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Brief Communication

Meiotic drive does not impede success in sperm competition in the stalk-eyed fly, *Teleopsis dalmanni*

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Abstract

Male X-linked meiotic drive systems, which cause the degeneration of Y-bearing sperm, are common in the Diptera. Sperm killing is typically associated with fitness costs that arise from the destruction of wildtype sperm and collateral damage to maturing drive sperm, resulting in poor success under sperm competition. We investigate X-linked meiotic drive fertility in the stalk-eyed fly, *Teleopsis dalmanni*. Drive male paternity was measured in double mating trials under sperm competition against a wildtype male. Drive males sired the same number of offspring as wildtype males, both when mated first or second. This is the first evidence that drive males can compete equally with non-drive males in double matings, challenging the assumption that drive males inevitably suffer reduced fertility. The finding is in accord with previous work showing that the number of sperm per ejaculate transferred to females during non-competitive single matings does not differ between drive and wildtype males, which is likely due to the adaptive evolution of enlarged testes in drive males. Future experiments will determine whether the competitive ability of drive males is maintained under higher rates of female remating likely to be experienced in nature.

Keywords: meiotic drive, stalk-eyed fly, sperm competition, multiple mating

Introduction

Meiotic drive causes the unequal transmission of genes to the next generation, violating Mendelian laws of segregation (Gershenson, 1928; Sandler & Novitski, 1957). In the extreme, the driver entirely excludes wildtype alleles and is transmitted to all offspring (Searle & de Villena, 2022; Wolf et al., 2022). X-linked drivers are common among Diptera species and lead to dysfunction of Y-bearing sperm and the production of female-only broods (Hurst & Pomiankowski, 1991; James & Jaenike, 1990; Jiggins et al., 1999; Newton et al., 1976; Policansky, 1974; Presgraves et al., 1997). Such a significant transmission advantage could potentially lead to population extinction due to the lack of males (Hamilton, 1967; Hatcher et al., 1999; Mackintosh et al., 2021). However, the fitness costs associated with carrying drive genes often result in negative frequency-dependent selection, which limits their spread (Finnegan et al., 2019; Lindholm et al., 2016).

One factor that strongly impacts the spread of meiotic drive genes is reduced fertility (Zanders & Unckless, 2019). Males with drive not only lose wildtype gametes but typically suffer pleiotropic "collateral damage" that reduces the activity or number of mature drive sperm, leading to poor outcomes, especially under sperm competition (Price & Wedell, 2008). This deficit is likely to be prominent in insects that possess reproductive organs specialized for long-term storage of viable sperm, increasing interactions between ejaculates (Parker, 1970). Evidence from sperm competition studies of X-linked meiotic drive systems in *Drosophila* species supports this prediction. In *Drosophila pseudoobscura*, SR drive males sire fewer offspring than standard males in double mating trials (Price et al., 2008a). Drive males have a disproportionally lower success both in their ability to defend against other sperm as the first (P1) male or to displace sperm already in storage as the second (P2) male (Price et al., 2008a). A similar pattern occurs in *Drosophila simulans* with reduced success in P1 and P2 positions for drive males, and preferential drive male sperm ejection from the female reproductive tract even without competition from the second male's sperm (Angelard et al., 2008; Atlan et al., 2004). It has been suggested that increased female polyandry evolves to undermine the success of drive sperm and an experimental evolution study in *D. pseudoobscura* and a double mating experiment in *Drosophila recens* support this possibility, linking the frequency of drive with the rate of multiple mating (Courret et al., 2019; Dyer & Hall, 2019; Haig & Bergstrom, 1995; Price et al., 2008b; Zeh & Zeh, 1997).

In this article, we investigate the association between X-linked meiotic drive and reduced male fertility using the X-linked SR meiotic drive system in the stalk-eyed fly, *Teleopsis dalmanni*. Stalk-eyed fly females store sperm in the spermathecae (long-term storage organs) after mating, before sperm migrate to the ventral receptacle, where they are individually packaged into pouches prior to release into the oviduct for fertilization of mature eggs (Kotrba, 1995; Presgraves et al., 1999). In several stalk-eyed fly species, the main mode of sperm competition is sperm mixing, rather than male precedence (Bellamy, 2012; Corley et al., 2006; Lorch et al., 1993; Wilkinson et al., 1998a). Double mating trials

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appear to confirm that drive males should be poor competitors as drive (SR) males sired fewer offspring than wildtype (ST) males (Wilkinson & Fry, 2001; Wilkinson et al., 2006). However, several factors raise concerns about a simplistic interpretation of these findings. The first study was an inter-population cross of Malaysian and Thai flies. It was carried out before genetic markers had been developed and used variation in leg color to assign parentage, which has an unknown error rate (Wilkinson & Fry, 2001). In addition, this study was in the conger *Teleopsis whitei* which may well have a different pattern of sperm competition than in *T. dalmanni*. The second study is in *T. dalmanni* and reported a lower SR male paternity using double mating trials (Wilkinson et al., 2006). However, this effect was limited to broods in which all offspring were sired by a single parent, that were less frequently fathered by SR males. There was no difference in SR and ST paternity in mixed paternity broods. In addition, this experiment only considered the competitive ability of SR males when mating second. This means that defensive traits of SR sperm and ejaculate were not assessed, so it is unclear whether the lack of success of SR males is general or limited to lower sperm precedence when mating second.

In addition, a profound challenge arises from recent findings that SR males transfer similar numbers of sperm per ejaculate (Meade et al., 2019, 2020). This was measured in females both in the spermathecae and the ventral receptacle after matings with SR or ST males, as well as after up to three sequential matings by a single male (Meade et al., 2019). Furthermore, when egg counts were used to measure fertility after single matings, it did not differ for females mated to SR or ST males (Meade et al., 2020). Dissection of adult SR males reveals that they have greatly enlarged testes, which allow sperm delivery and fertility to be maintained despite the destruction of sperm caused by meiotic drive (Meade et al., 2019, 2020). This challenges the conventional view that drive males are weak competitors, and specifically, the finding of a competitive deficit of drive males in double mating trials.

Here, the competitive success of SR males was measured in a standard sperm competition assay using reciprocal double mating trials in which the SR male mated first followed by the ST male, or vice versa. This allowed an assessment of the SR male's success in both the offensive and defensive role and revealed whether there is first or last male sperm precedence. Even though multiple mating well above two is the norm in *T. dalmanni* stalk-eyed flies (Baker, 2001; Baker et al., 2003; Chapman et al., 2005), the simplicity of the double mating trial allows clear assessment of whether SR sperm suffer a disadvantage in competition with ST sperm when the two males mate equally. The offspring arising from these trials were collected and genotyped at the larval stage to determine the proportion of offspring sired by SR males. This enabled the study to avoid confounds in paternity share relating to egg to adult viability differences, which have recently been shown to disadvantage SR-carrying larvae (Finnegan et al., 2019).

Methods

Stock populations

Flies for the standard stock (ST-stock) population carry only the wildtype X chromosome (X^{ST}). They were collected (by S. Cotton and A. Pomiankowski) in 2005 from the Ulu Gombak valley, Peninsular Malaysia (3°19′N 101°45′E). They have since been maintained in high-density cages (>200 individuals) to minimize inbreeding and are regularly monitored to ensure they do not contain the meiotic drive.

The meiotic drive stock (SR-stock) population is composed of females that are homozygous for a sex-ratio-distorting X chromosome (X^{SR}). They were derived from flies collected in 2012 (by A. Cotton and S. Cotton) from the same location as the ST-stock. X^{SR}/Y males produce 100% female offspring due to transmission distortion. The X^{SR} female stock is maintained by crossing X^{SR}/X^{SR} females with X^{ST}/Y males to produce X^{SR}/Y drive males, who are then mated to the X^{SR}/X^{SR} females to generate the next generation of the SR-stock females. The outcrossing to ST males from the ST-stock ensures that the two stocks only differ in their X chromosomes and are homogenized for autosomal content.

Both stock populations were kept at 25 °C, with a 12:12 hr dark:light cycle and fed puréed sweetcorn twice weekly. Fifteen-minute artificial dawn and dusk periods were created by illumination from a single 60W bulb at the start and end of the light phase.

Experimental populations

Experimental ST (X^{ST}/Y) and SR males (X^{SR}/Y) were drawn from the ST-stock and SR-stock, respectively. They were housed separately in cages of ~50 individuals until sexually mature, in groups of similar age (6–8 weeks). ST-stock females were added to these cages at an equal sex ratio for > 3 days to allow males to mate. The females were then removed and discarded. Experimental males were then kept in single-sex groups for a further 3–6 days to allow their accessory glands to return to full size (Rogers et al., 2005).

Experimental ST females (X^{ST}/X^{ST}) were drawn from the ST-stock. All experimental females were virgins, 6–8 weeks old, and had reached sexual maturity (Baker et al., 2003). ST females were anesthetized on ice and their eyespans were measured (see below method) to exclude small flies and limit variation in size and fecundity that could influence sperm allocation strategies in males (Cotton et al., 2015). Only large females with an eyespan > 5.4 mm were used in mating trials (range 5.4–5.8 mm).

Sperm competitiveness of SR and ST males

Mating trials were conducted to measure the competitiveness of SR and ST males. On the day preceding each assay, experimental females were housed singly in 500 ml clear plastic containers with a moist cotton wool base. On the trial day, a single male was added to each container ~15 min after dawn, as this is the period during which mating is most likely (Chapman et al., 2005). Males were allowed to mate, defined as a copulation lasting ≥ 30 s, as durations shorter than this are usually insufficient for sperm transfer (Cotton et al., 2015; Rogers et al., 2006). The mating duration was recorded. If no mating was observed after 15 min, the male was moved to a new container with a new female. If mating still did not occur after a further 15 min, the male was discarded. The original unmated female was used again and placed with another male. If this did not result in a copulation after 15 min, the female was discarded.

A second mating was performed 24 hr later, following the same protocol. Again, if the male failed to copulate with the female after 30 min, he was replaced, and if a mating still did not occur, the female was discarded. The mating failure rate was extremely low: one ST male failed to mate on day 1 (P1), one SR male failed to mate on day 2 (P2), and one female was

discarded as she failed to mate with any male. Females were mated either to an SR male followed by an ST male, or an ST male followed by an SR male. Once females had been double mated, the containers were lined with a fresh moist cotton wool base and 1tsp puréed sweetcorn to collect eggs, which was renewed every 2–3 days for 2 weeks. This kept larval density low, maximizing survival. Bases were stored in Petri dishes at 25 °C. In total, 62 females were successfully mated twice: 30 to an SR male first and 32 to an ST male first. For ease, these matings were carried out in two batches, 1 week apart.

After mating, experimental males were removed and frozen, and their eyespan and thorax length were measured under a Leica microscope using ImageJ (v1.46; Schneider et al., 2012). Eyespan was defined as the distance between the outer tips of the eyes (Hingle et al., 2001). Thorax length was defined as the distance ventrally from the anterior tip of the prothorax along the midline, to the joint between the metathoracic legs and the thorax (Rogers et al., 2008).

Progeny genotyping

Petri dishes were examined for larvae one week after collection. Larvae that had developed to be large enough to be seen by eye were transferred to a 96-well plate. Each Petri dish was then examined daily to collect the remaining growing larvae until there was no further evidence of their presence. Each well of the plate contained 100 µl digestion solution (20 mM EDTA, 120 mM NaCl, 50 mM Tris-HCL, 1% SDS, pH 8.0) and 4 µl proteinase K (10 mg ml^{-1}). A standard protocol was adapted to extract and purify DNA from larvae (see Supplementary Information 1 for details; protocol from Burke et al., 1998). The X-linked INDEL marker *comp162710* was used to identify offspring of ST and SR fathers, due to its reported accuracy in determining phenotype (>90%; Meade et al., 2019). X^{ST} carries a large allele (286 bp), whereas X^{SR} carries a small allele (201 bp).

Nine females produced no offspring. A further two females produced low numbers of offspring (2, 6), of which none and one were successfully genotyped, respectively. Overall, in 7 of 31 cases, the mating order was P1 ST—P2 SR, and in 4 of 31 cases, the mating order was P1 SR—P2 ST. There was no mating order effect on failure to produce genotyped offspring (Fisher exact test $p = .508$). These 11 females were removed from further analysis.

Not all offspring collected over the 2-week period were genotyped for logistical reasons. On average, 39.8 (range 0–116) offspring were collected, and 21.9 (range 0–59) offspring were genotyped per female; a total of 1,161 successful PCRs. The 96-well plates were genotyped without regard to the offspring of particular females as they were collected on particular days. This approach led to a high correlation between offspring production and genotyping ($\rho = 0.872$, $n = 51$, $p < .001$).

Statistical methods

All tests were carried out in R version 4.1.2 (R Core Team, 2021). To test if mating order or genotype affected the number of offspring sired by each male, P1:P2 offspring (the number of offspring sired by P1 relative to the number of offspring sired by the P2 male) or ST:SR offspring (the number of offspring sired by the ST relative to the number of offspring sired by the SR male) were fitted as the response variable in generalized linear models (GLMs) with a binomial error distribution. The response variables were coded using the R *cbind* function. Count data of offspring sired by each male was used in the binomial analysis rather than one male's paternity proportion to account for the variable sample size of offspring assigned to each male (larger sample sizes provide better estimates), as used by others (Dobler et al., 2022). It is not possible to treat mating order and genotype in a single "global" model combining genotype and mating order as each female's offspring are derived from only two males who have both a genotype and mating order. Hence, the binomial analysis (y_1, y_2) enters offspring either according to mating order (y_1 = P1, y_2 = P2) or genotype (y_1 = ST, y_2 = SR) in two separate analyses. As the GLMs were over-dispersed, a quasi-binomial error distribution was used. Tests were repeated excluding females that had ≤10 offspring genotyped. The number of larvae collected and the batch in which the matings were performed were assessed as potential confounding variables. In addition, the data were split in two, considering offspring number of SR/ST or in the P1/P2 role, with linear models on genotype. In order to assess the power of the experiment to detect differences in mating order or genotype, the same GLM statistic was calculated with up to a 10-fold increase in sample size on re-sampled data (with replacement). One thousand repeats were performed at each sample size, and the resulting GLM statistics examined for evidence of difference in paternity due to mating order or genotype (see Supplementary Information 4 for detailed method description and code).

The effect of male thorax length (a proxy for body size) and relative eyespan (the variation in eyespan after controlling for thorax length) were also considered in the analysis. Both traits are strongly condition dependent and indicators of male genetic and phenotypic quality (Cotton et al., 2015; David et al., 2000; Howie et al., 2019). Whether these male trait sizes differed between genotypes was tested by fitting thorax length and relative eyespan as the response variable in linear models. In addition, whether mating duration differed by mating order and genotype was tested by fitting mating duration as a response variable in linear models, and by its inclusion as a fixed effect in GLMs with the number of offspring sired by each male. Full statistical analyses are reported in the Supplementary Informations 2 and 3.

Results

Male fertility

In total, 62 females were reciprocally mated to males of each genotype. Fifty-one females had offspring (between 4–59) that were successfully genotyped (27 P1 SR—P2 ST and 24 P1 ST—P2 SR matings), and of these, 47 females had ≥10 genotyped offspring (23 P1 SR—P2 ST and 24 P1 ST—P2 SR). For two of the reciprocal matings, one mating was 29 s in duration; these matings were included in the subsequent analysis as, in both cases, the male in question produced offspring.

The distribution of proportions sired by the two males was flat, including offspring broods that were exclusively sired by either the P1 or P2 male (Figure 1A) or by either the ST or SR male (Figure 1B), with means around equality (mean ± *SD* P2 male = 0.522 ± 0.327, SR male = 0.575 ± 0.316). Using offspring numbers (rather than proportions), there was no effect of mating order ($F_{1,49} = 1.307$, $p = .259$; Figure 2A) or genotype ($F_{1,49} = 0.196$, $p = .660$; Figure 2B) on the number of offspring sired by each

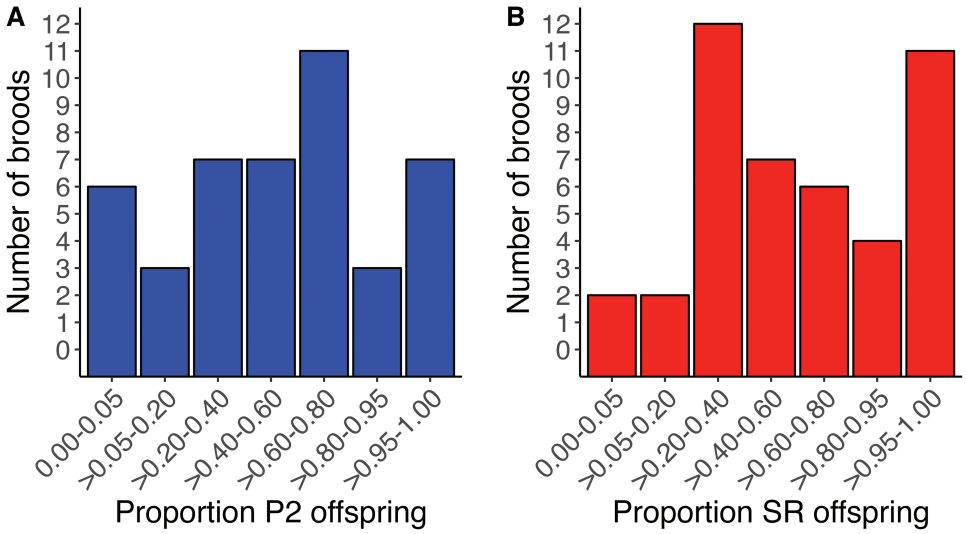

Figure 1. (A) The distribution of P2, the proportion of offspring sired by the second male, is shown per brood (blue). (B) The distribution of the proportion of offspring sired by the SR male is shown per brood (red).

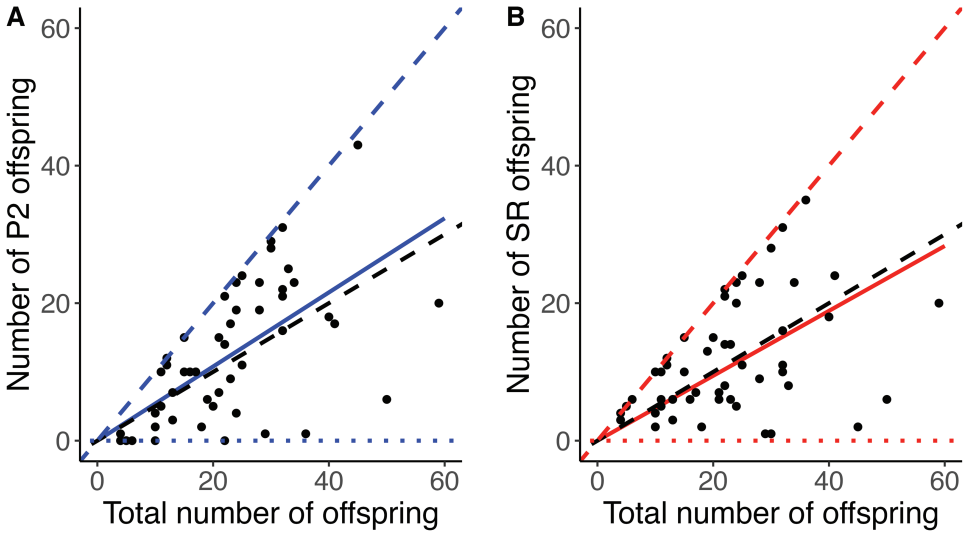

Figure 2. (A) Points correspond to the number of P2 offspring against the total number of offspring per brood. The solid blue line represents the regression of the number of P2 offspring against the total number of offspring (β = 0.539; intercept constrained to zero). The blue dashed line represents P2 = 1.000 (all P2 offspring), the black dashed line represents P2 = 0.500 (equal P1 and P2 offspring), and the blue dotted line represents P2 = 0.000 (all P1 offspring). (B) Points correspond to the number of SR offspring against the total number of offspring per brood. The solid red line represents the regression of the number of SR offspring against the total number of offspring (β = 0.472; intercept constrained to zero). The red dashed line represents SR = 1.000 (all SR offspring), the black dashed line represents SR = 0.500 (equal SR and ST offspring), and the red dotted line represents SR = 0.000 (all ST offspring).

male. Nor was there an effect of genotype when the data were split in halves, either with the SR male in the P1 role ($F_{1,49}$ = 0.002, p = .963), or in the P2 role ($F_{1,49}$ = 0.434, p = .513). An additional test added the total number of offspring collected as a covariate as it varied between females (mean ± *SD*; 48.196 ± 22.735 offspring; range 6–116 offspring), but its inclusion did not alter the main effects of mating order or genotype (p > .05; see Supplementary Information 2, Figure S2). Likewise, the main effects were unchanged when batch number was included as a covariate (p > .05; see Supplementary Information 2). The results of these tests were also unchanged after the exclusion of the four females that had less than 10 offspring genotyped (see Supplementary Information 3).

In 11 of the 47 cases with ≥10 offspring genotyped, one male sired more than 0.95 of the offspring, with no difference between male mating position (four sired by the P1 male, and seven sired by the P2 male, $F_{1,9}$ = 0.986, p = .351) or male genotype (eight sired by the SR male and three were sired by the ST male, $F_{1,9}$ = 0.841, p = .383). When these extreme cases were excluded, there was still no effect of mating order ($F_{1,32}$ = 0.094, p = .761) or male genotype ($F_{1,32}$ = 0.589, p = .448) on the number of offspring sired.

To assess the power of the data to detect differences, the data were resampled (with replacement) using a 1–10-fold increase in sample size compared to the original data (1,000 repeats for each fold increase, Supplementary Information 4). As expected, the fraction of runs with significant differences

(at $p < .05$) increased with sample size. The increase was marked for mating order with a P2 advantage evident at a 4-fold increase in sample size (95% confidence interval [CI] 0.207–4.567 in favor of P2). However, the increase was minor for genotype, and there was no advantage to either genotype even with a 10-fold increase in sample size (95% CI −0.789–3.667 in favor of SR).

Male trait size and mating duration

Thorax length was smaller in SR than ST males (mean ± *SE*, SR = 2.190 ± 0.023 mm, $N = 49$, ST = 2.297 ± 0.025 mm, $N = 50$; $F_{1,97} = 9.783$, $p = .002$). Eyespan is strongly colinear with thorax ($F_{1,97} = 167.242$, $p < .001$) and was likewise smaller in SR males (SR = 7.304 ± 0.111 mm, ST = 7.897 ± 0.115 mm; $F_{1,97} = 13.766$, $p < .001$; Supplementary Information 2, Figure S1). However, the relative eyespan did not differ between genotypes ($F_{1,96} = 3.734$, $P = 0.056$; Supplementary Information 2, Figure S1). As thorax length differed between genotypes, it was added as a covariate, but there was still no effect of mating order ($F_{1,44} = 1.161$, $p = .287$) or male genotype ($F_{1,44} = 0.369$, $p = .547$) on the number of offspring sired by each male.

Mating duration did not differ with mating order (mean ± *SE*, P1 = 63.94 ± 3.43 s, P2 = 73.45 ± 3.43 s; $F_{1,100} = 0.943$, $p = .334$) or genotype (ST = 60.82 ± 2.36 s, SR = 76.57 s ± 9.42 s, $F_{1,100} = 2.627$, $p = .108$). Mating duration did not affect the number of offspring sired by the P2 male ($F_{1,48} = 0.022$, $p = .882$), but P1 males with a shorter mating duration sired a greater number of offspring ($F_{1,48} = 4.082$, $p = .049$). Mating duration did not affect the number of offspring sired by the SR male ($F_{1,48} = 0.246$, $p = .622$) or the ST male ($F_{1,48} = 3.366$, $p = .073$). Given its inconsistent effect on the number of offspring sired, the mating durations of the two males were added as covariates, but there was still no effect of mating order ($F_{1,47} = 1.208$, $p = .277$) or genotype ($F_{1,47} = 0.071$, $p = .791$) on the number of offspring sired.

Discussion

Our study provides little support for the idea that males carrying X-linked meiotic drive are at a disadvantage under sperm competition due to sperm loss and other deleterious effects of meiotic drive on sperm function (Courret et al., 2019; Verspoor et al., 2020). Here, the paternity of SR males did not differ from ST males overall, nor in the P1 or P2 positions considered separately. This challenges the general pattern which has been reported across the Diptera (Dyer & Hall, 2019; Hurst & Pomiankowski, 1991; James & Jaenike, 1990; Jiggins et al., 1999; Newton et al., 1976; Policansky, 1974; Presgraves et al., 1997; Price et al., 2008a). It is also in opposition to previous evidence of lower drive male paternity in stalk-eyed fly double-mating experiments, which were discussed in the Introduction (Wilkinson & Fry, 2001; Wilkinson et al., 2006). Our results are robust to a number of potential confounding factors: matings were performed between flies from the same population, offspring paternity was assessed using highly accurate genetic markers, larvae were used to assess paternity—which reduces the impact of lower egg-adult viability in SR females—and double matings were carried out with SR males in the first and second mating position to reliably assess sperm precedence. Furthermore, the findings here align with those of Meade et al. (2019, 2020), who showed that sperm numbers transferred to females and the resulting fertility do not differ in single matings by SR and ST males.

Our results do not invalidate previous findings, which likely reflect genuine experimental differences. The study of Wilkinson and Fry (2001) was carried out on the closely related species *T. whitei*, which also carries X-linked SR meiotic drive that is thought to have evolved prior to the divergence of these two species (Meier & Baker, 2002; Presgraves et al., 1997). Genetic markers for drive have not been identified in *T. whitei* (G. S. Wilkinson, personal communication), implying a small inversion is associated with drive in this species, unlike the multiple inversions that cover most of the *T. dalmanni* SR X chromosome (Christianson et al., 2011; Paczolt et al., 2017; Reinhardt et al., 2014, 2023; Wilkinson et al., 2006). This means that few X-linked genes are in linkage disequilibrium with those that control drive, potentially limiting the possibility of compensatory testes enlargement and explaining why *T. whitei* drive males have reduced fertility under sperm competition. The second study of Wilkinson et al. (2006) used a similar double mating design in *T. dalmanni* (although only with SR males in the P2 role). As in this study, it reported no difference between SR and ST success in mixed paternity broods. However, in single-parent broods (where only one male fathered offspring), there were 11 from the ST male and only three from the SR male (rate 14/40 = 35%). In this study, we found the pattern was reversed with three from the ST male and eight from the SR male (rate 11/51 = 22%). There were experimental design differences that might be important. In particular, Wilkinson et al. (2006) took experimental males from mixed sex cages with no control over prior mating, whereas we kept males without females for several days to allow their accessory glands to return to full size (Rogers et al., 2005). This could explain the higher rate of single-parent broods in Wilkinson et al. (2006). However, combining across these two studies, we conclude that there can be little confidence that there is a large deficit in SR male single-parent broods. This is consistent with previous work, which showed no difference in the failure rate of sperm transfer to the spermatheca of females mated once either to ST or SR males (Meade et al., 2019).

In line with earlier work on sperm competition in stalk-eyed flies, there was no effect of mating order on paternity, suggesting that the sperm of the first and second male simply mix and there is no sperm precedence in *T. dalmanni* (Bellamy, 2012; Corley et al., 2006; Wilkinson & Fry, 2001). Corley et al. (2006) found evidence of a trimodal P2 distribution, centered around equal paternity as well as a strong bias to either the first or second male (double matings with ST males). This contrasts with the flat distribution shown here (Figure 1). The difference could be due to the multiple mating design used by Corley et al. (2006), in which each female was mated three times with the first and second males. A trimodal pattern was also reported in a double mating design in the distantly related South African stalk-eyed fly species *Diasemopsis meigenii*, where extreme paternity bias was explained by the failure of sperm transfer after a single copulation (Bellamy, 2012). Whatever the explanation, none of these studies support the idea of a competitive advantage associated with mating position in stalk-eyed flies.

The lack of difference found in this study may be limited by sample size ($n = 51$), like all statistical comparisons. We addressed this by re-sampling the data with up to a 10-fold inflation in sample size. This increased the likelihood of finding

a mating order difference (favoring P2 at a 4-fold increase in sample size) to a much greater extent than a genotypic difference (no difference even at a 10-fold increase in sample size). Given that these comparisons rely on the same distribution of the data, they allow us to conclude that if there is a difference in the paternity gain due to genotype, it is of a lower order than that relating to mating order, and there is no evidence to support the hypothesis of a competitive disadvantage associated with drive (if anything, the data favors a SR advantage). Our approach is not wholly satisfactory as re-sampling maintains the distribution of offspring genotyped per female, which was variable (95% confidence range 19–26), although to some extent this is accounted for by the binomial tests. A re-sampling of this distribution would inevitably require further assumptions and end-up being contrived. We adopted an approach that maintains the distribution of offspring genotyped per female to frame our conclusions within the limitations of the data collected.

In this study of *T. dalmanni*, sperm competition was assessed under low-stress conditions. Virgin females were mated to two males separated by a 24-hr period. Experimental males were not virgins but had been kept for several days in single-sex groups. The objective was to assess SR and ST males under standardized conditions as a first step to understanding how SR males perform under sperm competition. This is a highly specific experiment, designed to test whether a male gains an advantage after a single competitive mating, either because there is first/last male precedence or variation due to genotype. In the wild, competitive conditions are more complex. Males form leks with multiple females at dusk and then mate in a short period at dawn before dispersal, with occasional matings interspersed during daylight hours (Chapman et al., 2005; Cotton et al., 2010, 2015; Wilkinson et al., 1998b). Females mate repeatedly in a life span that can extend over several months (Reguera et al., 2004; Wilkinson et al., 1998b). Multiple matings are required to maxise fertility as males transfer low numbers of sperm per ejaculate (Meade et al., 2019; Rogers et al., 2006; Wilkinson et al., 2005; Baker, 2001), and sperm usage leads to a quick drop in female fertility over time (Meade et al., 2017; Wilkinson et al., 1998a). Future experiments need to assess the success of single SR and ST male matings in females with a background of multiple mating, closer to the conditions found in nature. There may be differences when female sperm storage organs are saturated compared to the situation with double mating when females are below maximal fertility (Baker, 2001). In addition, it will be important to assess the effect of the mating rate, which is lower in SR males (Meade et al., 2020; Rogers et al., 2008; Wilkinson et al., 2003). SR males may be less able to compete in populations at high density where there are multiple opportunities to mate, even though sperm transfer does not differ between genotypes in sequential matings over a short period of time (Meade et al., 2019). These further studies will provide a more comprehensive assessment of sperm competition as a factor contributing to the fertility of drive males and its consequences for the frequency of SR in wild populations.

In summary, we demonstrate that meiotic drive is not always associated with male fertility reduction under conditions of sperm competition, even though drive destroys half of carrier-male sperm. The lack of a fertility cost potentially contributes to the relatively high frequency of meiotic drive in *T. dalmanni*, which is around 20% in wild populations (Cotton et al., 2014; Paczolt et al., 2017; Wilkinson et al., 2003). This pattern is unlike other species where drive males do poorly under sperm competition and the spread of drive is reliant on a high frequency of monandrous matings (Courret et al., 2019; Dyer and Hall, 2019; Price et al., 2008b). The absence of a fertility cost is likely an evolved response to the loss of sperm caused by meiotic drive, which is supported by the finding in *T. dalmanni* that drive male testes are larger at eclosion, have higher growth rates and are considerably enlarged at maturity (Bradshaw et al., 2022; Meade et al., 2020). We provide strong evidence against the consensus that drive males are outperformed by non-drive males under sperm competition—which suggests that other species should be investigated for evidence of mitigation of drive fertility costs.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

The data that support the findings of this study are openly available in the Dryad at https://doi.org/10.5061/dryad.mkkwh713g.

Author contributions

S.B., L.M., and A.P. conceived the study. S.B. and L.M. carried out experiments and S.B. collected the data. S.B., L.M., and A.P. analyzed the data. S.B. and A.P. wrote the paper. All authors read, reviewed, and agreed on the submitted version.

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