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Title: Does meiotic drive alter male mate preference?

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Running title: Male mate preference and meiotic drive

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, 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 while 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**

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 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 (X^{SR}) 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 X^{SR} 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 and Presgraves 2012; Sutter and Lindholm 2015). Reinhardt et al. (2014) showed that there are almost a thousand fixed differences between SR and ST X-linked genes in *T. dalmanni*, but only 11 for autosomal genes, consistent with mutation accumulation on X^{SR}. There is some evidence for reduced genetic quality of SR. Males and females carrying the X^{SR} chromosome have reduced egg-to-adult viability (Finnegan et al. 2019a), 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 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 X-linked or influenced by X-linked factors because they only differ in their X chromosomes with freely recombining autosomes. 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 will 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 colourful 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 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.

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 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. 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, >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.

Experimental flies

Experimental males were collected from egg-lays, a petri dish 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.

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.

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 *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. 2019a; Paczolt et al. 2017). *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. 2019a). Males with large *ms395* alleles and small *comp162710* alleles were classed as SR. Where markers gave conflicting signals, genotype was assigned on the basis of *comp162710* allele size.

Statistical analysis – Genotype and male preference

Model outputs are reported in the Supplementary Information. In the first experiment, we 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.

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 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.

Statistical analysis – Mating frequency

The effect of genotype on mating frequency in the first experiment was reported previously (Meade et al. 2019b). 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.

RESULTS

Genotype and male preference

In the first experiment, males showed a preference for large females when genotypes were pooled ($Pref$ mean ± SE = 0.3637 ± 0.056; t = 6.287, P < 0.0001, n = 162). Males preferred large females in their first ($Pref$ mean ± SE = 0.4321 ± 0.0711, P < 0.0001, n = 162), second ($Pref$ mean ± SE = 0.3030 ± 0.0832, P = 0.0006, n = 132) and third mating ($Pref$ mean ± SE = 0.4257 ± 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: $Pref$ mean ± SE = 0.1803 ± 0.1269, n = 61, P = 0.2000; fifth mating: $Pref$ mean ± SE = 0.2593 ± 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 ($Pref$ mean ± SE = 0.3970 ± 0.080, t = 4.959 P < 0.0001, n = 81) and ST males ($Pref$ mean ± SE = 0.3367 ± 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 $Pref$ mean ± SE = 0.5062 ± 0.0964, $P < 0.0001$, $n = 81$; ST $Pref$ mean ± SE = 0.3684 ± 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, $\chi^2_1 = 1.8069$, $P = 0.1789$, $n = 193$).

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, $Pref$ mean ± SE = 0.1132 ± 0.0970, P = 0.2853, n = 107; fifth mating, $Pref$ mean ± SE = 0.2500 ± 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 ($Pref$ mean ± SE = 0.4110 ± 0.0618, t = 3.840, P = 0.0003, n = 89). Medium males ($Pref$ mean ± SE = 0.1919 ± 0.0828, t = 1.910, P = 0.0611, n = 63) and small males showed no preference ($Pref$ mean ± SE = -0.0702 ± 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 ($Pref$ mean ± SE = 0.2508 ± 0.0887, t = 4.153, P = 0.0001, n = 69) and ST males ($Pref$ mean ± SE = 0.2156 ± 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 (1st mating $F_{1,174} = 0.3541$, $P = 0.5525$; 2nd mating $F_{1,154} = 2.4044$, $P = 0.1230$; 3rd 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, $\chi^2_1 = 5.6826$, $P = 0.05835$).

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 ± 0.2268, medium mean ± SE = 3.6031 ± 0.2165, small mean ± SE = 2.1600 ± 0.2414; $\chi^2_{1,173} = 13.4863$, $P = 0.0005$).

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, we 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 SR and ST 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, 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 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. We 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.

We deliberately designed the experiments 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. Our design 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, our 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 our design masked differences in male mate preference between the two genotypes.

Our attempt to mimic wild conditions is complicated by the recent discovery of 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.

While there was no difference in the preference of SR and ST males, we 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. 2019b), 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. 2019b).

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 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. 2019b). 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. 2019b). 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 X^{SR} 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.

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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 F.C. collected the data. S.R.F. and A.P. analysed 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 Finnegan et al. 2019b.

Supplementary data doi:10.5061/dryad.2f8v175

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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.

Figure 1

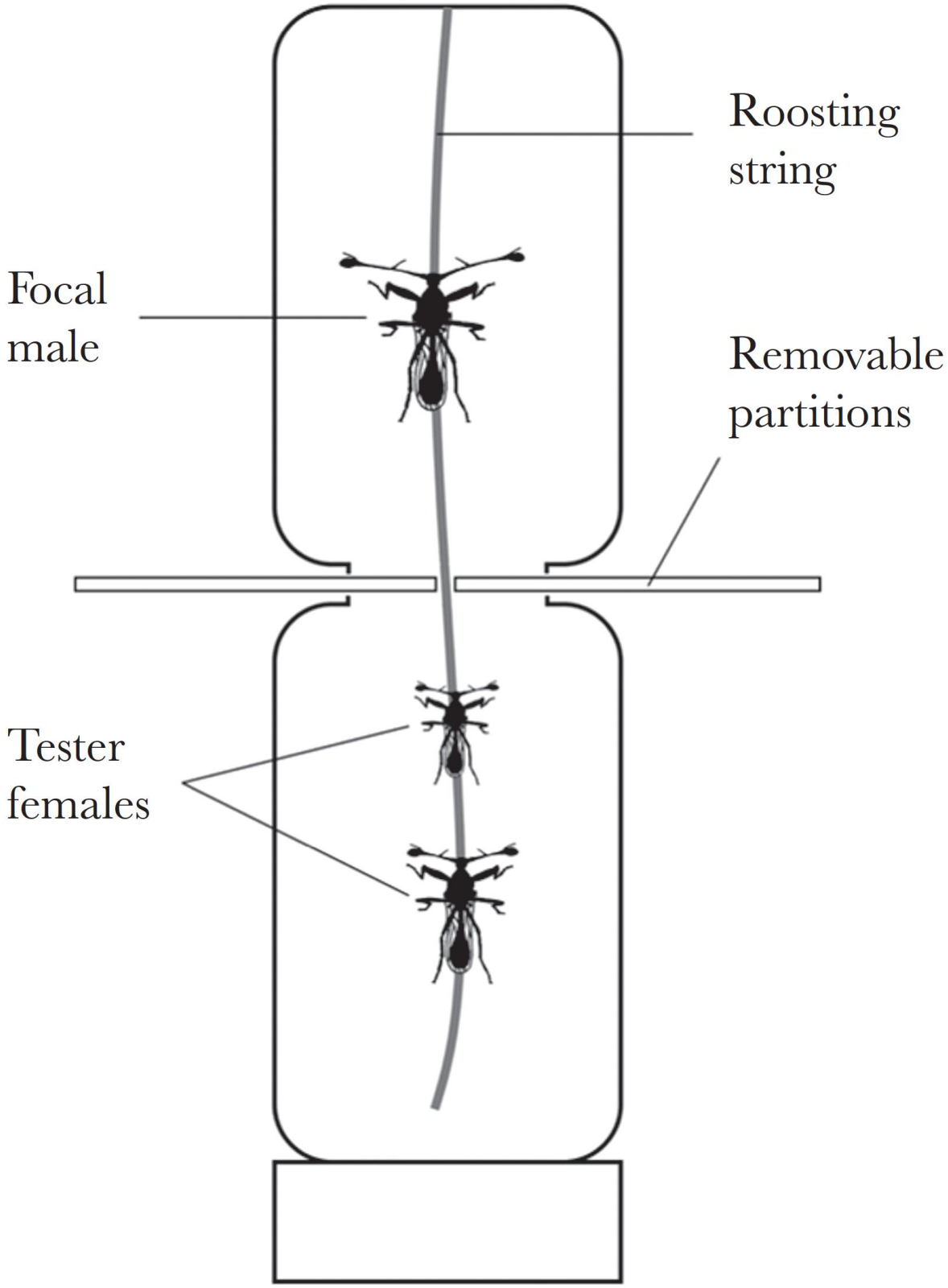
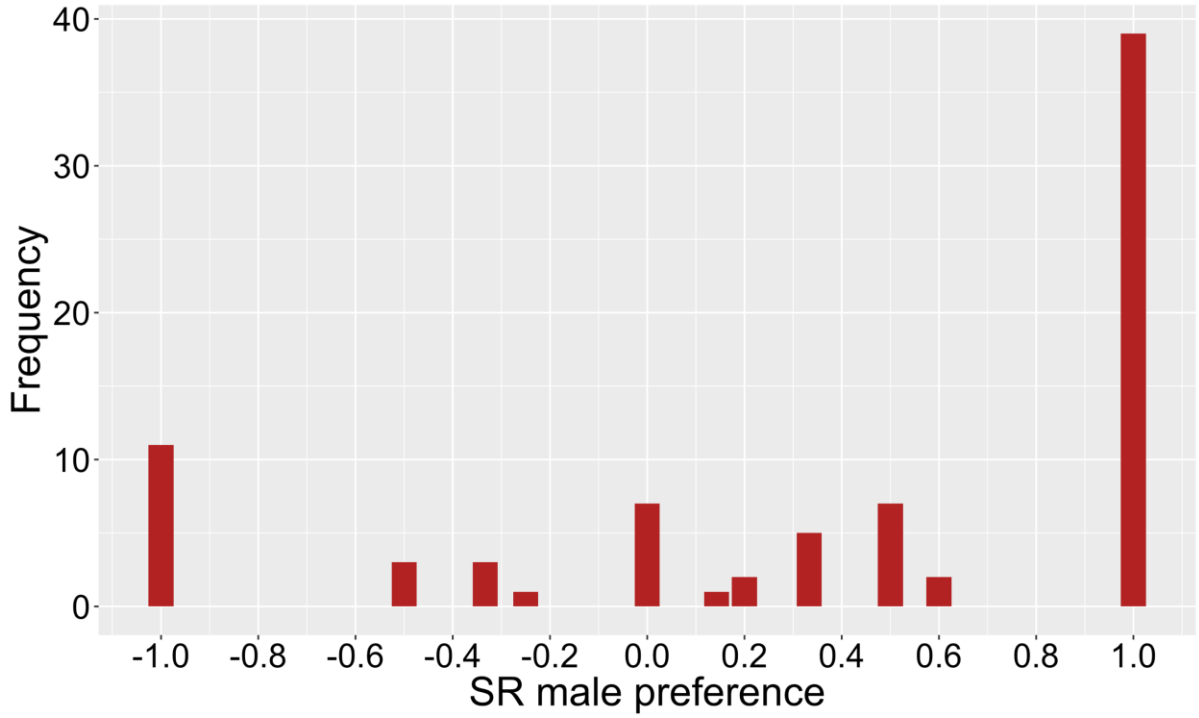
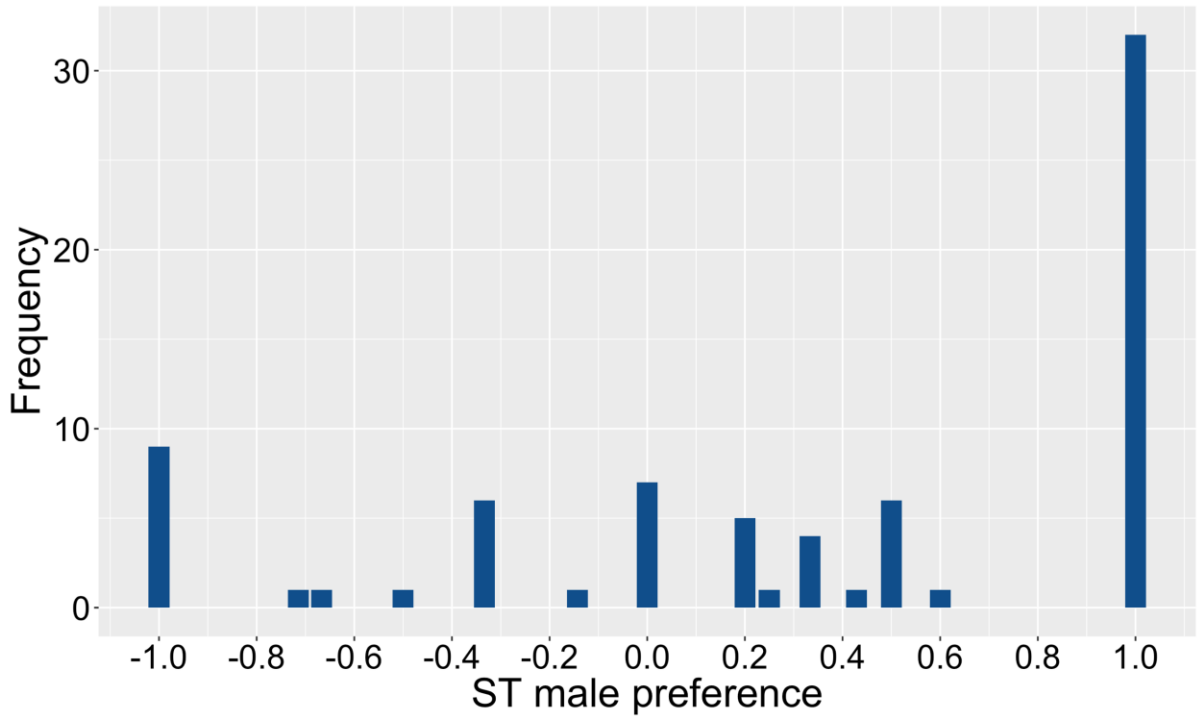

Roosting string
Focal male
Removable partitions
Tester females

Figure 2


40
30
20
10
0
Frequency
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
SR male preference


30
20
10
0
Frequency
-1.0
-0.8
-0.6
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-0.2
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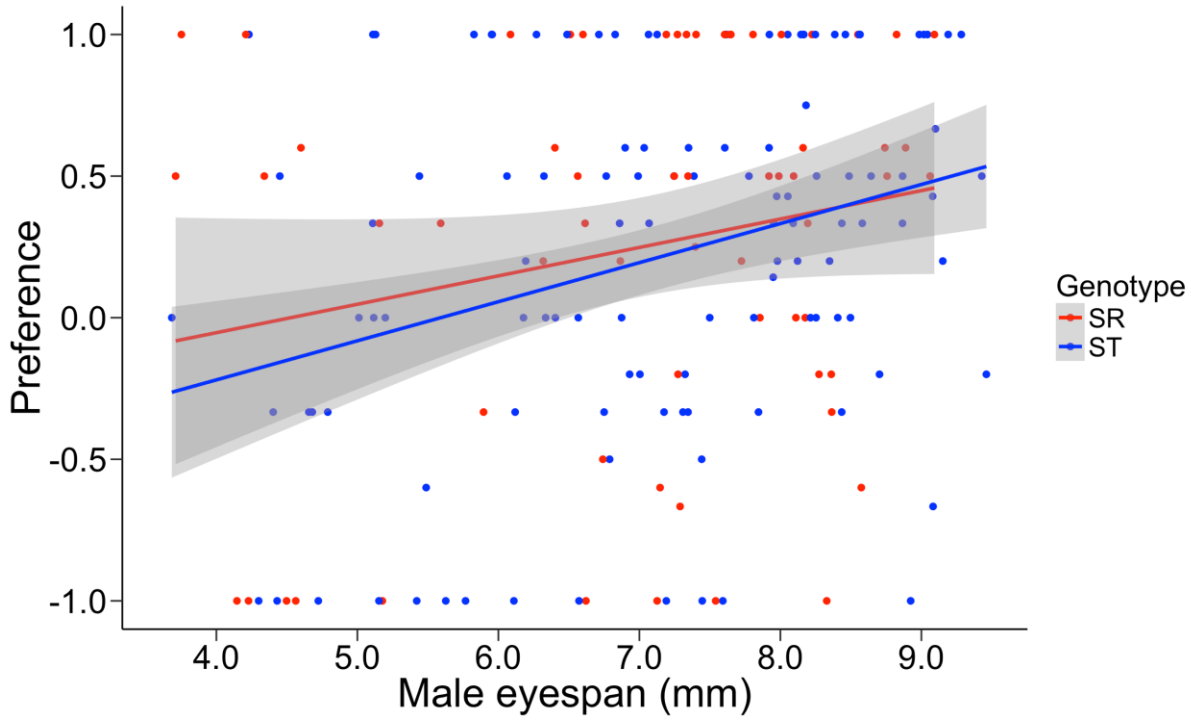
0.2
0.4
0.6
0.8
1.0
ST male preference

Figure 3


1.0
0.5
0.0
-0.5
-1.0
Preference
4.0
5.0
6.0
7.0
8.0
9.0
Male eyespan (mm)
Genotype
SR
ST