

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

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3 Sam Ronan Finnegan^a, Nathan Joseph White^{a,b}, Dixon Koh^a, M. Florencia Camus^a,

4 Kevin Fowler^a and Andrew Pomiankowski^{a,ct}

5

6 ^a Department of Genetics, Evolution and Environment, University College London, Gower

7 Street, London, WC1E 6BT, UK

8 ^b Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield,

9 S10 2NT UK

10 ^c CoMPLEX, University College London, Gower Street, London, WC1E 6BT, UK

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12 [†] **Corresponding author:** Andrew Pomiankowski, ucbhpom@ucl.ac.uk

13 **Tel:** +44 (0) 20 76797697,

14 **Andrew Pomiankowski Orcid:** 0000-0002-5171-8755

15 **Sam Finnegan Orcid:** 0000-0001-6893-7068

16 **Nathan White Orcid:** 0000-0002-0898-760X

17 **M Florencia Camus Orcid:** 0000-0003-0626-6865

18 **Kevin Fowler Orcid:** 0000-0001-9737-7549

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21 Teleopsis

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24 **Abstract**

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

40 **Introduction**

41 Meiotic drivers are selfish genetic elements that subvert the standard mechanisms of
42 gametogenesis to promote their own transmission [1]. During meiosis, a driver disables or
43 prevents the maturation of gametes that contain the non-driving element [1,2]. In extreme
44 cases, drive can reach 100% transmission to the next generation. In male heterogametic
45 species, drivers are most frequently found on the X-chromosome [3], commonly known as
46 '*Sex-Ratio*' or SR [4]. These drivers target developing sperm carrying the Y chromosome,
47 causing their dysfunction, which results in strongly female biased broods.

48

49 SR is predicted to spread rapidly due to its transmission advantage. When homozygous
50 female fitness is not greatly reduced, SR could potentially spread to fixation and cause
51 population collapse and extinction through massive sex ratio imbalance [5,6]. Empirical
52 evidence for this is limited to laboratory environments where drive causes extinction in
53 small populations [7-9] and a single putative example under natural conditions [10]. More
54 typically, studies in wild populations find that drive exists as a low-frequency polymorphism
55 [10-12], with persistence that can span over a million years [13,14]. In order for SR to persist
56 as a polymorphism, there must be frequency-dependent selection, allowing spread when
57 rare but retarding further increases in frequency as drive becomes more common. The
58 selective counter forces that fulfil this requirement may act in males or females but in
59 general they are not well understood. We discuss potential causes of selection first in males
60 and then females in the following sections.

61

62 Selection on male viability may be associated with the drive chromosome. It is likely to
63 operate in a frequency-independent manner and as a consequence will not have a
64 stabilizing effect on the frequency of drive [15,16]. But it has been suggested that there will
65 be negative frequency-dependent selection on male fertility [17]. This has intuitive appeal
66 because the spread of SR causes the population sex ratio to become increasingly female
67 biased. In such a population, the average male mating rate will increase. If SR male fertility
68 increases at a lower rate than non-drive (ST) male fertility when males mate multiply (for
69 instance because SR males are sperm limited), then a polymorphism could be stabilised [17].
70 Decreased male fertility under multiple mating is a general feature observed in many drive
71 systems [17-19]. However, for this effect alone to prevent SR fixation, SR male fertility must
72 fall to less than half that of ST males as the mating rate increases [17], a condition not met
73 in a number of species that nonetheless are found with stable SR polymorphism [16]. A
74 related suggestion is that SR males may be out-competed at higher mating rates, motivated
75 by some evidence that SR males are poor sperm competitors [20-22]. However, the strength
76 of sperm competition weakens as SR spreads, as this reduces the number of competitor
77 males in the population, which seems unlikely to exert a stabilizing effect on SR frequency.
78 SR males may do poorly in other forms of male-male competition if SR is generally
79 associated with poor performance. Such effects are likely to decrease as drive spreads and
80 males become rare, again making it unlikely that this form of selection will stabilize drive.
81 Models that combine the effects of decreased male fertility and reduced sperm competitive
82 ability on SR frequency dynamics find they can lead to a stable polymorphism [23]. But this
83 equilibrium can be destabilised by perturbations in either the population sex ratio or the
84 frequency of SR. In particular, given a meta-population of small demes, slight fluctuations in

85 SR frequency are likely to cause drive to spread to fixation, resulting in population extinction
86 [24].
87
88 Suppressors are another selective force operating in males that limits the spread of drive
89 alleles. Most obviously, selection favours the evolution of suppression on chromosomes
90 targeted by drivers for dysfunction. In an SR system with complete drive, if resistance is
91 linked to the Y-chromosome, it restores transmission to Mendelian levels, while non-
92 resistant Y-chromosomes are not transmitted at all [25]. Y-linked suppressors are therefore
93 expected to spread quickly even if they have deleterious side effects [26]. Unlinked
94 suppressors will also be favoured because drive in males causes gamete loss and is often
95 associated with dysfunction amongst the surviving, drive-carrying sperm. Reduced sperm
96 number is likely to reduce organismal fertility. Additionally, as SR spreads it causes the
97 population sex ratio to become female-biased, providing a further advantage to suppressors
98 as they increase the production of male offspring, which have higher reproductive value
99 than female offspring [27, 28]. The spread of suppressors reduces the advantage of drive
100 and could lead to its loss. But both types of suppressors are under negative frequency-
101 dependent selection, because a lower frequency of drive reduces selection in their favour.
102 Under some circumstances this could lead to a stable polymorphism at the drive locus. Y-
103 linked and autosomal suppressors of SR drive have been detected in a number of species
104 including *Drosophila simulans*, *D. affinis*, *D. subobscura*, *D. quinara*, *D. mediopunctata* and
105 *Aedes aegypti* [29]. The evolution of suppressors can be remarkably rapid. For example, in
106 the Paris SR system of *D. simulans*, the increase of SR from less than 10% to more than 60%
107 in a mere five years has been matched by a similar increase in suppressor frequency over
108 the same time period [30]. While suppressors are common, they are not universal and have

109 not been detected against SR drive in *D. pseudoobscura*, *D. recens* and *D. neotestacea* [29].
110 In these systems, other factors are therefore necessary to explain extant SR polymorphism.
111
112 Another force that may prevent SR fixation is reduced fitness of female carriers [31]. As
113 male X-linked drive causes defects in spermatogenesis, there is no obvious mechanistic
114 carry-over to female oogenesis. Likewise, examples of meiotic drive in female
115 gametogenesis, which affect the biased segregation of chromosomes into the egg or polar
116 bodies, show no carry-over to segregation bias in male gamete production [2]. For selection
117 to act against female carriers, the drive locus must either have direct pleiotropic fitness
118 effects or be in linkage with alleles that impact fitness. Linkage is a plausible explanatory
119 factor given that drive systems are often located in genomic regions with low recombination
120 rates, such as in inversions [32-35]. If the inversion is at low frequency, it will rarely be
121 homozygous and the recombination rate among SR chromosomes will be low. Inversions
122 also severely limit the exchange of genes with the homologous region on the standard
123 chromosome (as this requires a double cross-over within the inverted region [36,37]). The
124 consequence is that low frequency inversions will be subject to weak selection and suffer
125 the accumulation of a greater mutation load [34,38]. Recessive viability and sterility effects
126 are expected as they will not be evident in females until the frequency of drive is high
127 enough for homozygotes to be common. In contrast, hemizyosity in males means recessive
128 and dominant effects are always expressed and will be more strongly selected against. In
129 general, SR inversions are expected to be enriched for sexually antagonistic alleles that
130 benefit the sex in which drive occurs [39]. This means that we expect that loss of fitness will
131 be greater in females and likely to be recessive. These effects are likely to produce relevant
132 frequency dependence that restricts fixation of drive. Severe reductions in female viability

133 and fertility in SR homozygotes, along with SR heterozygotes, have been reported in several
134 *Drosophila* species [31,34,40]. But it is surprising how rarely viability effects of drive in
135 either sex have been studied, compared to fertility effects in males [41]. These deleterious
136 consequences are likely to build up and lead to a reduction in SR frequency through time
137 [34].

138

139 Large-scale chromosomal inversions are not a universal feature of SR, however. Inversions
140 are not present in the Paris SR system in *D. simulans* [29]. Despite this, SR must be weakly
141 deleterious in this species as it is rapidly declining in frequency in populations that have
142 recently become completely suppressed [42]. The deleterious effects of the Paris SR
143 chromosome must arise due to deleterious effects caused by the drive genes themselves or
144 a tightly linked region. The genetically distinct Winters SR system in the same species also
145 lacks association with an inversion [43]. It persists despite having been completely
146 suppressed for thousands of years, suggesting it does not cause any pleiotropic fitness
147 deficit [43]. These are the only well characterised examples of meiotic drive not being
148 associated with inversions, so this feature may be a rarity.

149

150 Another aspect operating in females concerns behavioural resistance to the spread of SR.
151 Laboratory experiments suggest that increased levels of polyandry can be selected as a
152 defence mechanism against SR [22]. This benefit arises when drive male sperm are weak
153 competitors against wildtype male sperm [41]. Recent modelling work shows that polyandry
154 helps prevent invasion of SR, but cannot prevent fixation of drive alone [44]. As drive
155 spreads, additional matings have a lower probability of involving wildtype males, so the
156 disadvantage to drive sperm declines. There needs to be positive frequency-dependent

157 costs to achieve a stable polymorphism [44], for instance, when homozygous females have
158 lower viability than heterozygotes. If a stable polymorphism can evolve, the frequency of
159 drive should decline with the rate of female remating. There is evidence in favour of this
160 idea in *D. neotestacea* which exhibits a stable cline in SR frequency that correlates
161 negatively with the frequency of polyandry [10], and a similar pattern has been reported in
162 *D. pseudoobscura* [11]. Alternatively, females may simply avoid mating with SR males
163 [45,46]. In stalk-eyed flies, females prefer to mate with males with large eyespan [47,48], a
164 trait that is reduced in SR males [47,49,50]. Sexual selection may therefore be acting in this
165 species to limit the spread of SR. However, this form of selection against drive is likely to be
166 restricted to a sub-set of species with drive, as it requires the linkage of SR with a
167 conspicuous trait subject to mate choice [46]. Another potential example is the autosomal *t*-
168 locus system in mice which is proposed to be detectable in mate choice through olfaction
169 [51] but this preference has not been confirmed [52]. A counter example is in *D.*
170 *pseudoobscura*, where females do not avoid mating with SR males, though there would be
171 considerable benefit to doing so [53].

172

173 In this study, we determine the effect of SR meiotic drive on viability in the Malaysian stalk-
174 eyed fly, *Teleopsis dalmanni*. Our objective was to assess whether there is a SR-linked
175 deleterious mutation load leading to higher developmental mortality before adult eclosion.
176 Populations of this species carry SR at a moderate level of ~20% but with considerable
177 variation among populations [14,54,55]. SR resides within a large paracentric inversion (or
178 inversions) that covers most of the X chromosome [49]. There is no recombination between
179 SR and ST haplotypes [14] and the lower frequency of SR in the wild means SR homozygous
180 recombination events are relatively rare (at 20%, the recombination rate of SR is a quarter

181 that of ST). SR is absent from a cryptic species of *T. dalmanni* estimated to have diverged ~1
182 Mya. X-linked meiotic drive is also present in the more distantly related species *T. whitei*,
183 which diverged on order 2-3.5 Mya [14,56]. But to what extent the mechanism or genetic
184 basis is conserved remains to be established.

185

186 The ancient origin of the X^{SR} chromosome and limited recombination across the X^{SR}
187 chromosome are predicted to have led to the accumulation of deleterious alleles.

188 Consistent with a lack of recombination, there are 955 fixed sequence differences between
189 transcripts linked to X^{SR} and X^{ST} [57]. The main evidence for a deleterious effect of X^{SR} on
190 fitness is the reduced eyespan of SR males [47,50]. Male eyespan is an exaggerated, highly
191 condition-dependent trait used in female mate choice [47,58], as well as signalling between
192 males [59,60], which reflects male genetic and phenotypic quality [58,61,62]. However, in a
193 series of experiments Wilkinson et al. [63] found little direct evidence that the SR reduces
194 fitness components. Although larval viability was not directly assessed, progeny production
195 showed no difference between SR and ST homozygous females [63]. Another study
196 compared offspring genotypes of heterozygous females mated to ST males, and reported
197 little deviation from expected assuming no viability selection differences [49]. Adult survival
198 did not vary with genotype in either males or females [63]. There was no evidence for a
199 deleterious effect of X^{SR} on female fecundity, rather heterozygotes were more productive,
200 suggesting overdominance [63]. However, sample size in these experiments was small, and
201 fecundity/fertility results were based on progeny counts which are confounded by genotype
202 effects on larval survival. The only significant detriment reported was in SR male fertility
203 which was reduced when males were allowed to mate with large numbers of females (eight)
204 for 24 hours [63]. However, a further experiment that measured male fertility through

205 counts of fertile eggs (avoiding any confounding impact of larval survival), failed to show any
206 difference between SR and ST male fertility [64].

207

208 To better understand these previous results, we were motivated to explicitly test for
209 differences in larval survival. Our experimental design was similar to that used in early
210 investigations of *D. pseudoobscura* [31,40]. Controlled crosses were carried out to produce
211 eggs with all possible SR and ST male and female genotypes. These were reared together to
212 ensure exposure to similar environmental variation. The sample size was large to maximize
213 our power to detect genotypic survival differences. Offspring were genotyped at adult
214 eclosion, yielding observed genotype ratios in order to estimate the selection coefficients
215 operating against drive in both sexes. Our principal aims were to test whether the SR-drive
216 chromosome causes viability loss during egg-to-adult development, and whether fitness
217 effects are recessive or sex-limited.

218

219 **Methods**

220 *Fly stocks and maintenance*

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

227

228 A meiotic drive stock was created using flies collected from the same location in 2012 [50].
229 Meiotic drive is maintained in this stock by following a standard protocol [54,65]. Females
230 heterozygous for the drive chromosome are mated to males from the standard stock. It is
231 expected that half their male offspring will inherit the drive chromosome. All male offspring
232 are crossed to three females from the standard stock and the sex ratio of their progeny
233 scored. Males that sire all-female broods of at least 15 individuals are considered to be
234 carriers of meiotic drive. In the meiotic drive stock, drive strength is 100% percent, and no
235 males are produced by X^{SR}/Y males carrying the drive chromosome [65]. Progeny from drive
236 males are female heterozygotes for the drive chromosome. They are subsequently mated to
237 standard males, and the process is repeated.

238

239 Experimental crosses

240 To generate the five possible genotypes of both females (X^{ST}/X^{ST} , X^{SR}/X^{ST} , X^{SR}/X^{SR}) and males
241 (X^{ST}/Y , X^{SR}/Y), two crosses were performed (Figure 1). In Cross A, drive males (X^{SR}/Y) were
242 mated to heterozygous females (X^{SR}/X^{ST}). This cross produces X^{SR}/X^{SR} and X^{SR}/X^{ST} female
243 zygotes in equal proportions. In Cross B, standard males (X^{ST}/Y) were mated to heterozygous
244 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 female
245 zygotes in equal proportions. Experimental males were collected from the drive stock that
246 were approximately 50:50 X^{ST}/Y and X^{SR}/Y males. They were crossed to standard stock
247 females (X^{ST}/X^{ST}) and one larva per male was genotyped to define the paternal genotype.
248 Experimental females heterozygous for drive (X^{SR}/X^{ST}) were collected from crosses between
249 drive males and females from the standard stock.

250

251 Individual males were placed with three virgin females in 500ml pots. Females that died
252 during the experiment were replaced, but males were not. 25 Cross A and 50 Cross B pots
253 were set-up. The base of each pot was lined with moistened cotton wool covered with blue
254 tissue paper to aid egg visualisation. The cotton bases were removed for egg collection and
255 replaced three times per week. Fertilised eggs were identified under light microscopy as
256 those that showed signs of development (e.g. segmental striations, development of
257 mouthparts; [66]) and transferred to a 90mm petri dish containing a large cotton pad
258 moistened with 15ml of water and 2.5ml of food. Three different food conditions were used
259 that varied in their corn content: 25% corn, 50% corn, and 75% corn. In each mixture the
260 remainder was made up with a sucrose solution (25% sucrose/water w/w). To ensure the
261 sucrose solution had a similar viscosity to puréed corn, an indigestible bulking agent was
262 added (methylcellulose, 3% w/w; [67]). 4 eggs from Cross A and 8 eggs from Cross B were
263 transferred to each petri dish. This gives the five possible genotypes (X^{ST}/X^{ST} , X^{SR}/X^{ST} , X^{SR}/X^{SR} ,
264 X^{ST}/Y , X^{SR}/Y) in an expected 1:2:1:1:1 ratio (Table 1). Prior to the end of development, six
265 Petri dishes were placed inside a large cage and all eclosing adult flies were collected. The
266 cage was used as a level of analysis of the relative egg-to-adult viability of different
267 genotypes in the subsequent analyses.

268

269 Genotyping

270

271 DNA was extracted in 96-well plates using a modification of a standard isopropanol
272 precipitation protocol ([68]; see electronic supplementary material, S1 Methods for full
273 protocol). DNA was PCR-amplified in 96-well plates, using forward and reverse primers for
274 *comp162710* an indel marker with small alleles (201bp) indicating the presence of the drive

275 chromosome and large alleles (286bp) indicating the presence of the standard chromosome
276 (GS Wilkinson, personal communication; [65]).

277

278 Statistical analysis

279

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

285

286 Egg-to-adult viability of each genotype

287 In the first analysis, the number of eclosed adult flies of each genotype was compared to the
288 number expected at the level of the cage. Each cage contained six petri dishes with 12 eggs,
289 producing a maximum of 72 flies. Genotyping effort varied across cages and sexes. The
290 expected number of each genotype was determined with respect to the genotyping effort
291 of the relevant sex for a particular cage. For example, if 24 males were collected from a
292 given cage, and 75% of these males were genotyped, then the expected number of X^{SR}/Y
293 individuals is $(24 \times 0.75) / 2 = 9$. Due to the nature of the experimental design, we expected
294 twice as many X^{SR}/X^{ST} females compared with X^{SR}/X^{SR} and X^{ST}/X^{ST} females. For example, in a
295 cage with 36 genotyped females we expected 18 X^{SR}/X^{ST} females and 9 each of the
296 remaining two female homozygotes. We then divided the observed number of flies of a
297 given genotype by the expectation for that genotype to obtain the cage estimate of egg-to-
298 adult viability. We then split the data by sex and analysed the relationship between egg-to-

299 adult viability and genotype using linear mixed-effect modelling with lme4 [69] in R [70].
300 Genotype and food condition were modelled as fixed effects and cage ID and collection date
301 as random effects. Significance of model terms was determined using the lmerTest R
302 package [71]. Mean viability measures were estimated using model terms.

303

304 Estimating the strength of selection against drive

305 In the second analysis, we estimated the strength of selection against drive using Bayesian
306 inference, separately for males and females. Cage survival frequencies for each genotype
307 were pooled. The probability of drawing the male genotype distribution was calculated for
308 values of the selection coefficient taken from a uniform prior distribution for $s_m = 0 - 1$, in
309 0.001 increments. We then used a binomial model to determine the likelihood of drawing
310 the observed number of X^{ST}/Y and X^{SR}/Y males for each value of s_m . As we used a uniform
311 prior, the posterior probability simplifies to the likelihood. The 95% and 99% credible
312 intervals were determined from the probability density. The probability of observing the
313 distribution of the three female genotypes was estimated under a multinomial where the
314 values of s_f and h (Table 1) were taken from a uniform prior distribution for every
315 combination of values of s_f and h ranging from 0 - 1, in 0.001 intervals. The 95% and 99%
316 credible intervals were determined in the same way as in males, and displayed as a two-
317 dimensional contour. Note that the probability of drawing X^{SR}/X^{ST} females was multiplied by
318 two because the experimental design was expected to generate twice as many
319 heterozygote eggs compared to all of the other genotypes. To determine if s_m and s_f were of
320 different strength, 1000 random samples each of s_m and s_f (taking h equal to its mode) were
321 drawn from the posterior distributions with probability of drawing a value equal to its
322 likelihood. A distribution of differences was obtained by subtracting the randomly drawn s_f

323 values from the randomly drawn s_m values. A z-score was calculated to determine if this
324 distribution is different from zero.

325

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

331

332 **Results**

333 Effect of food condition

334 Food condition had no overall effect on the egg-to-adult viability of males ($F_{2,72} = 0.1085$, $P =$
335 0.8973) or females ($F_{2,54} = 0.1552$, $P = 0.8566$), nor did it alter the genotype response
336 (genotype-by-condition interaction, males $F_{2,79} = 0.8026$, $P = 0.4518$; females $F_{4,116} = 0.2044$,
337 $P = 0.9355$). So, offspring counts were pooled across food conditions within sexes in the
338 following analyses.

339

340 Egg-to-adult viability of each genotype

341 From a total of 96 cages, each containing 72 eggs, we collected a total of 1065 males and
342 2500 females, of which 798 and 1272 were genotyped respectively. Male genotype had a
343 significant effect on egg-to-adult viability, with X^{SR}/Y males showing significantly reduced
344 viability ($F_{1,81} = 11.7296$, $P < 0.001$). X^{ST}/Y males had a mean viability of 0.5412, and X^{SR}/Y
345 males had a mean viability of 0.4036 (Figure 2). Genotype also had a significant effect on
346 egg-to-adult viability in females ($F_{2,120} = 4.7593$, $P = 0.0103$). Mean viability was 0.6294 in

347 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-
348 hoc comparison test revealed that the viability of X^{ST}/X^{ST} females was greater than X^{SR}/X^{SR}
349 females ($P = 0.0104$), while X^{SR}/X^{ST} females had intermediate viability, but not different from
350 either homozygote ($X^{SR}/X^{ST} - X^{SR}/X^{SR}$ comparison: $P = 0.2949$; $X^{SR}/X^{ST} - X^{ST}/X^{ST}$ comparison: P
351 $= 0.3293$; Figure 2).

352

353 Estimating the strength of selection against drive

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

358

359 The posterior probability of each combination of the female selection parameters s_f and h
360 values is shown in Figure 4. The modal values are $s_f = 0.242$ and $h = 0.511$, with the bivariate
361 95% and 99% credible interval displayed as a two-dimensional contour (Figure 4). The
362 probability of the modal s_f value compared to the null hypothesis of no viability selection
363 against drive in females has a Bayes Factor $BF_{10} = 572.89$. The strength of selection against
364 drive in males and females (s_f and s_m ; setting h to its modal value), did not differ between
365 the sexes ($|z| = 0.3785$, $\alpha = 0.01$ $P = 0.7047$).

366

367 In the pairwise comparison of individual female genotypes there was a difference between
368 the egg-to-adult viability of X^{ST}/X^{ST} and X^{SR}/X^{ST} females, with a selection coefficient mode =
369 0.126 with a 95% credible interval = 0.007 – 0.232 and a 99% credible interval = -0.017 –
370 0.261. A similar difference was observed in the comparison of X^{SR}/X^{ST} and X^{SR}/X^{SR} , with a

371 selection coefficient mode = 0.138 with a 95% credible interval of 0.008 – 0.252 and a 99%
372 credible interval of -0.038 – 0.287.

373

374 **Discussion**

375 Due to their two-fold transmission advantage in males, X chromosomes that exhibit sex-
376 *ratio* meiotic drive (X^{SR}) potentially can spread to fixation and cause population extinction
377 [5,6]. Despite this, several meiotic drive systems exist in broadly stable polymorphisms
378 [10,11,55]. This suggests that there are costs of carrying the X^{SR} chromosome. In the stalk-
379 eyed fly system, the X^{SR} chromosome contains a large inversion [49], which is expected to
380 accumulate deleterious mutations as they are less efficiently removed by recombination
381 than those of the X^{ST} chromosome. This mutation load is expected to lead to a decrease in
382 fitness of the X^{SR} chromosome. Here, controlled crosses were used to estimate one
383 component of fitness, egg-to-adult viability, of meiotic drive genotypes. There was a
384 reduction in viability linked to X^{SR} in both males and females. In X^{SR} hemizygous males this
385 was $s_m = 21\%$ (Figure 3) and in X^{SR} homozygous females $s_f = 24\%$ (Figure 4). The negative
386 effect of X^{SR} in females was largely additive ($h \sim 0.5$), with heterozygotes being
387 intermediate in viability compared to homozygotes. The estimates of selection (s_m and s_f) do
388 not differ between the sexes. This probably reflects a lack of sexual dimorphism in fitness at
389 the larval stage. In *D. melanogaster*, egg-to-adult viability measured for particular genotypes
390 is strongly positively correlated across the sexes, whereas adult reproductive success is
391 typically negatively correlated [72,73].

392

393 In the experiment, individual males of known genotype, either SR or ST, were crossed with
394 heterozygous females. Eggs were collected and combined in groups of 6 petri dishes each

395 containing 12 eggs. The eggs were visually inspected for signs of development, so as to be
396 able to exclude the possibility that differential fertility of the two paternal genotypes (i.e. SR
397 or ST) affected the subsequent output of adult flies. In addition, a pilot experiment showed
398 equal levels of SR and ST male fertility in conditions similar to those used here (electronic
399 supplementary material, Table S8). The combination of eggs from the two crosses were
400 expected to generate all five genotypes in an even ratio, except for heterozygous females
401 which were expected at double the number of the other genotypes. The objective was to
402 standardise competition between genotypes. It is hard to estimate whether this objective
403 was attained, as only surviving adults were genotyped. The observed adult genotype
404 frequencies were compared to infer genotype-specific survival in the egg-to-adult stage. The
405 number of flies genotyped was sufficiently large ($N_m = 798$, $N_f = 1272$) to give reasonable
406 assurance of the accuracy of the estimates. Even with this sizeable sample, the bounds on
407 the estimates of s_m , s_f and h remain large (Figure 3-4) but we can be confident that drive is
408 associated with loss of viability in both sexes. Our results contrast with a prior study
409 showing that adult lifespan is independent of SR genotype in males and females [63],
410 revealing a difference between larval and adult genotypic effects. This previous study also
411 suggested that larval survival is independent of SR genotype [63]. The reasons for this
412 difference are unclear; there could be differences that relate to food and housing, the
413 mixture of genotypes undergoing larval competition or the SR haplotype used as those in
414 Wilkinson et al. [63] cause less than 100% transmission distortion. This suggests that further
415 investigation is warranted in a number of directions.

416

417 This is the first study showing a reduction in SR viability in stalk-eyed flies. Similar methods
418 have been applied previously in *D. pseudoobscura* [31,32,40]. Wallace [40] observed strong

419 selection against X^{SR} in both sexes. In high density populations, Beckenbach [32] found a
420 reduction in X^{SR}/Y viability but no viability effect on homozygous X^{SR} female viability. In
421 contrast, Curtsinger and Feldman [31] report stronger selection against homozygous X^{SR}
422 females. Comparisons of these three studies provides strong evidence to suggest that
423 viability selection is density-dependent, as reduction in X^{SR} viability was greatest under high
424 density [40], and a lack of differential viability was observed in another experiment carried
425 out at low density [32]. In the present study, stalk-eyed fly larvae were cultured under low
426 density and provided with an excess of food. Future work will need to determine whether
427 varying levels of food stress enhance or restrict the deleterious effect of the X^{SR}
428 chromosome.

429

430 Strong viability selection against the X^{SR} chromosome, as found here under laboratory
431 conditions, may play a key role in determining the equilibrium level of the SR polymorphism
432 in the wild. There are several other factors that could be involved in determining SR
433 frequency, such as suppressors, polyandry and various forms of sexual behaviour which we
434 discuss further here. First, in *D. simulans*, SR commonly co-occurs with suppressors which
435 restrict the transmission advantage [43,74]. Although early work on the stalk-eyed fly drive
436 system suggested that there were suppressors [47], this has not been sustained by further
437 work, either on the autosomes or Y chromosomes [14]. Second, polyandry may evolve to
438 limit the spread of SR [22]. Polyandry is the norm in *T. dalmanni* [55,66], and there is
439 evidence that SR male sperm does less well under sperm competition [63] and may suffer
440 from interactions with non-sperm ejaculate components produced by standard males
441 (though this has only been shown in the related species *T. whitei*, [21]). But it has not been

442 shown whether variation in the degree of polyandry correlates with SR frequency in natural
443 populations of stalk-eyed flies.

444

445 Third, it has long been suggested that mate choice may play a role in determining the
446 frequency of drive [51]. This may be important in stalk-eyed flies as they are canonical
447 examples of sexual selection driven by mate choice [75,76]. In *T. dalmanni*, drive males are
448 expected to attract fewer females as they have reduced eyespan, and hence mate less often
449 [47,50]. However, there is as yet no evidence in stalk-eyed flies that the strength of female
450 mate preference has been enhanced in populations subject to drive. Nor has there been
451 investigation of whether females that carry SR show alterations in their mating behaviour. A
452 related consideration is male mate preference [77] which has been shown to be an
453 important behavioural adaptation in *T. dalmanni* favouring male matings with fecund
454 females [78]. A recent study reported that SR had no direct effect on male mate choice [79].
455 However, the strength of male mate preference positively covaries with male eyespan. As
456 drive males have smaller eyespan [50], we expect they will be less discriminating in their
457 mate choice [79].

458

459 Finally, measurements of sperm number per mating report that SR males deliver as many
460 sperm as ST males, and a single mating with a SR male results in the same female fertility as
461 a mating with a ST male [65]. Whether this pattern carries over to situations where a male
462 can mate with multiple females is less clear. One experiment showed no difference between
463 SR and ST males [64], whereas another experiment found lower fertility in SR males [63]
464 when multiple females were allowed to mate freely with a single male for a day. The cause
465 of this difference is unclear, but drive males have been shown to have lower mating rates

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

470

471 The number of different factors set out above make it difficult to predict whether they are
472 sufficient to explain the observed frequency of ~20% [14,55]. Many could act as stabilizing
473 forces which restrict the spread of drive in a frequency-dependent manner. Future work
474 should aim to examine these factors, in combination with the intensity of egg-to-adult
475 viability selection measured here, in a modelling framework in order to predict the
476 evolutionary outcomes. This needs to be coupled to better estimation of ecological and
477 demographic parameters across local populations of *T. dalmanni* in which SR frequency is
478 known to be highly variable [50].

479

480

481

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487 with the statistical analysis.

488

489 **Data accessibility statement.** Raw data available from the Dryad Digital Repository:

490 doi:10.5061/dryad.kc49jk1

491

492

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

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684 meiotic drive alter male mate preference? *Submitted*.
685

686 **Figure legends**

687

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

695

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

698

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

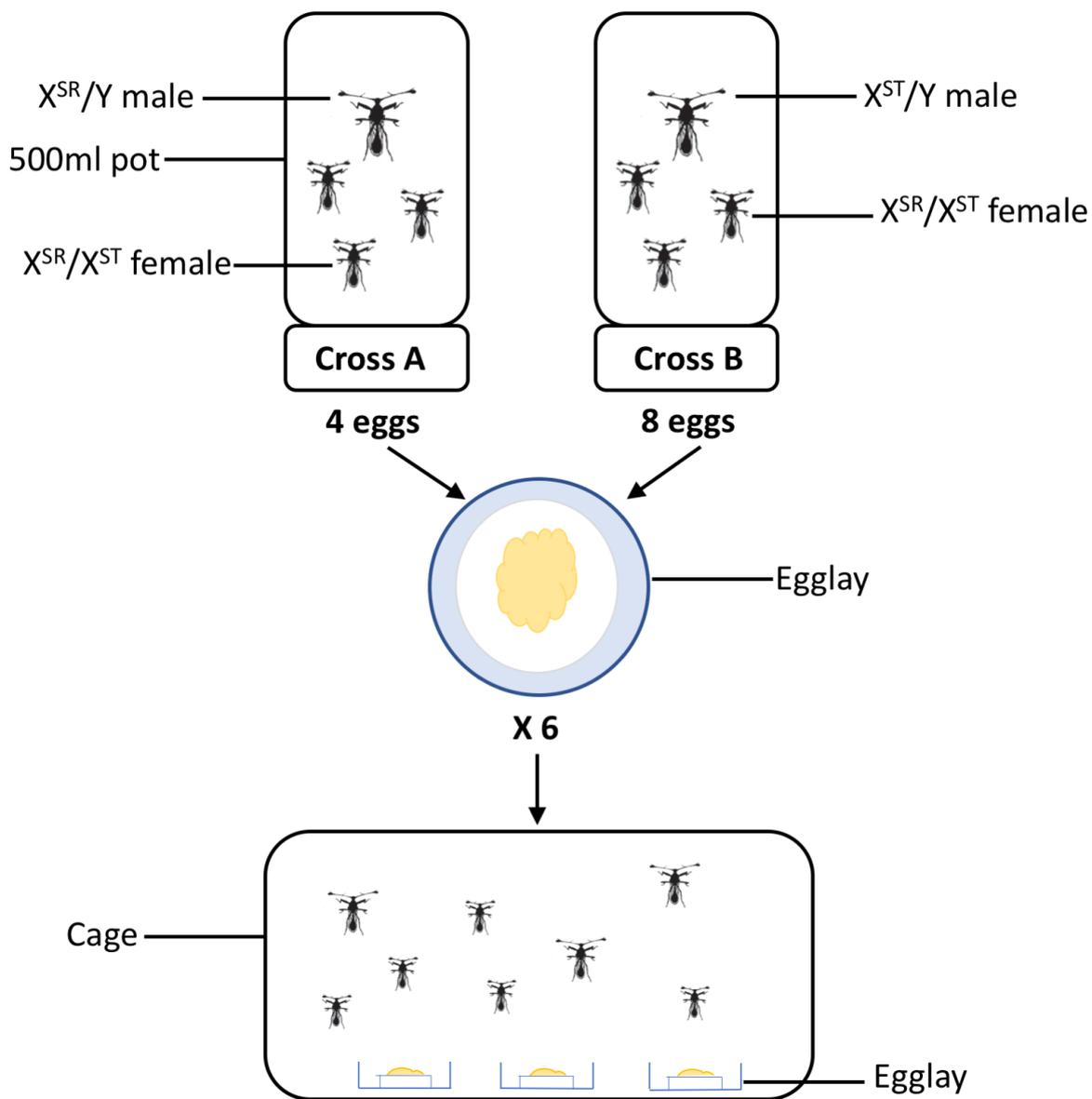
702

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

707

708 **Figures**

709 Figure 1:

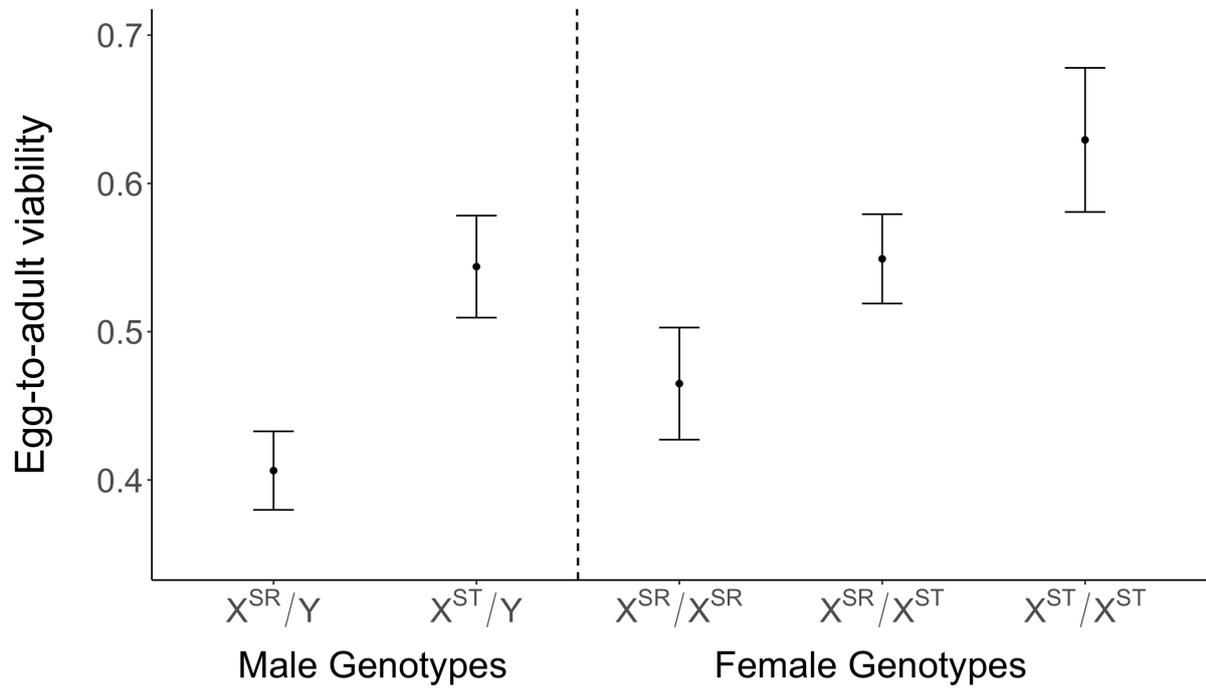


710

711

712 Figure 2:

713

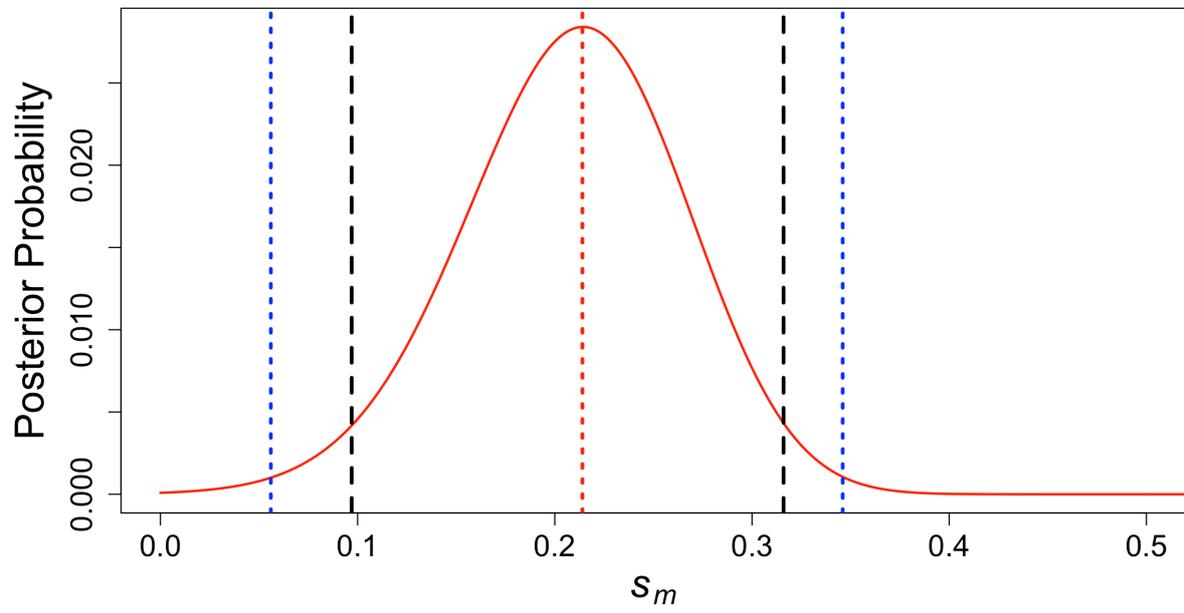


714

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716 Figure 3:

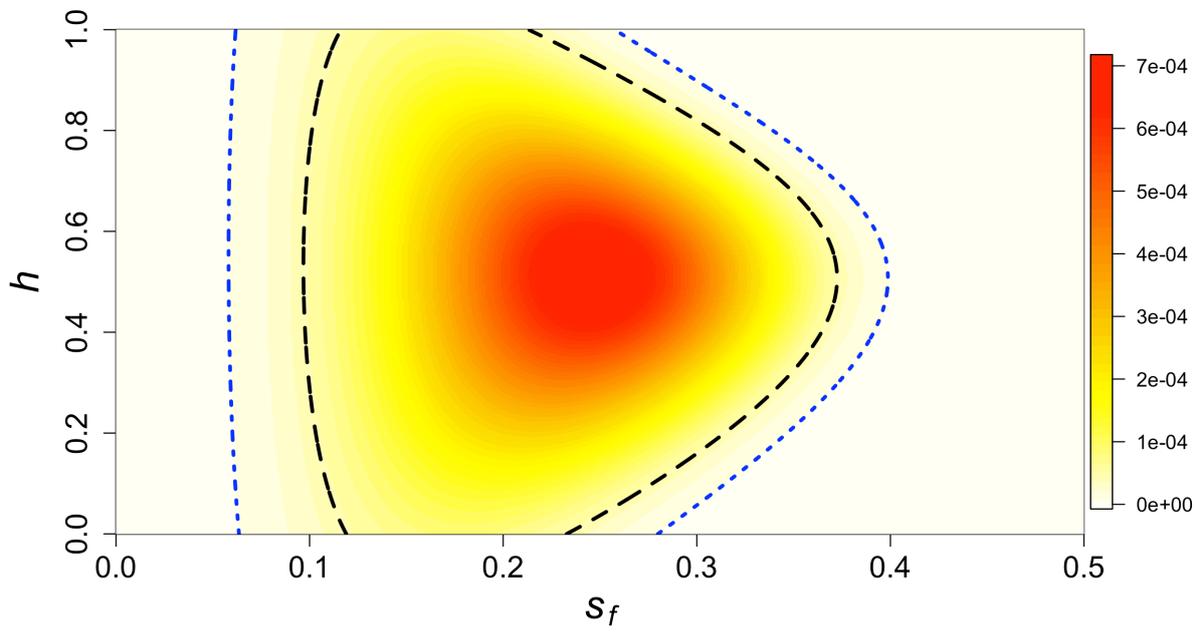
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719

720 Figure 4:



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722

723

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

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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:

```
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	0.0144249	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:

```
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	116.00000	4.6824068	0.0110758
Condition	0.0275940	0.0137970	2	53.53907	0.1551592	0.8566625
Genotype:Condition	0.0727005	0.0181751	4	116.00000	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:

```
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

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Genotype	0.7757225	0.7757225	1	81	11.72957	0.0009681

Supplementary table S4

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

```
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

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Genotype	0.8239569	0.4119784	2	120	4.759265	0.0102556

Supplementary table S5

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

```
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^{SR}/Y genotype is used as the comparison, so its egg-to-adult viability is the model intercept term, 0.40633. The viability of X^{ST}/Y (labelled as simply GenotypeXY in the model), is calculated by adding the intercept term and the effect term together: $0.40633 + 0.13755 = 0.54388$.

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:

	diff	lwr	upr	p adj
SRX-SRSR	0.0841424	-0.0481157	0.2164006	0.2916928
XX-SRSR	0.1643466	0.0320885	0.2966048	0.0104345
XX-SRX	0.0802042	-0.0520539	0.2124623	0.3260922

Fertility trial - Supplementary table S8

Below are the results of a trial designed to test the fertility of eggs laid by X^{SR}/X^{ST} females crossed to X^{SR}/Y (Cross A) and X^{ST}/Y (Cross B) males. One day old eggs were collected and counted, then allowed to develop for a further five days. After five days of development, the vast majority of fertilised eggs have hatched, and the remainder 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.

Date	Cross	Pot.ID	Total.eggs	Unfert	Fert	Percent.Fert
15-Nov	A	A1	12	3	9	0.7500000
15-Nov	A	A2	131	12	119	0.9083969
15-Nov	A	A3	76	6	70	0.9210526
15-Nov	B	B1	81	8	73	0.9012346
15-Nov	B	B2	67	6	61	0.9104478
15-Nov	B	B3	40	4	36	0.9000000
21-Nov	A	A1	43	4	39	0.9069767
21-Nov	A	A2	89	4	85	0.9550562
21-Nov	A	A3	76	3	73	0.9605263
21-Nov	B	B1	85	8	77	0.9058824
21-Nov	B	B2	105	8	97	0.9238095
21-Nov	B	B3	34	3	31	0.9117647
23-Nov	A	A1	90	0	90	1.0000000
23-Nov	A	A2	69	3	66	0.9565217
23-Nov	A	A3	43	3	40	0.9302326
23-Nov	B	B1	57	4	53	0.9298246
23-Nov	B	B2	49	0	49	1.0000000
23-Nov	B	B3	42	0	42	1.0000000
17-Dec	A	A1	59	2	57	0.9661017
17-Dec	A	A2	69	2	67	0.9710145
17-Dec	A	A3	35	0	35	1.0000000
17-Dec	B	B1	84	0	84	1.0000000
17-Dec	B	B2	58	1	57	0.9827586
17-Dec	B	B3	52	3	49	0.9423077
19-Dec	A	A1	47	0	47	1.0000000
19-Dec	A	A2	134	4	130	0.9701493
19-Dec	A	A3	13	2	11	0.8461538
19-Dec	B	B1	99	8	91	0.9191919
19-Dec	B	B2	29	3	26	0.8965517
19-Dec	B	B3	34	0	34	1.0000000

Cross	Total.eggs	Total.Unfertilised	Fertility
A	986	48	0.9513185
B	916	56	0.9388646

Data accessibility

Raw and processed data are available on the Dryad Digital Repository: [doi:10.5061/dryad.kc49jk1](https://doi.org/10.5061/dryad.kc49jk1)