cross B. Then, we adjusted the thorax length and eyespan of all cross B flies by this percentage difference. This allowed us to directly compare female genotypes unique to each cross.

After controlling for cross in this way, we replicate earlier models by showing that genotype affects absolute but not residual eyespan, and genotype affects thorax length.

**Absolute eyespan:**

<table>
<thead>
<tr>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Residual eyespan:**

<table>
<thead>
<tr>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

263
We used Tukey post-hoc comparison tests to compare the absolute eyespan of female genotypes in the cross-controlled dataset, separately for each treatment.

In low stress, $X^{ST}X^{ST}$ females have significantly larger eyespan ($\text{mean } \pm \text{se} = 5.74013 \pm 0.0425\text{mm}$) than $X^{SR}X^{SR}$ females ($\text{mean } \pm \text{se} = 5.7096 \pm 0.0347\text{mm}$), and $X^{ST}X^{SR}$ females ($\text{mean } \pm \text{se} = 5.7118 \pm 0.0285\text{mm}$) are intermediate.

In high stress, heterozygous $X^{SR}X^{ST}$ females are largest ($\text{mean } \pm \text{se} = 4.2422 \pm 0.0315\text{mm}$), larger than $X^{ST}X^{ST}$ ($\text{mean } \pm \text{se} = 4.1328 \pm 0.0420\text{mm}$) but not $X^{SR}X^{SR}$ ($\text{mean } \pm \text{se} = 4.0958 \pm 0.0399\text{mm}$) homozygotes.

We also used Tukey post-hoc comparison tests to compare the thorax lengths of female genotypes in the cross-controlled dataset, separately for each treatment.

In low stress, as for absolute eyespan, homozygous standard $X^{ST}X^{ST}$ females have the largest thorax ($\text{mean } \pm \text{se} = 2.3202 \pm 0.0182\text{mm}$), homozygous drive $X^{SR}X^{SR}$ females have the smallest ($\text{mean } \pm \text{se} = 2.2909 \pm 0.0139\text{mm}$), and heterozygous $X^{ST}X^{SR}$ are intermediate ($\text{mean } \pm \text{se} = 2.3030 \pm 0.0123\text{mm}$).

In high stress, heterozygous $X^{SR}X^{ST}$ females have the largest thorax ($\text{mean } \pm \text{se} = 1.8036 \pm 0.0131\text{mm}$), significantly larger than either homozygous genotype ($X^{ST}X^{ST}$ mean $\pm$ se = 1.7321 ± 0.0162mm, $X^{SR}X^{SR}$ mean $\pm$ se = 1.7219 ± 0.0158mm).

### C.1.19 Supplementary tables S26-28

<table>
<thead>
<tr>
<th>term</th>
<th>comparison</th>
<th>estimate</th>
<th>conf.low</th>
<th>conf.high</th>
<th>adj.p.value</th>
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<tbody>
<tr>
<td>Genotype</td>
<td>SRX-SRSR</td>
<td>0.0475560</td>
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<td>0.1292332</td>
<td>0.3580762</td>
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<tr>
<td>Genotype</td>
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<tr>
<td>Genotype</td>
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<td>-0.0002429</td>
<td>0.1876226</td>
<td>0.0507668</td>
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</tbody>
</table>

### C.1.20 Supplementary tables S29-30

<table>
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<th>term</th>
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<th>adj.p.value</th>
</tr>
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<td>Genotype</td>
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<tr>
<td>Genotype</td>
<td>XX-SRSR</td>
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<tr>
<td>Genotype</td>
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</tbody>
</table>

C.1.19 Supplementary tables S26-28

We used Tukey post-hoc comparison tests to compare the absolute eyespan of female genotypes in the cross-controlled dataset, separately for each treatment.

In low stress, $X^{ST}X^{ST}$ females have significantly larger eyespan ($\text{mean } \pm \text{se} = 5.74013 \pm 0.0425\text{mm}$) than $X^{SR}X^{SR}$ females ($\text{mean } \pm \text{se} = 5.7096 \pm 0.0347\text{mm}$), and $X^{ST}X^{SR}$ ($\text{mean } \pm \text{se} = 5.7118 \pm 0.0285\text{mm}$) females are intermediate.

<table>
<thead>
<tr>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
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<td>76.4501950</td>
<td>2054.895527</td>
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<tr>
<td>Dev.time</td>
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<td>1.5245415</td>
<td>1.5245415</td>
<td>40.977967</td>
</tr>
<tr>
<td>Genotype</td>
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<td>7.676202</td>
</tr>
<tr>
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<tr>
<td>Residuals</td>
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<td>39.5849844</td>
<td>0.0372039</td>
<td>NA</td>
</tr>
</tbody>
</table>

C.1.20 Supplementary tables S29-30

We also used Tukey post-hoc comparison tests to compare the thorax lengths of female genotypes in the cross-controlled dataset, separately for each treatment.

In low stress, as for absolute eyespan, homozygous standard $X^{ST}X^{ST}$ females have the largest thorax ($\text{mean } \pm \text{se} = 2.3202 \pm 0.0182\text{mm}$), homozygous drive $X^{SR}X^{SR}$ females have the smallest ($\text{mean } \pm \text{se} = 2.2909 \pm 0.0139\text{mm}$), and heterozygous $X^{ST}X^{SR}$ are intermediate ($\text{mean } \pm \text{se} = 2.3030 \pm 0.0123\text{mm}$).

<table>
<thead>
<tr>
<th>term</th>
<th>comparison</th>
<th>estimate</th>
<th>conf.low</th>
<th>conf.high</th>
<th>adj.p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
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<tr>
<td>Genotype</td>
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<tr>
<td>Genotype</td>
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<td>0.0829781</td>
<td>0.0894728</td>
</tr>
</tbody>
</table>

C.1.20 Supplementary tables S29-30

We also used Tukey post-hoc comparison tests to compare the thorax lengths of female genotypes in the cross-controlled dataset, separately for each treatment.

In low stress, as for absolute eyespan, homozygous standard $X^{ST}X^{ST}$ females have the largest thorax ($\text{mean } \pm \text{se} = 2.3202 \pm 0.0182\text{mm}$), homozygous drive $X^{SR}X^{SR}$ females have the smallest ($\text{mean } \pm \text{se} = 2.2909 \pm 0.0139\text{mm}$), and heterozygous $X^{ST}X^{SR}$ are intermediate ($\text{mean } \pm \text{se} = 2.3030 \pm 0.0123\text{mm}$).

<table>
<thead>
<tr>
<th>term</th>
<th>comparison</th>
<th>estimate</th>
<th>conf.low</th>
<th>conf.high</th>
<th>adj.p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>SRX-SRSR</td>
<td>0.0850067</td>
<td>0.0371611</td>
<td>0.1328523</td>
<td>0.0001015</td>
</tr>
</tbody>
</table>
In the second experiment, where eggs from cross A and cross B were combined together, absolute female eyespan depended on food treatment and eyespan, but not their interaction.

m1 <- lm(data = subset(N3_F, !is.na(Eyespan) & !is.na(Genotype)),
formula = Eyespan ~ Treatment + Genotype + Treatment:Genotype)

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
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<th>Mean Sq</th>
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<tr>
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<td>0.2528537</td>
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<tr>
<td>Residuals</td>
<td>446</td>
<td>74.0542612</td>
<td>0.1660409</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

To compare the absolute eyespan of female genotypes we used Tukey post-hoc comparison tests, separately for each treatment.

In low stress, homozygous standard X<sup>ST</sup>X<sup>ST</sup> females have the largest absolute eyespan (mean ± se = 6.0766 ± 0.0252), followed by X<sup>SR</sup>X<sup>ST</sup> heterozygotes (mean ± se = 5.9246 ± 0.0352), with X<sup>SR</sup>X<sup>SR</sup> homozygotes possessing the smallest absolute eyespan (mean ± se = 5.9888 ± 0.02659).

<table>
<thead>
<tr>
<th>term</th>
<th>comparison</th>
<th>estimate</th>
<th>conf.low</th>
<th>conf.high</th>
<th>adj.p.value</th>
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</thead>
<tbody>
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<tr>
<td>Genotype</td>
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<td>0.2565997</td>
<td>0.0020531</td>
</tr>
<tr>
<td>Genotype</td>
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<td>0.0878786</td>
<td>0.0097151</td>
<td>0.1854723</td>
<td>0.0872406</td>
</tr>
</tbody>
</table>

In high stress, heterzygous X<sup>SR</sup>X<sup>ST</sup> females females had the largest absolute eyespan (mean ± se = 4.4860 ± 0.0577), followed by homozygous standard X<sup>ST</sup>X<sup>ST</sup> (mean ± se = 4.4860 ± 0.0577), with homozygous drive X<sup>SR</sup>X<sup>SR</sup> (mean ± se = 4.2596 ± 0.0691).

<table>
<thead>
<tr>
<th>term</th>
<th>comparison</th>
<th>estimate</th>
<th>conf.low</th>
<th>conf.high</th>
<th>adj.p.value</th>
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<tr>
<td>Genotype</td>
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</table>
C.2 Supplementary figure S1

Thorax length of $X^{SR}X^{SR}$ (red), $X^{SR}X^{ST}$ (green), and $X^{ST}X^{ST}$ (blue) females, in high stress and low stress food conditions, after adjusting for cross. The percentage difference in thorax length of Cross A and Cross B $X^{SR}X^{ST}$ females was calculated, and the thorax length of all Cross B females was multiplied by this difference. Points show mean ± one standard error.
Appendix D

Does meiotic drive alter male mate preference?

Does meiotic drive alter male mate preference?

Sam Ronan Finnegan, Leslie Nitsche, Matteo Mondani, M. Florencia Camus, Kevin Fowler, and Andrew Pomiankowski

INTRODUCTION

Male mate preferences have been demonstrated across a range of species, including the Malaysian stalk-eyed fly, Teleopsis dalmani. This species is subject to sex-ratio (SR), an X-linked male meiotic driver, which causes the dysfunction of Y-sperm and the production of all-female broods. While there has been work considering female avoidance of meiotic drive males, the mating decisions of drive-bearing males have not been considered previously. Drive males may be less able to bear the cost of choice as SR is associated with a low-frequency inversion that causes reduced organ size. Drive males may also experience weaker selection for preference maintenance if they are avoided by females. Using binary choice trials, across two experiments, we confirmed male preference for large (fecund) females but found no evidence that the strength of male preference differs between drive and standard males. We showed that large eyespan males displayed strong preference for large females, whereas small eyespan males showed no preference. Taken together, these results suggest that, even though meiotic drive is associated with lower genetic quality, it does not directly interfere with male mate preference among available females. However, as drive males tend to have smaller eyespan (albeit only ~5% on average), this will to a minor extent weaken their strength of preference.

Key words: condition-dependent, male mate preference, mate choice, meiotic drive, sexual selection, stalk-eyed fly.
males with the opportunity to mate selectively. The direct cost of male preference is likely to be small as a male can easily compare females that settle on his lek. In addition, in the dawn period, there is typically no competition for mating as only the harems male mates. However, there may be costs related to the mating rate. Mating is associated with a temporary reduction in accessory gland size, and these organs do not recover to pre-mating size for around 24 h (Rogers et al. 2005). In a study of the correlates of mating frequency, the majority of males (76.1%) presented with six females were unable to mate with all of them within an hour (Rogers et al. 2005), considerably longer than the early morning period of mating in the field (Cotton et al. 2010). These data suggest that males suffer limits to their daily mating capacity, which probably extends across days. In addition, females are observed to fly off leks during the dawn period, whether they have mated or not (Pomiankowski A, personal observation). A male preoccupied mating with one female loses the opportunity to mate with others. Males are likely to benefit from exercising mate preference because females vary in fecundity. In the wild and the laboratory, female fecundity is positively correlated with body size and nutritional status (David et al. 1998; Cotton et al. 2010, 2013). Female eyespan is a likely target trait for male preference. In field samples, female eyespan is predictive of fecundity even after controlling for body size, with which it strongly covaries (Cotton et al. 2010). Indeed, male mate preference for large eyespan and high fecundity has been reported in this species under both laboratory and field conditions (Cotton et al. 2015). Together, this evidence suggests that females vary in reproductive quality in ways that will affect male fitness and the costs of male preference are unlikely to outweigh the potential benefits.

Here, we investigate the effect of sex-ratio (SR), X-linked meiotic drive, on male mate preference in T. dalmanni. SR systems are common in flies, causing male carriers to produce female-biased broods (Jarvik 2001; Lindholm et al. 2016). In stalk-eyed flies, the SR X chromosome (XSR) exists at moderate frequencies: ~20% (Wilkinson et al. 2003; Cotton et al. 2014; Paczolt et al. 2017). The gene controlling meiotic drive is located in a large paracentric inversion covering most of the XSR chromosome (Johns et al. 2009; Paczolt et al. 2017). Low-frequency inversions are associated with reduced recombination rates and are subject to weaker natural selection and the accumulation of deleterious mutations (Hoffmann and Räscar 2008; Kirkpatrick 2010). In several drive systems, this results in reduced viability (Curtis and Feldman 1980; Beckenbach 1996; Larracuent and Frösgraves 2012; Sutter and Lindholm 2015). Reinhart et al. (2014) showed that there are almost a thousand fixed differences between SR and ST (the wildtype X chromosome) X-linked genes in T. dalmanni, but only 11 for autosomal genes, consistent with mutation accumulation on XSR. There is some evidence for reduced genetic quality of SR. Males and females carrying the XSR chromosome have reduced egg-to-adult viability (FinneGAN et al. 2019b), even though adult longevity is not affected (Wilkinson et al. 2006). In addition, SR males have repeatedly been shown to have reduced eyespan both in laboratory (Wilkinson et al. 1998; Johns et al. 2005; Meade et al. 2019b) and wild populations (Cotton et al. 2014). This association probably arises because male eyespan is highly condition dependent and reflects environmental (David et al. 1998; Cotton et al. 2004) and genetic quality (David et al. 2000; Bellamy et al. 2013).

Previous work has not investigated whether meiotic drive affects sexual preference. The X chromosome is likely to be a favorable location for the evolution of preference genes (Kirkpatrick and Hall 2004) and there is some evidence that sex-linked preferences are common (Morales-Alvaro 2019). In T. dalmanni, differences in mate preference between SR and ST males are expected to be influenced by X-linked factors because they do not differ in autosomal content. A number of arguments lead to the prediction that SR males will show weaker mate preference than ST males. Female mate preferences are often costly condition-dependent traits, with the highest quality females showing the strongest preference for the most attractive males (Cotton et al. 2006). For example, female three-spined sticklebacks (Gasterosteus aculeatus) from high condition families display strong preference for male red throat coloration, whereas females from low condition families do not (Bakker et al. 1999). In T. dalmanni, if male mate preference is costly, low-condition SR males may be less able to bear this cost, leading to weaker SR male preferences for high-value females (Howie and Pomiankowski 2018). A more direct association may arise due to linkage of preference alleles to the XSR inversion. Given greater mutational decay on the XSR chromosome, SR males would be expected to display weaker preferences for high-quality females. A third possibility arises from the association of SR with reduced male eyespan. Theoretical work suggests that visual perception improves as eyespan increases (Burkhhardt and de la Mote 1983). Small eyespan may limit the ability of males to discriminate among females. Female mate preference in female stalk-eyed flies shows an association between eyespan and visual discrimination (Hingle et al. 2001a), and this may well extend to males. A final possibility is that, because females prefer to roost and mate with males of large eyespan (Wilkinson and Reillo 1994; Wilkinson and Dodson 1997; Hingle et al. 2001a; Cotton et al. 2016), ST males on average attract fewer females to their leks. This could result in weaker selection for mate preference among SR males if they have less opportunity for choice. A potential example is the two-spotted goby, Gobiusculus flavescens, where large attractive males prefer to mate with colorful females, but small less attractive males express no preference, despite equal courtship effort (Amundsen and Forsgren 2003).

To assay male mate preference, we used simple binary choice trials (Cotton et al. 2015) to measure the strength of male preference in stalk-eyed flies. In two experiments, SR and ST males were presented with two females, one large and one small, and allowed to mate freely during a short time period. Two females is the mean number observed in the wild on male–female leks (Cotton et al. 2016). The design aimed to mimic, under controlled conditions, the sex ratio and time-frame under which male preference is expressed in the wild. In the first experiment, focal male eyespan was constrained to lie within a narrow range of trait values to test whether the genotypic differences between SR and ST males cause differences in mating behavior independent of male eyespan. In the second experiment, focal male eyespan was unconstrained and drawn from its natural distribution to determine the direct effect of eyespan and its association with genotype (SR and ST) on mate preference.

METHODS

Source populations

A stock population was obtained from Ulu Gombak in Malaysia (3°19’N 101°45’E) in 2005 (by Sam Cotton and A.P.). It is maintained at 25°C on a 12:12 hour light-dark cycle at high population
density. This population’s males are only standard (i.e., wildtype), and it is designated the ST stock as it does not contain individuals carrying the X<sup>M</sup> drive chromosome.

In 2012, a further collection was made of male flies from the same location (by Alison Cotton and Sam Cotton) and used to create an SR stock population that maintains the X<sup>N</sup> chromosome, following a standard protocol (Presgraves et al. 1997; Meade et al. 2019a). Briefly, individual males from the SR population are housed with three ST stock females and mate freely. Their offspring sex ratio is scored. Males siring female-biased broods (>90%, female offspring and >15 total offspring) are designated SR (X<sup>SR</sup>/Y), and their female progeny are, therefore, carriers of the SR chromosome (X<sup>SR</sup>/X<sup>SR</sup>). Progeny from other males, which are likely to be ST, are discarded. The resulting heterozygous females are then mated with ST stock males (X<sup>ST</sup>/Y), producing SR (X<sup>SR</sup>/Y) and ST (X<sup>ST</sup>/Y) males in an expected 1:1 ratio. These males are crossed to three ST stock females, and the process is repeated (i.e., keeping the progeny of X<sup>SR</sup>/Y males and discarding those of X<sup>ST</sup>/Y males). The regular crossing with ST stock males and females homogenizes the autosomes, Y chromosome, wildtype ST chromosome, and mitochondrial genes across the two stock populations. In other respects, the SR and ST stocks were kept under similar conditions.

Experimental flies
Experimental males were collected from egg-lays, a petri dish containing moisturized cotton wool and ~15g pureed sweetcorn, placed into SR stock cages. The petri dishes were removed after 3 days and, subsequently, the enclosed adults were collected after 3–4 weeks. Eyespan was measured as the distance between the outermost edges of the eye bulbs (Cotton et al. 2004) using ImageJ (v1.5.0). In the first experiment, males were standardized to a narrow range of eyespan (7.5–8.5 mm) to minimize any potential effect of variation in male eyespan on female behavior. Males were housed in large cages (35 × 22 × 20 cm), with a similar number of stock females for them to mate at a normal rate prior to the mating assay. Experimental females were collected from the ST stock population and their eyespan measured. Females used in the experiment were defined as large (eyespan ≥8 mm) or small (eyespan ≤5.4 mm) following Rogers et al. (2006) and Cotton et al. (2015). Intermediate size females were discarded. Large adult females were fed high-quality food consisting of 100% pureed corn. Small adult females were fed low-quality food consisting of 20% pureed corn and 80% sugar solution (25% sugar w/v) with the addition of an indigestible bulking agent (3% carboxymethylcellulose w/v) to make the viscosity similar to that of the high-quality food (Rogers et al. 2008; Cotton et al. 2015). The two diets were used to amplify differences in fecundity between the size classes of experimental female (Cotton et al. 2015). Previous work with more extreme dietary differences shows that diet does not affect the rate of female mating (Hingle et al. 2001b). The two classes of female were housed with stock males to allow them to mate at a normal rate.

In the second experiment, males were reared from egg-lays collected from SR stock cages with variable amounts of corn (between 1.5 and 15 g) to generate size variation in eyespan and therefore the procedures used were similar to the first experiment. One exception was that both types of female, large and small, were fed the same high-quality food as adults. This ensured that the assays of male preference were independent of any differences in fecundity brought about by dietary manipulation.

Male mating assays
Male flies were presented with a choice of large and small females in mating chambers (Figure 1; Cotton et al. 2015). Mating chambers were set up in the afternoon prior to each assay. Males were placed in the top compartment, with one large and one small female placed in the bottom compartment. Interactions between males and females were prevented during this period by a cardboard partition placed between the compartments. At dusk on the assay day, the partition was removed and the mating chambers were observed for 30 min. The number of copulations with each size-class and the order of mating were recorded. A successful copulation was defined as intromission lasting more than 30 s as copulations shorter than this duration do not result in spermatozoa transfer (Rogers et al. 2006). Males that attempted to mate but were unsuccessful were presented with a different set of one large and one small female and observed for an additional 30 min. After completion of the assay, focal males were frozen and stored in ethanol. Females were isolated in individual 500-mL pots for 2 days before being returned to population cages, ensuring that no females were used in assays on consecutive days.

Genotyping
The experimenters were blind to the genotype of experimental males as this was inferred post hoc by genotyping. DNA was extracted using a standard protocol (see Supplementary Methods) and two markers were used to distinguish SR and ST males. Microsatellite mos395 has a bimodal distribution where large (>218 bp) alleles are strongly associated with SR (Johns et al. 2005; Cotton et al. 2014; Paczolt et al. 2017; Meade et al. 2019a). CompH2710 is an indel marker with a small allele (201 bp) found in

![Figure 1](https://example.com/image1.png)
SR males and a large allele (286 bp) found in ST males (Wilkinson GS, personal communication), which has been used previously as an SR marker (Moran et al. 2019a). Males with large sn395 alleles and small comp162710 alleles were classified as SR. Where markers gave conflicting signals, genotype was assigned on the basis of comp162710 allele size.

Statistical analysis—genotype and male preference
All statistical analyses were carried out in R (R Core Team 2016). Model outputs are reported in the Supplementary Information. In the first experiment, we analyzed the effect of genotype on the number of copulations with each size class of female using logistic regression, weighted by the total number of copulations carried out by each male, with a quasibinomial error structure to account for overdispersion. The intercept term in this model determines whether males show preference for either size class of females. The data were also split by genotype and the same model was run to determine if SR and ST males preferred large females. For comparison with earlier work (Cotton et al. 2015), mate preference for each individual male was assessed using an index based on the proportion of total copulations with the large female, \( \text{Pref} = \frac{(C_L - C_S)}{(C_L + C_S)} \), where \( C_L \) and \( C_S \) are the number of copulations with the large and small females, respectively. Preference values range \( \pm 1 \) and are symmetric about 0. For an individual male, a value \( \geq 0 \) indicates preference for large females, and \( \leq 0 \) indicates preference for small females. Preference in each consecutive mating was assessed using binomial tests on the number of copulations with large and small females, on the pooled data set, and SR and ST males separately. The effect of genotype on the number of copulations with large and small females was analyzed for each consecutive mating using generalized linear models (GLMs) with quasibinomial error distributions.

Statistical analysis—eyespan and male preference
The second experiment allowed us to consider whether male eyespan had an effect on mating preference and its interaction with male genotype. First, the effects of male eyespan, genotype, and their interaction were modeled for the number of copulations with each size class of female in a generalized linear model, weighted by the total number of copulations carried out by each male, with a quasibinomial error structure. Then, males were split into three eyespan categories: small (eyespan <6.0 mm), medium (eyespan 6.0-7.5 mm), and large (eyespan >7.5 mm). The effect of eyespan category, genotype, and their interaction on the number of copulations with each size class of female was analyzed in a generalized linear model with a quasibinomial error distribution. The difference in mean preferences of each size group was assessed using the ttest function of the multcomp package in R. The effect of genotype on thorax length and eyespan was analyzed in a linear model. Other tests were carried out as in the first experiment.

Statistical analysis—mating frequency
The effect of genotype on mating frequency in the first experiment was reported previously (Moran et al. 2019a). Here, we combined data across both experiments to examine how the total number of matings by each male was affected by genotype in generalized linear models with Poisson error distribution. We then analyzed the
effect of eyespan on mating frequency using data from the second experiment, in which there was variation in male eyespan.

RESULTS
Genotype and male preference
In the first experiment, males showed a preference for large females when genotypes were pooled (Pref mean ± standard error [SE] = 0.3637 ± 0.056; \( n = 628 \), \( P < 0.0001 \), \( a = 162 \)). Males preferred large females in their first (Pref mean ± SE = 0.4321 ± 0.0711, \( P < 0.0001 \), \( a = 162 \)); second (Pref mean ± SE = 0.3330 ± 0.0832, \( P = 0.0006 \), \( a = 123 \)); and third mating (Pref mean ± SE = 0.4257 ± 0.0904, \( P < 0.0001 \), \( a = 101 \)). For subsequent matings, there was no male preference for large females, in large part reflecting the reduced sample size (fourth mating: Pref mean ± SE = 0.1903 ± 0.1269, \( n = 61 \), \( P = 0.2096 \), fifth mating: Pref mean ± SE = 0.2593 ± 0.1894, \( a = 27 \), \( P = 0.2478 \)). The preference of SR and ST males did not differ from each other (GLM: \( t = 0.150, P = 0.8808, a = 157 \)). Preference was for large eyespan females in both SR (Pref mean ± SE = 0.3970 ± 0.0889, \( t = 4.959 P < 0.0001 \), \( a = 81 \)) and ST males (Pref mean ± SE = 0.3367 ± 0.0806, \( t = 4.098, P = 0.0001, a = 76 \); Figure 2). Across consecutive copulations, SR and ST males preferred large females in the first (SR Pref mean ± SE = 0.5062 ± 0.0964, \( P = 0.0001, a = 81 \)); ST Pref mean ± SE = 0.3681 ± 0.1073, \( t = 2.201, P = 0.0418, a = 76 \); second (SR Pref mean ± SE = 0.3333 ± 0.1227, \( P = 0.013, a = 60 \), ST Pref mean ± SE = 0.2647 ± 0.1178, \( P = 0.0385, a = 68 \)); and third (SR Pref mean ± SE = 0.3900 ± 0.1526, \( P = 0.0007, a = 40 \); ST Pref mean ± SE = 0.4737 ± 0.1177, \( P = 0.0005, a = 57 \)) mating and did not differ in the strength of their preference across these copulations (first mating: \( F_{1,112} = 9.1017, P = 0.0414 \); second mating: \( F_{1,112} = 0.1623, P = 0.6878 \); third mating: \( F_{1,112} = 0.0226, P = 0.9667 \); SR and ST males did not differ in the frequency of failing to mate at least once (SR: \( 22/383, \text{ST: } 13/49, t^2 = 1.8069, P = 0.1789, a = 193 \)).

Eyespan and male preference
In the second experiment, larvae were exposed to variable amounts of food during development. Adult males showed considerable variation in eyespan (mean ± standard deviation [SD] = 7.026 ± 1.495 mm, range 3.625-9.461 mm). Eyespan was strongly colinear with body size (i.e., thorax length, \( F_{1,113} = 788.5, P < 0.0001 \)), but did not differ with genotype \( (F_{1,113} = 0.3322, P = 0.5655) \), nor was there a difference in the allometric slope of eyespan on body size with genotype \( (F_{1,113} = 0.0014, P = 0.9706 \) Supplementary Figure S1). As before, when individuals from both genotypes were pooled, males showed a preference for large females overall (Pref mean ± SE = 0.2344 ± 0.0494, GLM: \( t = 7.044, P < 0.0001 \), \( a = 178 \)); and in the first (Pref mean ± SE = 0.3371 ± 0.0707, \( P = 0.0001 \), \( a = 176 \)), second (Pref mean ± SE = 0.2785 ± 0.0767, \( P = 0.0003 \), \( a = 158 \)), and third matings (Pref mean ± SE = 0.2593 ± 0.0831, \( P = 0.0033, a = 135 \)). Again, there was no male preference for large females in subsequent matings as sample size fell (fourth mating, Pref mean ± SE = 0.1132 ± 0.0970, \( P = 0.2853, a = 167 \), fifth mating, Pref mean ± SE = 0.2509 ± 0.1220, \( P = 0.0599, a = 64 \)). Male eyespan had a strong positive effect on mating preference \( (F_{1,114} = 3.8333, P = 0.0166 \); Figure 3). When males were split into three groups based on eyespan (large >7.5 mm, medium
Figure 2
Frequency distribution of male preference values for SR (top) and ST (bottom) males from the first experiment. Preference is given by 
\[ \text{Pref} = \frac{(C_L - C_S)}{(C_L + C_S)} \]
where \(C_L\) and \(C_S\) are the number of copulations with large and small females, respectively. Positive values indicate preference for mating with large females, and negative values indicate preference for mating with small females.

Figure 3
Line graph showing the regression of male preference (Pref) on eyespan for ST and SR males from the second experiment. Shaded areas represent 95% confidence intervals.
In this study of stalk-eyed flies, we found that males show preference for large eye-span females. This mirrors previous laboratory and field studies in *T. dalmanni* (Cotton et al. 2015). As in other species, the likely benefit of this preference derives from mating with higher fecundity females (Olson 1993; Down and Montgomery 2004; Byrne and Rice 2006; Reading and Buckrell 2007). Female eye-span reliability indicates fecundity among field-caught stalk-eyed flies, where it explains a significant amount of variation in ovarian egg number, even after controlling for body size (Cotton et al. 2010, 2015).

There was no difference between drive (SR, and wildtype (ST)) males in their strength of preference. In order to compare genotypes independent of differences in size, eye-span was restricted to a narrow range at the large end (7.5–9.5 mm) of the distribution. Male eye-span is a highly condition-dependent trait, sensitive to both environmental (David et al. 2000; Cotton et al. 2004) and genetic stress (Wilkinson et al. 1998; Relans et al. 2003). By placing limits on the eye-span of experimental males, we may have inadvertently picked out SR and ST males of equivalent high condition and, thereby, masked differences between the genotypes. This may be a problem as NO is a restricted resource for a mature sperm, and SR males with higher condition may have a stronger preference (SR Pref mean ± SE = 0.7072 ± 0.1263, t = 0.0404, P = 0.6890, n = 56).

As in the first experiment, there was no difference in the strength of preference according to genotype (*F*$_1$,375 = 0.6657, *P* = 0.4139). Both SR (Pref mean ± SE = 0.8008 ± 0.0987, t = 4.153, *P* = 0.0001, n = 69; and ST males (Pref mean ± SE = 0.9096 ± 0.0806, t = 5.464, *P* < 0.0001, n = 63). After correcting for the effect of eye-span group (large, medium, and small eye-span), there was still no effect of genotype on the strength of preferences (all *P* > 0.4, nor any interaction between eye-span group and genotype (*F*$_1$,375 = 0.7830, *P* = 0.3874). Both SR and ST males preferred large females in the first mating (SR Pref mean ± SE = 0.8737 ± 0.1181, t = 0.0041, n = 61; ST Pref mean ± SE = 0.9258 ± 0.0899, t = 0.0019, n = 114), second (SR Pref mean ± SE = 0.4236 ± 0.1218, t = 0.0018, n = 56; ST Pref mean ± SE = 0.1800 ± 0.0988, t = 0.0066, n = 100, and third (SR Pref mean ± SE = 0.3976 ± 0.0987, t = 0.0011, n = 47; ST Pref mean ± SE = 0.2935 ± 0.0161, t = 0.0007, n = 86) mating, and there was no difference in the strength of SR and ST preference across these matings (first mating SR Pref mean ± SE = 0.3541, *P* = 0.5252; second mating SR Pref mean ± SE = 0.0754, *P* = 0.5417; third mating SR Pref mean ± SE = 0.0809, *P* = 0.5936; and ST eye-span large: 9/89, median: 3/63, small: 10/38, *P* = 0.0538).

**Mating frequency**

SR males mated less often than ST males in the 30-min observation period SR mean ± SE = 26.127 ± 0.1445; ST mean ± SE = 36.267 ± 0.1392, *χ*$_2$ = 5.5672, *P* = 0.0183. Genotype had a strong effect on mating frequency in large eye-span flies (*χ*$_1$ = 8.9030, *P* = 0.0037) but not in medium (*χ*$_1$ = 0.4153, *P* = 0.5193) or small eye-span flies (*χ*$_1$ = 0.0001, *P* = 0.9915). In the second experiment, males with large and medium eye-span mated more frequently than small eye-span males (large mean ± SE = 3.8876 ± 0.2268, medium mean ± SE = 3.6031 ± 0.2163, small mean ± SE = 2.1600 ± 0.2414; *χ*$_1$ = 15.4863, *P* = 0.0003).

**DISCUSSION**

Male mate preferences have been observed across a range of species, even where initially unexpected, for example, in polygynous species that lack paternal care or other forms of direct male investment in offspring or mating partners (Edward and Chapman 2001). In this study of stalk-eyed flies, we found that males show preference for large eye-span females. This mirrors previous laboratory and field studies in *T. dalmanni* (Cotton et al. 2015). As in other species, the likely benefit of this preference derives from mating with higher fecundity females (Olson 1993; Down and Montgomery 2004; Byrne and Rice 2006; Reading and Buckrell 2007). Female eye-span reliability indicates fecundity among field-caught stalk-eyed flies, where it explains a significant amount of variation in ovarian egg number, even after controlling for body size (Cotton et al. 2010, 2015).
examination of male genitalia (Wilkinson GS, personal communication). Only T. dalmanni individuals were used in the experiments here. Previous field work (Cotton et al. 2010, 2014, 2015) was carried out in the Gombak valley in Malaysia where both species occur in sympathy (Pymiakowski A, unpublished data). It is not yet known how the presence/absence of meiotic drive affects patterns of sexual selection in the two species.

Although there was no difference in the preference of SR and ST males, we found that large eyespan males showed stronger preference and small eyespan males exhibited no preference. Vision is the dominant sensory mode for assessment of potential mates in stalk-eyed flies (Chapman et al. 2005, 2017). Because stereoscopic vision and visual acuity improve as eyespan increases (Burkhardt and de la Motte 1985; de la Motte and Burkhardt 1985), males with larger eyespan will be better able to distinguish differences between females and express stronger preference, as has been found for female mate preference in T. dalmanni (Hingl et al. 2001a). Mean eyespan is smaller in SR than ST males (Wilkinson et al. 1998; Cotton et al. 2014; Meade et al. 2015a), and field samples show that males with smaller eyespan attract fewer females to their lek sites (Cotton et al. 2010). On average SR males will attract fewer females to their leks, and have fewer opportunities for choice. However, the magnitude of this effect may be small as the eyespan difference between SR and ST laboratory-reared males is only ~5% (Meade et al. 2015b).

We predicted that SR males would have weak preference if male choice is costly and condition dependent but this is not supported by the data. The absence of male–male competition at dawn when most mating takes place (Cotton et al. 2010) and the short amount of time before female lek departure do not point to obvious male preference costs associated with distinguishing between females that have already settled at a lek site. Smaller eyespan may mean that SR males may have fewer opportunities to choose between females and lose out to rival males in establishing ownership of favorable lek sites. But when SR males do attract multiple females, they will likely benefit from preferential mating with large females (leading to fecundity benefits) just like ST males.

A further observation was a lower mating frequency in SR males with large eyespan (compared to ST males with large eyespan), although this had no effect on their preference. Previous work in T. dalmanni has linked mating rate to accessory gland size, the organ that produces nonsperm components of the ejaculate (Baker et al. 2003; Rogers et al. 2005), and SR males have smaller accessory gland size (Meade et al. 2015a). This deficit may arise due to a greater allocation of resources to testes, which are enlarged in large eyespan SR males, presumably to compensate for the destruction of sperm by meiotic drive (Meade et al. 2015b). A lower mating frequency was also observed in small eyespan males compared to those with medium and large eyespan, suggesting that SR males behave like these lower quality males. How this different aspect of male mating behavior affects fitness needs further work.

This is the first study of how meiotic drive influences male mating preference. It has wider significance as drive is associated with lower genetic quality due to mutation accumulation in the X0 inversion. But there was no weakening in the strength of drive male preference. Our results suggest that the expression of male mate preference is not condition dependent (Cotton et al. 2006). Male (and female) mate preference may not incur significant costs when there are multiple females (males) to choose between. This contrasts with other aspects of male mating behavior, such as attracting females and warding off competitors, which are likely to be costly and condition dependent. We observed a reduction in preference as male eyespan decreased and this is likely to affect drive males more as their eyespan on average is reduced. To fully gauge the impact of these findings, further work on male choice will focus on whether the expected reduced eyespan of drive males impacts their ability to dominate lek sites and attract females.

**SUPPLEMENTARY MATERIAL**

Supplementary data are available at *Behavioral Ecology* online.

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**Authors’ Contributions** S.R.F., K.F., and A.P. conceived and designed the study. S.R.F., L.N., M.M., and P.G. collected the data. S.R.F. and A.P. analyzed the data and wrote the paper with input from K.F.

**Data Accessibility** Analyses reported in the article can be reproduced using the data provided by Fimegan et al. 2015a.

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Meiotic drive reduces egg-to-adult viability in stalk-eyed flies


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Meiotic drive reduces egg-to-adult viability in stalk-eyed flies

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A number of species are affected by Sex-Ratio (SR) meiotic drive, a selfish genetic element located on the X-chromosome that causes dysfunction of Y-bearing sperm. SR is transmitted to up to 100% of offspring, causing extreme sex ratio bias. SR in several species is found in a stable polymorphism at a moderate frequency, suggesting there must be strong frequency-dependent selection resisting its spread. We investigate the effect of SR on female and male egg-to-adult viability in the Malaysian stalk-eyed fly, Teleopsis dalmanni. SR meiotic drive in this species is old, and appears to be broadly stable at a moderate (approx. 20%) frequency. We use large-scale controlled crosses to estimate the strength of selection acting against SR in female and male carriers. We find that SR reduces the egg-to-adult viability of both sexes. In females, homozygous females experience greater reduction in viability ($s_f = 0.242$) and the deleterious effects of SR are additive ($h = 0.511$). The male deficit in viability ($s_m = 0.214$) is not different from that in homozygous females. The evidence does not support the expectation that deleterious side effects of SR are recessive or sex-limited. We discuss how these reductions in egg-to-adult survival, as well as other forms of selection acting on SR, may maintain the SR polymorphism in this species.

1. Introduction