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

Sam Ronan Finnegan a, Nathan Joseph White a,b, Dixon Koh a, M. Florencia Camus a,
Kevin Fowler a and Andrew Pomiankowski a,c†

a Department of Genetics, Evolution and Environment, University College London, Gower
Street, London, WC1E 6BT, UK
b Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield,
S10 2NT UK
c CoMPLEX, University College London, Gower Street, London, WC1E 6BT, UK

† Corresponding author: Andrew Pomiankowski, ucbhpom@ucl.ac.uk
Tel: +44 (0) 20 76797697,

Andrew Pomiankowski Orcid: 0000-0002-5171-8755
Sam Finnegan Orcid: 0000-0001-6893-7068
Nathan White Orcid: 0000-0002-0898-760X
M Florencia Camus Orcid: 0000-0003-0626-6865
Kevin Fowler Orcid: 0000-0001-9737-7549

Keywords: drive, selfish genetic element, sex ratio, sexual selection, SR, stalk-eyed fly,
Teleopsis
Running Head: Meiotic drive viability

Electronic Supplementary Material is available online at...
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. 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 (~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.
**Introduction**

Meiotic drivers are selfish genetic elements that subvert the standard mechanisms of gametogenesis to promote their own transmission [1]. During meiosis, a driver disables or prevents the maturation of gametes that contain the non-driving element [1,2]. 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 [3], commonly known as 'Sex-Ratio' or SR [4]. 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 [5,6]. Empirical evidence for this is limited to laboratory environments where drive causes extinction in small populations [7-9] and a single putative example under natural conditions [10]. More typically, studies in wild populations find that drive exists as a low-frequency polymorphism [10-12], with persistence that can span over a million years [13,14]. 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. We 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 [15,16]. But it has been suggested that there will be negative frequency-dependent selection on male fertility [17]. 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 multiply (for instance because SR males are sperm limited), then a polymorphism could be stabilised [17].

Decreased male fertility under multiple mating is a general feature observed in many drive systems [17-19]. 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 [17], a condition not met in a number of species that nonetheless are found with stable SR polymorphism [16]. A related suggestion is that SR males may be out-competed at higher mating rates, motivated by some evidence that SR males are poor sperm competitors [20-22]. 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 [23]. 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 [24].

Suppressors are another selective force operating in males that limits 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 [25]. Y-linked suppressors are therefore expected to spread quickly even if they have deleterious side effects [26]. 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 [27, 28]. 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 [29]. 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 [30]. While suppressors are common, they are not universal and have
not been detected against SR drive in *D. pseudoobscura*, *D. recens* and *D. neotestacea* [29].

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 [31]. 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 [2]. 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 [32-35]. 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 [36,37]). The consequence is that low frequency inversions will be subject to weak selection and suffer the accumulation of a greater mutation load [34,38]. 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 [39]. This means that we expect that loss of fitness will be greater in females and likely to be recessive. These effects are likely to 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 [31,34,40]. But it is surprising how rarely viability effects of drive in either sex have been studied, compared to fertility effects in males [41]. These deleterious consequences are likely to build up and lead to a reduction in SR frequency through time [34].

Large-scale chromosomal inversions are not a universal feature of SR, however. Inversions are not present in the Paris SR system in D. simulans [29]. 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 [42]. The deleterious effects of the Paris SR chromosome must arise due to deleterious effects caused by 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 [43]. It persists despite having been completely suppressed for thousands of years, suggesting it does not cause any pleiotropic fitness deficit [43]. 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 [22]. This benefit arises when drive male sperm are weak competitors against wildtype male sperm [41]. Recent modelling work shows that polyandry helps prevent invasion of SR, but cannot prevent fixation of drive alone [44]. 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 [44], 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 [10], and a similar pattern has been reported in
*D. pseudoobscura* [11]. Alternatively, females may simply avoid mating with SR males
[45,46]. In stalk-eyed flies, females prefer to mate with males with large eyespan [47,48], a
trait that is reduced in SR males [47,49,50]. 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 [46]. Another potential example is the autosomal t-
locus system in mice which is proposed to be detectable in mate choice through olfaction
[51] but this preference has not been confirmed [52]. 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 [53].

In this study, we determine the effect of SR meiotic drive on viability in the Malaysian stalk-
eyed fly, *Teleopsis dalmanni*. Our 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 ~20% but with considerable
variation among populations [14,54,55]. SR resides within a large paracentric inversion (or
inversions) that covers most of the X chromosome [49]. There is no recombination between
SR and ST haplotypes [14] 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 ~1181 Mya. X-linked meiotic drive is also present in the more distantly related species *T. whitei*, which diverged on order 2-3.5 Mya [14,56]. But to what extent the mechanism or genetic basis is conserved remains to be established.

The ancient origin of the X<sub>SR</sub> chromosome and limited recombination across the X<sub>SR</sub> 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<sub>SR</sub> and X<sub>ST</sub> [57]. The main evidence for a deleterious effect of X<sub>SR</sub> on fitness is the reduced eyespan of SR males [47,50]. Male eyespan is an exaggerated, highly condition-dependent trait used in female mate choice [47,58], as well as signalling between males [59,60], which reflects male genetic and phenotypic quality [58,61,62]. However, in a series of experiments Wilkinson et al. [63] found little direct evidence that the SR reduces fitness components. Although larval viability was not directly assessed, progeny production showed no difference between SR and ST homozygous females [63]. Another study compared offspring genotypes of heterozygous females mated to ST males, and reported little deviation from expected assuming no viability selection differences [49]. Adult survival did not vary with genotype in either males or females [63]. There was no evidence for a deleterious effect of X<sub>SR</sub> on female fecundity, rather heterozygotes were more productive, suggesting overdominance [63]. 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 [63]. 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 [64].

To better understand these previous results, we were motivated to explicitly test for differences in larval survival. Our experimental design was similar to that used in early investigations of *D. pseudoobscura* [31,40]. 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 our 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. Our 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.

**Methods**

*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 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 [50]. Meiotic drive is maintained in this stock by following a standard protocol [54,65]. 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 [65]. Progeny from drive males are female heterozygotes for the drive chromosome. They are subsequently mated to standard males, and the process is repeated.

**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; [66]) 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; [67]). 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.

**Genotyping**

DNA was extracted in 96-well plates using a modification of a standard isopropanol precipitation protocol ([68]; see electronic supplementary material, S1 Methods for full protocol). DNA was PCR-amplified in 96-well plates, using forward and reverse primers for *comp162710* 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, personal communication; [65]).

Statistical analysis

We 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 electronic supplementary material, Table S1-S7.

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, we 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 we expected 18 $X^{SR}/X^{ST}$ females and 9 each of the remaining two female homozygotes. We 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. We then split the data by sex and analysed the relationship between egg-to-
adult viability and genotype using linear mixed-effect modelling with lme4 [69] in R [70].

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 [71]. Mean viability measures were estimated using model terms.

*Estimating the strength of selection against drive*

In the second analysis, we 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. We 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 we 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 \( s_f \)
values from the randomly drawn $s_m$ values. A z-score was calculated to determine if this distribution is different from zero.

We 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 $s_f$ to a single term with a uniform prior. The process was repeated to compare drive heterozygotes ($X^{SR}/X^{ST}$) and homozygotes ($X^{SR}/X^{SR}$).

**Results**

**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.

**Egg-to-adult viability of each genotype**

From a total of 96 cages, each containing 72 eggs, we 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).

**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$_{10} = 321.79$.

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
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 [5,6]. Despite this, several meiotic drive systems exist in broadly stable polymorphisms [10,11,55]. 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 [49], which is expected to accumulate deleterious mutations as they are less efficiently removed by recombination than those of 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 ($h \sim 0.5$), 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 [72,73].

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 (electronic 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 $s_m$, $s_f$ and $h$ remain large (Figure 3-4) but we can be confident that drive is associated with loss of viability in both sexes. Our results contrast with a prior study showing that adult lifespan is independent of SR genotype in males and females [63], revealing a difference between larval and adult genotypic effects. This previous study also suggested that larval survival is independent of SR genotype [63]. 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. [63] 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 [31,32,40]. Wallace [40] observed strong
Selection against $X^{SR}$ in both sexes. In high density populations, Beckenbach [32] found a reduction in $X^{SR}$/Y viability but no viability effect on homozygous $X^{SR}$ female viability. In contrast, Curtsinger and Feldman [31] report stronger selection against homozygous $X^{SR}$ females. Comparisons of these three studies provides strong evidence to suggest that viability selection is density-dependent, as reduction in $X^{SR}$ viability was greatest under high density [40], and a lack of differential viability was observed in another experiment carried out at low density [32]. In the present study, stalk-eyed fly larvae were cultured under low density and provided with an excess of 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 we discuss further here. First, in D. simulans, SR commonly co-occurs with suppressors which restrict the transmission advantage [43,74]. Although early work on the stalk-eyed fly drive system suggested that there were suppressors [47], this has not been sustained by further work, either on the autosomes or Y chromosomes [14]. Second, polyandry may evolve to limit the spread of SR [22]. Polyandry is the norm in T. dalmanni [55,66], and there is evidence that SR male sperm does less well under sperm competition [63] 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, [21]). 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 [51]. This may be important in stalk-eyed flies as they are canonical examples of sexual selection driven by mate choice [75,76]. In T. dalmanni, drive males are expected to attract fewer females as they have reduced eyespan, and hence mate less often [47,50]. 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 [77] which has been shown to be an important behavioural adaptation in T. dalmanni favouring male matings with fecund females [78]. A recent study reported that SR had no direct effect on male mate choice [79]. However, the strength of male mate preference positively covaries with male eyespan. As drive males have smaller eyespan [50], we expect they will be less discriminating in their mate choice [79].

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 [65]. 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 [64], whereas another experiment found lower fertility in SR males [63] 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 [64], and this could conceivably have contributed to lower fertility in females mated to SR males. As mentioned previously, P2 experiments indicate that SR males are poor sperm competitors with ST males which must arise from reasons other than numerical sperm transfer from the male [63].

The number of different factors set out above make it difficult to predict whether they are sufficient to explain the observed frequency of ~20% [14,55]. 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 [50].
Acknowledgements. SRF is supported by a London NERC DTP PhD Studentship (NE/L002485/1). AP is supported by Engineering and Physical Sciences Research Council grants (EP/F500351/1, EP/I017909/1), KF and AP by NERC grants (NE/G00563X/1, NE/R010579/1). The authors thank Rebecca Finlay for her help in maintaining the laboratory stocks, Dr Lara Meade for advice on experimental design and Prof Mark Thomas for help with the statistical analysis.


Author contributions. The research project was conceived by SRF, NJW, KF and AP. The experiment was carried out by SRF and NJW, with genotyping by SRF, DK and MFC. The data was analysed by SRF, NJW and AP, and the paper written by SRF and AP.
References


70. R Core Team. 2018. R: A language and environment for statistical computing. R
Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-
project.org/.

71. Kuznetsova A, Brockhoff PB and Christensen RHB. 2017. ImerTest Package: Tests in

USA. 98, 1671-1675.

between population fitness and sexual dimorphism in seed beetles. Proc. R. Soc. B
277, 1345-1352.

Drosophila simulans: co-occurrence of a meiotic drive and a suppressor of drive. J
Evol Biol 8, 283-300.

Dimorphism in Stalk-eyed Flies (Diopsidae). Naturwissenschaften 72, 204-206.

76. Burkhardt D, de la Motte I. 1988. Big ‘antlers’ are favoured: female choice in stalk-
eyed flies (Diptera, Insecta), field collected harems and laboratory experiments. J.


female eyespan and fecundity in the stalk-eyed fly Teleopsis dalmanni. Behav. Ecol.
26, 376-385.
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<sup>SR</sup>/X<sup>SR</sup> and X<sup>SR</sup>/X<sup>ST</sup> females, in equal proportions. Cross B produces X<sup>SR</sup>/Y and X<sup>ST</sup>/Y males and X<sup>ST</sup>/X<sup>ST</sup> and X<sup>SR</sup>/X<sup>ST</sup> 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 egglays 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<sub>m</sub>). 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<sub>f</sub>) 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.
Figures

Figure 1:
Figure 2:

Egg-to-adult viability

<table>
<thead>
<tr>
<th>Male Genotypes</th>
<th>Female Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X^{SR}/Y$</td>
<td>$X^{ST}/Y$</td>
</tr>
<tr>
<td>$X^{SR}/X^{SR}$</td>
<td>$X^{SR}/X^{ST}$</td>
</tr>
<tr>
<td>$X^{ST}/X^{ST}$</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3:
Figure 4:
Electronic supplementary material

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

**Authors:** Sam Ronan Finnegan, Nathan Joseph White, Dixon Koh, M. Florencia Camus, Kevin Fowler and Andrew Pomiankowski

**Journal:** Proceedings of the Royal Society B

DOI: 10.1098/rspb.2019.1414
Supplementary Methods - DNA Extraction and Genotyping Protocol

DNA was extracted by isopropanol precipitation in 96-well plates. Half a fly thorax was added to a well containing 4ul Proteinase K (10 mg.ml-1) and 100ul DIGSOL (25mM NaCl, 1mM EDTA, 10mM Tris–Cl pH 8.2), mechanically lysed, and incubated overnight at 55C. The following day, 35ul of 4M ammonium acetate was added and plates were left on ice for 5 minutes before being centrifuged at 4500RPM at 4C for 40 minutes. 80ul of supernatant was then aspirated into a new 96-well plate containing 80ul of isopropanol. The precipitate was discarded. Samples were then centrifuged again at 4500RPM and 4C for 40 minutes to precipitate the DNA. The supernatant was then discarded, 100ul 70% ethanol was added, and samples were spun again at 4500RPM and 4C for 20 minutes. The supernatant was once again discarded and plates were left to air-dry for 45 minutes at room temperature. Finally, 30ul 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 1ul of dried DNA, 1ul of primer mix (consisting of the forward and reverse primers of comp162710 at a concentration of 0.2uM) and 1ul 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 4ul ethidium bromide. PCR products were diluted with 3ul ultrapure water and 2ul of gel loading dye was added. 4ul 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, personal communication; Meade et al. 2019).
Model outputs

Supplementary table S1

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

\[
\texttt{m1 <- lmer(data=Male_Survival, formula = W ~ Genotype*Condition + (1|Cage_ID) + (1|Collection_Date))}
\]

|                  | 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 | 2.7344174 | 0.0077113 |
| ConditionL       | 0.0769641| 0.0720155  | 147.04295| 1.0687149 | 0.2869495 |
| ConditionM       | 0.0308253| 0.0730934  | 148.96913| 0.4217254 | 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 |

|                  | Sum Sq   | Mean Sq   | NumDF | DenDF | F value | Pr(>|F|) |
|------------------|----------|-----------|-------|-------|---------|----------|
| Genotype         | 0.7431435| 0.7431435 | 1     | 79.00000 | 11.1821885 | 0.0012649 |
| Condition        | 0.0142479| 0.0072124 | 2     | 72.97766 | 0.1085266 | 0.8972995 |
| Genotype:Condition | 0.1066840| 0.0533420 | 2     | 79.00000 | 0.8026450 | 0.4517624 |

Supplementary table S2

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

\[
\texttt{m1 <- lmer(data=Female_Survival, formula = W ~ Genotype*Condition + (1|Cage_ID) + (1|Collection_Date))}
\]

|                  | Estimate | Std. Error | df       | t value | Pr(>|t|) |
|------------------|----------|------------|----------|---------|----------|
| (Intercept)      | 0.4577565| 0.0710439  | 127.3557 | 6.4432926 | 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 |

|                  | Sum Sq   | Mean Sq   | NumDF | DenDF | F value | Pr(>|F|) |
|------------------|----------|-----------|-------|-------|---------|----------|
| Genotype         | 0.8327350| 0.4163675 | 2     | 116.0000 | 4.6824068 | 0.0110758 |
| Condition        | 0.0275940| 0.0137970 | 2     | 53.53907 | 0.1551592 | 0.8566625 |
| Genotype:Condition | 0.0727005| 0.0181751 | 4     | 116.0000 | 0.2043948 | 0.9355153 |

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
m1 <- 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 | 10.685053 | 0.0000000 |
| GenotypeXY 0.1375502 | 0.0401625 | 81.00000 | 3.424845 | 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>

Supplementary table S4

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

```r
m1 <- 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 | 10.975046 | 0.0000000 |
| GenotypeSRX 0.0841424 | 0.0532743 | 120.00000 | 1.579420 | 0.1168722 |
| GenotypeXX 0.1643466 | 0.0532743 | 120.00000 | 3.084916 | 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>

Supplementary table S5

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

```r
m1 <- 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<sup>SR</sup>/Y genotype is used as the comparison, so its egg-to-adult viability is the model intercept term, 0.40633. The viability of X<sup>ST</sup>/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.

Supplementary table S6

The viability of each female genotype was estimated in the same way as above:
m1 <- 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 |

**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>
Fertility trial - Supplementary table S8

Below are the results of a trial designed to test the fertility of eggs laid by X<sup>SR</sup>/X<sup>ST</sup> females crossed to X<sup>SR</sup>/Y (Cross A) and X<sup>ST</sup>/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 of 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>0.75000000</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>
<td>61</td>
<td>0.9104478</td>
</tr>
<tr>
<td>15-Nov</td>
<td>B</td>
<td>B3</td>
<td>40</td>
<td>4</td>
<td>36</td>
<td>0.9000000</td>
</tr>
<tr>
<td>21-Nov</td>
<td>A</td>
<td>A1</td>
<td>43</td>
<td>4</td>
<td>39</td>
<td>0.9069767</td>
</tr>
<tr>
<td>21-Nov</td>
<td>A</td>
<td>A2</td>
<td>89</td>
<td>4</td>
<td>85</td>
<td>0.9550562</td>
</tr>
<tr>
<td>21-Nov</td>
<td>A</td>
<td>A3</td>
<td>76</td>
<td>3</td>
<td>73</td>
<td>0.9605263</td>
</tr>
<tr>
<td>21-Nov</td>
<td>B</td>
<td>B1</td>
<td>85</td>
<td>8</td>
<td>77</td>
<td>0.9058824</td>
</tr>
<tr>
<td>21-Nov</td>
<td>B</td>
<td>B2</td>
<td>105</td>
<td>8</td>
<td>97</td>
<td>0.9238095</td>
</tr>
<tr>
<td>21-Nov</td>
<td>B</td>
<td>B3</td>
<td>34</td>
<td>3</td>
<td>31</td>
<td>0.9117647</td>
</tr>
<tr>
<td>23-Nov</td>
<td>A</td>
<td>A1</td>
<td>90</td>
<td>0</td>
<td>90</td>
<td>1.0000000</td>
</tr>
<tr>
<td>23-Nov</td>
<td>A</td>
<td>A2</td>
<td>69</td>
<td>3</td>
<td>66</td>
<td>0.9565217</td>
</tr>
<tr>
<td>23-Nov</td>
<td>A</td>
<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>
Data accessibility

Raw and processed data are available on the Dryad Digital Repository: doi:10.5061/dryad.kc49jk1