Adaptive maintenance of fertility in the face of meiotic drive

Lara Meade [a *], Sam Finnegan [a], Ridhima Kad [a], Kevin Fowler [a] & Andrew Pomiankowski [a, b]

[a] Department of Genetics, Evolution and Environment, University College London, Gower Street, London, WC1E 6BT, UK

[b] CoMPLEX, University College London, Gower Street, London, WC1E 6BT, UK

Keywords: accessory gland, multiple mating, sex ratio distorter, sperm competition, testis

Word count: 3048

Online-only elements: Appendix

Submitted to The American Naturalist as a Note

Short title: Fertility and meiotic drive

Abstract

Selfish genetic elements that gain a transmission advantage through the destruction of sperm have grave implications for drive male fertility. We report the first evidence for a male adaptation to this negative consequence of unsuppressed drive. In the X-linked SR meiotic drive system of a stalk-eyed fly, we found that drive males had greatly enlarged testes and so maintained high fertility despite the destruction of half their sperm. This was the case even when males were challenged with fertilising five females. Conversely, we observed an overt trade-off with mating frequency due to reduced allocation of resources to accessory glands. Body size and eyespan were also reduced, which are likely to impair viability, pre-copulatory competition and mating frequency. Gaining an understanding of the extent to which drivers promote the evolution of adaptive responses in the host is essential for predictions of equilibrium frequencies in wild populations.

30 **Introduction**

31

32 Meiotic drive genes gain a transmission advantage through manipulation of meiosis or
33 gametogenesis and are likely to have profound ecological and evolutionary consequences,
34 ranging from the evolution of sex determination systems and changes in karyotype, to
35 impacts on population persistence and sexual selection (Hurst and Werren 2001; Jaenike
36 2001; Werren 2011; Lindholm et al. 2016). Drivers have been uncovered in a wide range of
37 taxa, with a preponderance of linkage to the sex chromosomes in the heterogametic sex
38 (Hurst and Pomiankowski 1991; Jaenike 2001; Taylor and Ingvarsson 2003). When meiotic
39 drive occurs in males, it severely disrupts the maturation and fertilisation capacity of non-
40 carrier sperm (Burt and Trivers 2006; Lindholm et al. 2016). The consequent loss of
41 competent sperm imposes a fertility disadvantage to organismal fitness (Price and Wedell
42 2008), that is exaggerated under conditions of sperm competition (Taylor et al. 1999;
43 Angelard et al. 2008; Price et al. 2008*a*). The extent to which these and other detrimental
44 effects of sperm-killer drive promote adaptive responses in the host species has received
45 limited attention. There is an extensive literature on genetic elements that interfere and
46 suppress the action of drive (Atlan et al. 1997; Presgraves et al. 1997; Dyer 2012;
47 Larracuente and Presgraves 2012; Branco et al. 2013). For example, in *Drosophila* species,
48 suppressors of X-linked drive have been found on the Y chromosome (Carvalho et al. 1997;
49 Cazemajor et al. 1997; Branco et al. 2013) and throughout the rest of the genome (Carvalho
50 and Klaczko 1993; Atlan et al. 2003; Tao et al. 2007). A more recent suggestion is that drive
51 may promote the evolution of female polyandry in order to dilute the ejaculates of drive
52 males (Haig and Bergstrom 1995; Zeh and Zeh 1997; Wedell 2013; Holman et al. 2015).
53 There is some evidence for this from experimental evolution studies using populations

54 exposed to meiotic drive in *D. pseudoobscura* (Price et al. 2008*b*) and *Mus musculus*
55 (Manser et al. 2017), and from natural populations in which the rate of multiple mating
56 correlates positively with the frequency of drive in *D. pseudoobscura* (Price et al. 2014) and
57 *D. neotestacea* (Pinzone and Dyer 2013). Female mate choice may additionally evolve in
58 response to drive. In stalk-eyed flies, meiotic drive has been linked to small eyespan, which
59 may allow females to avoid mating with carrier males through assessing eyespan (Wilkinson
60 et al. 1998; Cotton et al. 2014). Female house mice could avoid mating with drive males
61 through detecting unique major histocompatibility alleles linked to the driving *t* complex
62 (Silver 1985; Lindholm et al. 2013), although evidence remains unclear (Lindholm and Price
63 2016).
64
65 Another, as yet unexplored, route by which males could adapt to drive is by increasing the
66 allocation of resources to sperm production, to offset the destructive effect of drive on
67 gametogenesis. Sperm production is positively correlated with testis size in multiple species
68 (Møller 1989; Gage 1994; Pitnick and Markow 1994; Pitnick 1996; Stockley et al. 1997; Fry
69 2006), and increased testis size is a well characterised evolutionary response to heightened
70 sperm competition favouring greater sperm production (Hosken and Ward 2001; Pitnick et
71 al. 2001; Simmons and García-González 2008; Gay et al. 2009). Such an adaptation in drive
72 males might plausibly occur if genes involved in testis development are physically linked
73 with the drive element. Meiotic drive elements are typically found within inversions or
74 other areas of low recombination (Lyon et al. 1988; James and Jaenike 1990; Johns et al.
75 2005; Dyer et al. 2007, 2013; Harvey et al. 2014; Reinhardt et al. 2014). These regions not
76 only keep driver and insensitive responder loci together but facilitate the spread of
77 modifiers that enhance transmission distortion (Hartl 1975; Larracuente and Presgraves

78 2012) and are predicted to be enriched for male beneficial sexually-antagonistic alleles
79 (Rydzewski et al. 2016). For similar reasons, alleles that enable increased investment in
80 testes could become associated with the drive haplotype.
81
82 We test this idea using the Malaysian stalk-eyed fly species *Teleopsis dalmanni*. This species
83 harbours SR, an X-linked driver, which produce strongly female-biased broods due the
84 destruction of Y-bearing sperm (Presgraves et al. 1997; Wilkinson and Sanchez 2001). The
85 X^{SR} drive chromosome is estimated to have diverged from a non-driving ancestor (X^{ST})
86 around 500,000 years ago (Paczolt et al. 2017), and is characterised by a large inversion(s)
87 covering most of the chromosome (Johns et al. 2005; Paczolt et al. 2017). X^{SR} is found at
88 appreciable frequencies (10 – 30%) across populations and generations (Wilkinson et al.
89 2003; Cotton et al. 2014) but appears to lack genetic suppressors (Reinhold et al. 1999;
90 Wolfenbarger and Wilkinson 2001; Paczolt et al. 2017). The mating system in *T. dalmanni* is
91 highly promiscuous. Females join male lek sites at dusk with matings occurring the following
92 dawn (Wilkinson et al. 1998; Chapman et al. 2005; Cotton et al. 2015). Females prefer to
93 join the roosting sites and mate with males that have large eyespan (Wilkinson and Reillo
94 1994; Cotton et al. 2010). Sperm competition is the norm, and ejaculates compete in a
95 raffle, without precedence for first or last males (Corley et al. 2006). This means that there
96 has been ample time and selective opportunity for adaptive responses to evolve in males
97 carrying the drive chromosome.
98
99 To test for male adaptive changes in response to drive we determined whether SR and
100 standard (ST) males differed in their reproductive (testis and accessory gland size) and
101 morphological traits (eyespan and body size). Testis size predicts the amount of sperm

102 found within female storage (Fry 2006). Accessory glands produce all non-sperm
103 components of the ejaculate, and their size is associated with male mating frequency (Baker
104 et al. 2003; Rogers et al. 2005*a*, 2005*b*). Body size and eyespan are also important
105 predictors of male mating frequency (Small et al. 2009; Cotton et al. 2010). We determined
106 SR male fertility under a situation maximizing the importance of sperm number. Males were
107 allowed a 10-hour period in which to mate with low or high numbers of females and the
108 number of fertilized eggs produced were counted. We then changed the experimental
109 design to one emphasizing the importance of mating frequency. Males were exposed to
110 females over a time-constrained 30-minute period, and the number of copulations that SR
111 males achieved was compared to that of ST males.
112
113 **Methods**
114
115 Details of stock collection and day-to-day upkeep can be found in the appendix.
116 Experimental males were taken from the SR-stock population, whose males are a ~50:50
117 mix of X^{SR} and X^{ST} genotypes. Experimental females were taken from the ST-stock
118 population, which lacks meiotic drive. Non-virgin males from the SR-stock were allowed to
119 mate freely with either one or five virgin ST-stock females, over a period of 10 hours from
120 artificial dawn. Mated females were allowed to lay eggs for 14 days, by which time most
121 females had stopped laying fertile eggs. Fecundity was recorded through egg counts, and
122 egg hatch was used as an estimate for fertility. On the following day, experimental males,
123 and a similar number of unmated males, were anaesthetised on ice and their testes and
124 accessory glands were removed (figure 1*A*) and photographed under differential
125 interference contrast microscopy. Organ area was measured at x50 magnification by tracing

126 the outline. Male eyespan (Hingle et al. 2001*a*) and a proxy for body size, thorax length,
127 (Rogers et al. 2008) were measured. The entire protocol was repeated in 16 batches.
128
129 In a second experiment, SR-stock males were introduced to two ST-stock non-virgin females
130 at artificial dawn. All copulations were counted over 30 minutes. A copulation was defined
131 as intromission lasting more than 30 seconds, as copulations shorter than this do not result
132 in spermatophore transfer (Rogers et al. 2006). Males that attempted to mate but were
133 unsuccessful (defined as following and attempted mounting, but no intromission) were
134 presented with a different set of females and observed for an additional 30 minutes. To
135 minimise any effects on mating frequency due to female choice, the experimental males
136 were standardised to a narrow range of eyespan (7.5 – 8.5 mm). This protocol was repeated
137 over 14 batches.
138
139 Males were genotyped using two X-linked INDEL markers, *comp162710* and *cnv395*, or a
140 microsatellite marker, *ms395*. Allele size of these markers reliably indicates the SR genotype
141 of the males in the laboratory population (Meade et al. 2018, *submitted*).
142
143 *Statistical analysis*
144
145 All tests were carried out in R version 3.32 (R Core Team 2016). We tested if male genotypes
146 differed in their morphological (body size and eyespan; linear models) and reproductive
147 traits (testis size and accessory gland size; linear mixed effects models). Differences in
148 relative trait sizes between genotypes, as well as in absolute trait sizes (models where body
149 size is excluded) are reported. Female total number of fertile eggs (Poisson generalised

150 linear mixed effects model (GLMM)) and proportion fertility (fertile eggs, non-fertile eggs;
151 binomial GLMM) are compared when mated to SR or ST males. We also tested if male
152 reproductive traits, and their interaction with male genotype, were important predictors of
153 fertility. Lastly we tested whether SR and ST males differed in their mating frequency over
154 30-minutes by comparing the likelihood that SR and ST males mate at all (binomial GLMM),
155 as well as the total number of copulations among males that mated at least once (Poisson
156 GLMM).
157
158 To avoid collinearity of male morphological and reproductive traits with body size, models
159 used residual values (Dormann et al. 2013). Reported P values were calculated with type II
160 tests, or type III tests where significant interactions were present (Fox and Weisberg 2011).
161 Where appropriate, batch was included as a random effect. Further details can be found in
162 the appendix.
163
164 **Results**
165
166 *SR trait size*
167
168 SR males had small body size (mean ± s.e. 2.29 ± 0.013 mm) compared to ST males (2.336 ±
169 0.009 mm; $F_{1,357} = 8.745$, $P = 0.003$; figure 1*B*). SR males also had small absolute ($F_{1,357} =$
170 42.631, $P < 0.001$; mean ± s.e. SR: 8.048 ± 0.046mm; ST: 8.402 ± 0.031mm; figure 1*B*) and
171 relative eyespan ($F_{1,355} = 0.713$, $P = 0.016$), especially when body size was small (body size by
172 genotype interaction $F_{1,355} = 4.175$, $P = 0.042$).
173

174 Despite their small size, SR testis size was large (mean ± s.e 1.94 ± 0.050 mm^2) compared to
175 ST males (1.54 ± 0.028 mm^2; $F_{1,280.16} = 73.796$, $P < 0.001$; figure 1*C*). SR males also had large
176 relative testis size ($F_{1,282.78} = 99.982$, $P < 0.001$). In contrast, SR males had small accessory
177 gland size (mean ± s.e. SR: 0.306 ± 0.011 mm^2; ST: 0.348 ± 0.010 mm^2; $F_{1,335.36} = 16.353$, $P <$
178 0.001; figure 1*D*), and this relationship remained true after accounting for their small body
179 size ($F_{1,334.03} = 7.801$, $P = 0.006$).
180
181 *SR fertility*
182
183 SR males did not differ from ST males in total (mean ± s.e. SR: 112.047 ± 8.290, ST: 107.053
184 ± 5.597; $\chi^2_1 = 2.416$, $P = 0.120$, $N = 215$; figure 2*A*-*B*) or proportion fertility (mean ± s.e. SR:
185 0.33 ± 0.024, ST: 0.767 ± 0.018; $\chi^2_1 = 2.469$, $P = 0.116$, $N = 215$) when held with females over
186 an extended 10-hour period. Males mating with five females achieved higher total fertility
187 than those mating with a single female ($\chi^2_1 = 43.698$, $P < 0.001$, $N = 215$), but were unable to
188 fertilise a higher proportion of eggs ($\chi^2_1 = 6.021$, $P = 0.014$, $N = 215$). The interaction
189 between mating group (one or five females) and genotype did not influence total ($\chi^2_1 =$
190 0.591, $P = 0.442$, $N = 215$) or proportion fertility ($\chi^2_1 = 1.377$, $P = 0.241$, $N = 215$).
191
192 Male testis size was an important predictor of fertility. Both total ($\chi^2_1 = 6.216$, $P = 0.013$, $N =$
193 165; figure 2*C*-*D*) and proportion fertility ($\chi^2_1 = 16.646$, $P < 0.001$, $N = 165$) were greater
194 amongst males with larger relative testis size. The addition of testis size did not alter the
195 relationship between genotype and total ($\chi^2_1 = 0.018$, $P = 0.895$, $N = 173$) or proportion
196 fertility ($\chi^2_1 = 0.260$, $P = 0.610$, $N = 173$). There was no interaction between testis size and
197 genotype predicting total ($\chi^2_1 = 0.164$, $P = 0.686$, $N = 173$) or proportion fertility ($\chi^2_1 = 0.617$,

198 P = 0.432, N = 173). Accessory gland size did not predict total (χ^2_1 = 0.032, P = 0.858, N =

199 165) or proportion fertility (χ^2_1 = 0.160, P = 0.689, N = 165).

200

201 *SR mating frequency*

202

203 A total of 493 copulations from 193 males were observed over the 30-minute mating trials.

204 SR males copulated (mean ± s.e. 2.75 ± 0.175, N = 81) fewer times on average than ST males

205 (3.55 ± 0.186, N = 76; χ^2_1 = 6.304, P = 0.012; figure 1*E*), but were not less likely to mate at

206 least once (SR: 81/104, ST: 76/89; χ^2_1 = 1.665, P = 0.197, N = 193).

207

208 **Discussion**

209

210 One of the main features of drive in males is reduced fertility due to the dysfunction of non-

211 carrier sperm. Here, we present evidence of an adaptive response to this deficit. Male *T.*

212 *dalmanni* carrying the X^{SR} drive chromosome have greatly enlarged testes (figure 1*C*), an

213 ~70% increase compared to standard males. This enables SR males to achieve fertility at a

214 level equivalent to that of standard males when given the opportunity to mate to a single

215 female over an extended period of 10 hours. When challenged with fertilising five females,

216 males fertilised a smaller proportion of their eggs, but SR males performed just as well as ST

217 males. These patterns are likely to arise because the production of sperm scales with testis

218 size, as seen in many insect species (Gage 1994; Pitnick and Markow 1994; Pitnick 1996;

219 Stockley et al. 1997), including *T. dalmanni* (Fry 2006). This interpretation is supported by

220 previous findings that SR male ejaculates contain similar numbers of sperm as ST males,

221 both after single and multiple matings (Meade et al. 2018, *submitted*), and that testis size is

222 an important predictor of male fertility. Despite the destruction of half their sperm, the
223 increased investment in sperm production of drive males allows them to deliver sufficient
224 sperm to achieve similar competitive ability to standard males.
225
226 We found a significant trade-off between testis and accessory gland size in drive males, as
227 their accessory glands were small, especially for their body size (figure 1*D*). Previous work
228 showed that male mating rate is phenotypically (Baker et al. 2003) and genetically linked
229 with accessory gland size (Rogers et al. 2005*a*). Here, when males were observed under
230 time constraint (30 minutes), SR mating frequency was low (figure 1*E*). Furthermore, SR
231 males were small and had small eyespan for their body size (figure 1*B*), which previous work
232 has shown is important in determining success in male-male antagonistic interactions
233 (Panhuis and Wilkinson 1999; Small et al. 2009) and mate choice (Panhuis and Wilkinson
234 1999; Hingle et al. 2001*b*; Cotton et al. 2010). Reduction in this suite of trait sizes will likely
235 have a serious impact upon male mating frequency. In the wild, males compete for roosting
236 sites while females assess males and roost with their chosen male overnight, with large
237 eyespan males attracting more females (Wilkinson and Reillo 1994; Cotton et al. 2010).
238 Males mate with females on their leks over a brief half hour period at dawn, after which
239 females depart if they fail to quickly gain a mating (AP, *personal observation*). Small and
240 unattractive SR males that cannot mate at a high enough rate will bias mating success
241 against them and toward the better endowed ST males.
242
243 Our study reveals that SR males adopt a different investment strategy in their reproductive
244 and somatic traits. A portion of their resources allocated to reproduction is wasted due to
245 meiotic drive, requiring increased investment in testes to raise sperm production. The main

246 route to this appears to be through the diversion of resource from the accessory glands. It
247 may also come from reduced investment in body size and eyespan. However small body size
248 and eyespan may arise from low genetic condition of drive males due to the accumulation
249 of deleterious mutations within the inversion(s) on X^{SR}. Inversions significantly suppress
250 recombination because pairing is partially inhibited during synapsis or because crossovers
251 give rise to unbalanced gametes (Kirkpatrick 2010). Stalk-eyed flies have three pairs of
252 chromosomes, and the SR inversion(s) covers nearly all of the X chromosome, meaning
253 approximately one third of all stalk-eyed fly genes are subject to weak natural selection
254 (Johns et al. 2005; Paczolt et al. 2017). This will have a negative effect on costly, condition-
255 dependent traits, like body size and eyespan (David et al. 2000; Cotton et al. 2004; Bellamy
256 et al. 2013). These views help to explain the investment patterns we observe. Our overall
257 hypothesis is that if drive males inevitably have small eyespan, they will be unattractive and
258 gain fewer mating opportunities. Consequently, investment in accessory glands which will
259 enable higher mating rates will give lower returns than diversion of resources into larger
260 testes, allowing SR males to produce ejaculates of equivalent size to those of standard
261 males, and be able to compete under the conditions of high sperm competition seen in
262 stalk-eyed flies. These ideas about the linkage of allocation trade-offs, condition and mating
263 rates need further investigation, in particular under the mating conditions that occur in the
264 wild.
265
266 Here we demonstrate for the first time that drive males can increase the production of
267 viable sperm and maintain fertility, despite sperm destruction, through investment in testes.
268 Other adaptive responses, such as genetic suppression, polyandry or female choice of non-
269 drive males, are likely to reduce the transmission advantage gained by drive, and should

lead to reductions in the equilibrium frequency that drive will reach (Hartl 1975; Taylor and Jaenike 2002; Holman et al. 2015). In sharp contrast, increased investment in sperm production will intensify, rather than prevent, the transmission of the driver, because the benefit to the individual male in gaining fertility is also beneficial to the drive element itself. We found that the increased investment in sperm production was associated with reduced accessory gland size and eyespan, traits that play a vital role in pre-copulatory competition and mating frequency. Whether these are general responses or particular to the stalk-eyed fly's development and life history is unclear. Increased investment in sperm production as a response to meiotic drive has neither been theoretically modelled nor empirically studied previously, but it will have implications for the spread and equilibrium frequency of drive.

Authors' contributions

All authors contributed to conceiving the project and methodology; LM and RK collected data on fertility and morphology; SF collected data on mating frequency; LM analysed the data; LM, KF and AP led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Acknowledgements

Funding was provided by a NERC Studentship held by LM, and by EPSRC (EP/F500351/1, EP/I017909/1) awards to AP and NERC grants (NE/G00563X/1, NE/R010579/1) to KF and AP.

Data accessibility

Data will be uploaded to the Dryad Digital Repository

Ethical statement

No ethical approval was required for this research

295 **Literature cited**

296

297 Angelard, C., C. Montchamp-Moreau, and D. Joly. 2008. Female-driven mechanisms,
298 ejaculate size and quality contribute to the lower fertility of sex-ratio distorter males in
299 *Drosophila simulans*. BMC Evolutionary Biology 8:326.

300 Atlan, A., C. Capillon, N. Derome, D. Couvet, and C. Montchamp-Moreau. 2003. The
301 evolution of autosomal suppressors of sex-ratio drive in *Drosophila simulans*. Genetica
302 117:47–58.

303 Atlan, A., H. Merçot, C. Landré, and C. Montchamp-Moreau. 1997. The sex-ratio trait in
304 *Drosophila simulans*: geographical distribution of distortion and resistance. Evolution
305 51:1886–1895.

306 Baker, R. H., M. Denniff, P. Futerman, K. Fowler, A. Pomiankowski, and T. Chapman. 2003.
307 Accessory gland size influences time to sexual maturity and mating frequency in the
308 stalk-eyed fly, *Cyrtodiopsis dalmanni*. Behavioral Ecology 14:607–611.

309 Bellamy, L., N. Chapman, K. Fowler, and A. Pomiankowski. 2013. Sexual traits are sensitive
310 to genetic stress and predict extinction risk in the stalk-eyed fly, *Diasemopsis meigenii*.
311 Evolution 67:2662–2673.

312 Branco, A. T., Y. Tao, D. L. Hartl, and B. Lemos. 2013. Natural variation of the Y chromosome
313 suppresses sex ratio distortion and modulates testis-specific gene expression in
314 *Drosophila simulans*. Heredity 111:8–15.

315 Burt, A., and R. Trivers. 2006. Genes in Conflict: The biology of selfish genetic elements.
316 Cambridge, MA: BelknapHarvard. Harvard University Press, Cambridge, CA, CA.

317 Carvalho, A. B., and L. B. Klaczko. 1993. Autosomal suppressors of sex-ratio in *Drosophila*
318 *mediopunctata*. Heredity 71 (Pt 5):546–551.

Carvalho, A. B., S. C. Vaz, and L. B. Klaczko. 1997. Polymorphism for Y-linked suppressors of sex-ratio in two natural populations of *Drosophila mediopunctata*. Genetics 146:891–902.

Cazemajor, M., C. Landré, and C. Montchamp-Moreau. 1997. The Sex-Ratio trait in *Drosophila simulans*: Genetic analysis of distortion and suppression. Genetics 147:635–642.

Chapman, T., A. Pomiankowski, and K. Fowler. 2005. Stalk-eyed flies. Current Biology 15:R533–R535.

Corley, L. S., S. Cotton, E. McConnell, T. Chapman, K. Fowler, and A. Pomiankowski. 2006. Highly variable sperm precedence in the stalk-eyed fly, *Teleopsis dalmanni*. BMC Evolutionary Biology 6:53.

Cotton, A. J., S. Cotton, J. Small, and A. Pomiankowski. 2015. Male mate preference for female eyespan and fecundity in the stalk-eyed fly, *Teleopsis dalmanni*. Behavioral Ecology 26:376–385.

Cotton, A. J., M. Földvári, S. Cotton, and A. Pomiankowski. 2014. Male eyespan size is associated with meiotic drive in wild stalk-eyed flies (*Teleopsis dalmanni*). Heredity 112:363–9.

Cotton, S., K. Fowler, and A. Pomiankowski. 2004. Condition dependence of sexual ornament size and variation in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). Evolution 58:1038–46.

Cotton, S., J. Small, R. Hashim, and A. Pomiankowski. 2010. Eyespan reflects reproductive quality in wild stalk-eyed flies. Evolutionary Ecology 24:83–95.

David, P., T. Bjorksten, K. Fowler, and A. Pomiankowski. 2000. Condition-dependent signalling of genetic variation in stalk-eyed flies. Nature 406:186–188.

343 Dormann, C. F., J. Elith, S. Bacher, C. Buchmann, G. Carl, G. Carré, J. R. G. Marquéz, et al.
344 2013. Collinearity: A review of methods to deal with it and a simulation study
345 evaluating their performance. Ecography 36:027–046.
346 Dyer, K. A. 2012. Local selection underlies the geographic distribution of sex-ratio drive in
347 *Drosophila neotestacea*. Evolution 66:973–984.
348 Dyer, K. A., M. J. Bray, and S. J. Lopez. 2013. Genomic conflict drives patterns of X-linked
349 population structure in *Drosophila neotestacea*. Molecular Ecology 22:157–169.
350 Dyer, K. A., B. Charlesworth, and J. Jaenike. 2007. Chromosome-wide linkage disequilibrium
351 as a consequence of meiotic drive. Proceedings of the National Academy of Sciences of
352 the United States of America 104:1587–1592.
353 Fox, J., and S. Weisberg. 2011. An R companion to applied regression (2nd ed.). Sage,
354 Thousand Oaks, CA.
355 Fry, C. L. 2006. Juvenile hormone mediates a trade-off between primary and secondary
356 sexual traits in stalk-eyed flies. Evolution and Development 8:191–201.
357 Gage, M. J. G. 1994. Associations between body size, mating pattern, testis size and sperm
358 lengths across butterflies. Proceedings of the Royal Society B: Biological Sciences
359 258:247–254.
360 Gay, L., D. J. Hosken, R. Vasudev, T. Tregenza, and P. E. Eady. 2009. Sperm competition and
361 maternal effects differentially influence testis and sperm size in *Callosobruchus*
362 *maculatus*. Journal of Evolutionary Biology 22:1143–1150.
363 Haig, D., and C. T. Bergstrom. 1995. Multiple mating, sperm competition and meiotic drive.
364 Journal of Evolutionary Biology 8:265–282.
365 Hartl, D. L. 1975. Modifier theory and meiotic drive. Theoretical Population Biology 7:168–
366 174.

367 Harvey, A. M., D. G. Rehard, K. M. Groskreutz, D. R. Kuntz, K. J. Sharp, P. K. T. Shiu, and T. M.
368 Hammond. 2014. A critical component of meiotic drive in *Neurospora* is located near a
369 chromosome rearrangement. Genetics 197:1165–1174.
370 Hingle, A., K. Fowler, and A. Pomiankowski. 2001*a*. The effect of transient food stress on
371 female mate preference in the stalk-eyed fly *Cyrtodiopsis dalmanni*. Proceedings of the
372 Royal Society B: Biological Sciences 268:1239–1244.
373 ———. 2001*b*. Size-dependent mate preference in the stalk-eyed fly *Cyrtodiopsis dalmanni*.
374 Animal Behaviour 61:589–595.
375 Holman, L., T. A. R. Price, N. Wedell, and H. Kokko. 2015. Coevolutionary dynamics of
376 polyandry and sex-linked meiotic drive. Evolution 69:709–720.
377 Hosken, D. J., and P. I. Ward. 2001. Experimental evidence for testis size evolution via sperm
378 competition. Ecology Letters 4:10–13.
379 Hurst, G. D. D., and J. H. Werren. 2001. The role of selfish genetic elements in eukaryotic
380 evolution. Nature Reviews Genetics 2:597–606.
381 Hurst, L. D., and A. Pomiankowski. 1991. Causes of sex ratio bias may account for unisexual
382 sterility in hybrids: A new explanation of Haldane's rule and related phenomena.
383 Genetics 128:841–858.
384 Jaenike, J. 2001. Sex chromosome meiotic drive. Annual Review of Ecology and Systematics
385 32:25–49.
386 James, A. C., and J. Jaenike. 1990. "Sex ratio" meiotic drive in *Drosophila testacea*. Genetics
387 126:651–6.
388 Johns, P. M., L. L. Wolfenbarger, and G. S. Wilkinson. 2005. Genetic linkage between a
389 sexually selected trait and X chromosome meiotic drive. Proceedings of the Royal
390 Society B: Biological Sciences 272:2097–2103.

391 Kirkpatrick, M. 2010. How and why chromosome inversions evolve. PLoS Biology 8.

392 Larracuente, A. M., and D. C. Presgraves. 2012. The selfish Segregation Distorter gene
393 complex of *Drosophila melanogaster*. Genetics. Genetics.

394 Lindholm, A. K., K. A. Dyer, R. C. Firman, L. Fishman, W. Forstmeier, L. Holman, H.
395 Johannesson, et al. 2016. The ecology and evolutionary dynamics of meiotic drive.
396 Trends in Ecology & Evolution 31:315–326.

397 Lindholm, A. K., K. Musolf, A. Weidt, and B. König. 2013. Mate choice for genetic
398 compatibility in the house mouse. Ecology and Evolution 3:1231–1247.

399 Lyon, M. F., J. Zenthon, E. P. Evans, M. D. Burtenshaw, and K. R. Willison. 1988. Extent of the
400 mouse *t* complex and its inversions shown by in situ hybridization. Immunogenetics
401 27:375–382.

402 Manser, A., A. K. Lindholm, L. W. Simmons, and R. C. Firman. 2017. Sperm competition
403 suppresses gene drive among experimentally evolving populations of house mice.
404 Molecular Ecology 5784–5792.

405 Meade, L., D. Dinneen, R. Kad, D. M. Lynch, K. Fowler, and A. Pomiankowski. 2018.
406 Compensation of ejaculate sperm number in male stalk-eyed flies carrying a selfish
407 meiotic drive element. Heredity *(Submitted)*.

408 Møller, A. P. 1989. Ejaculate size, testes size and sperm production in mammals. Functional
409 Ecology 3:91–96.

410 Paczolt, K. A., J. A. Reinhardt, and G. S. Wilkinson. 2017. Contrasting patterns of X-
411 chromosome divergence underlie multiple sex-ratio polymorphisms in stalk-eyed flies.
412 Journal of Evolutionary Biology 30:1772–1784.

413 Panhuis, T. M., and G. S. Wilkinson. 1999. Exaggerated male eye span influences contest
414 outcome in stalk-eyed flies (Diopsidae). Behavioral Ecology and Sociobiology 46:221–

415 227.

416 Pinzone, C. A., and K. A. Dyer. 2013. Association of polyandry and sex-ratio drive prevalence
417 in natural populations of *Drosophila neotestacea*. Proceedings of the Royal Society B:
418 Biological Sciences 280:20131397.

419 Pitnick, S. 1996. Investment in testes and the cost of making long sperm in *Drosophila*.
420 American Naturalist 148:57–80.

421 Pitnick, S., and T. A. Markow. 1994. Male gamertic strategies: sperm size, testis size, and the
422 allocation of ejaculate among successive mates by the sperm-limited fly *Drosophila*
423 *pachea* and its relatives. American Naturalist 143:785–819.

424 Pitnick, S., G. T. Miller, J. Reagan, and B. Holland. 2001. Males' evolutionary responses to
425 experimental removal of sexual selection. Proceedings of the Royal Society B: Biological
426 Sciences 268:1071–1080.

427 Presgraves, D. C., E. Severance, and G. S. Wilkinson. 1997. Sex chromosome meiotic drive in
428 stalk-eyed flies. Genetics 147:1169–80.

429 Price, T. A. R., A. J. Bretman, T. D. Avent, R. R. Snook, G. D. D. Hurst, and N. Wedell. 2008*a*.
430 Sex ratio distorter reduces sperm competitive ability in an insect. Evolution 62:1644–
431 1652.

432 Price, T. A. R., A. J. Bretman, A. C. Gradilla, J. Reger, M. L. Taylor, P. Giraldo-Perez, A.
433 Campbell, et al. 2014. Does polyandry control population sex ratio via regulation of a
434 selfish gene? Proceedings of the Royal Society B: Biological Sciences 281:20133259–
435 20133259.

436 Price, T. A. R., D. J. Hodgson, Z. Lewis, G. D. D. Hurst, and N. Wedell. 2008*b*. Selfish genetic
437 elements promote polyandry in a fly. Science 322:1241–1243.

438 Price, T. A. R., and N. Wedell. 2008. Selfish genetic elements and sexual selection: Their

439 impact on male fertility. Genetica 134:99–111.

440 R Core Team. 2016. R: A language and environment for statistical computing. R Foundation
441 for Statistical Computing, Vienna, Austria.

442 Reinhardt, J. A., C. L. Brand, K. A. Paczolt, P. M. Johns, R. H. Baker, and G. S. Wilkinson. 2014.
443 Meiotic drive impacts expression and evolution of X-linked genes in stalk-eyed flies.
444 PLoS Genetics 10:e1004362.

445 Reinhold, K., L. Engqvist, B. Misof, and J. Kurtz. 1999. Meiotic drive and evolution of female
446 choice. Proceedings of the Royal Society B: Biological Sciences 266:1341–1345.

447 Rogers, D. W., R. H. Baker, T. Chapman, M. Denniff, A. Pomiankowski, and K. Fowler. 2005*a*.
448 Direct and correlated responses to artificial selection on male mating frequency in the
449 stalk-eyed fly *Cyrtodiopsis dalmanni*. Journal of Evolutionary Biology 18:642–650.

450 Rogers, D. W., T. Chapman, K. Fowler, and A. Pomiankowski. 2005*b*. Mating-induced
451 reduction in accessory reproductive organ size in the stalk-eyed fly *Cyrtodiopsis*
452 *dalmanni*. BMC Evolutionary Biology 5:37.

453 Rogers, D. W., M. Denniff, T. Chapman, K. Fowler, and A. Pomiankowski. 2008. Male sexual
454 ornament size is positively associated with reproductive morphology and enhanced
455 fertility in the stalk-eyed fly *Teleopsis dalmanni*. BMC Evolutionary Biology 8:236.

456 Rogers, D. W., C. A. Grant, T. Chapman, A. Pomiankowski, and K. Fowler. 2006. The influence
457 of male and female eyespan on fertility in the stalk-eyed fly, *Cyrtodiopsis dalmanni*.
458 Animal Behaviour 72:1363–1369.

459 Rydzewski, W. T., S. A. Carioscia, G. Liévano, V. D. Lynch, and M. M. Patten. 2016. Sexual
460 antagonism and meiotic drive cause stable linkage disequilibrium and favour reduced
461 recombination on the X chromosome. Journal of Evolutionary Biology 29:1247–1256.

462 Simmons, L. W., and F. García-González. 2008. Evolutionary reduction in testes size and

463 competitive fertilization success in response to the experimental removal of sexual
464 selection in dung beetles. Evolution 62:2580–2591.
465 Small, J., S. Cotton, K. Fowler, and A. Pomiankowski. 2009. Male eyespan and resource
466 ownership affect contest outcome in the stalk-eyed fly, *Teleopsis dalmanni*. Animal
467 Behaviour 78:1213–1220.
468 Stockley, P., M. J. G. Gage, G. A. Parker, and A. P. Møller. 1997. Sperm competition in fishes:
469 The evolution of testis size and ejaculate characteristics. American Naturalist 149:933–
470 954.
471 Tao, Y., J. P. Masly, L. Araripe, Y. Ke, and D. L. Hartl. 2007. A *sex-ratio* meiotic drive system in
472 *Drosophila simulans*. I: An autosomal suppressor. PLoS Biology 5:e292.
473 Taylor, D. R., and P. K. Ingvarsson. 2003. Common features of segregation distortion in
474 plants and animals. Genetica 117:27–35.
475 Taylor, D. R., M. J. Saur, and E. Adams. 1999. Pollen performance and sex-ratio evolution in a
476 dioecious plant. Evolution 53:1028–1036.
477 Taylor, J. E., and J. Jaenike. 2002. Sperm competition and the dynamics of X chromosome
478 drive: Stability and extinction. Genetics 160:1721–1731.
479 Wedell, N. 2013. The dynamic relationship between polyandry and selfish genetic elements.
480 Philosophical Transactions of the Royal Society B: Biological Sciences 368:20120049.
481 Werren, J. H. 2011. Selfish genetic elements, genetic conflict, and evolutionary innovation.
482 Proceedings of the National Academy of Sciences of the United States of America
483 108:10863–10870.
484 Wilkinson, G. S., D. C. Presgraves, and L. Crymes. 1998. Male eye span in stalk-eyed flies
485 indicates genetic quality by meiotic drive suppression. Nature 391:276–279.
486 Wilkinson, G. S., and P. R. Reillo. 1994. Female choice response to artificial selection on an

exaggerated male trait in a stalk-eyed fly. Proceedings of the Royal Society B: Biological Sciences 255:1–6.

Wilkinson, G. S., and M. I. Sanchez. 2001. Sperm development, age and sex chromosome meiotic drive in the stalk-eyed fly, *Cyrtodiopsis whitei*. Heredity 87:17–24.

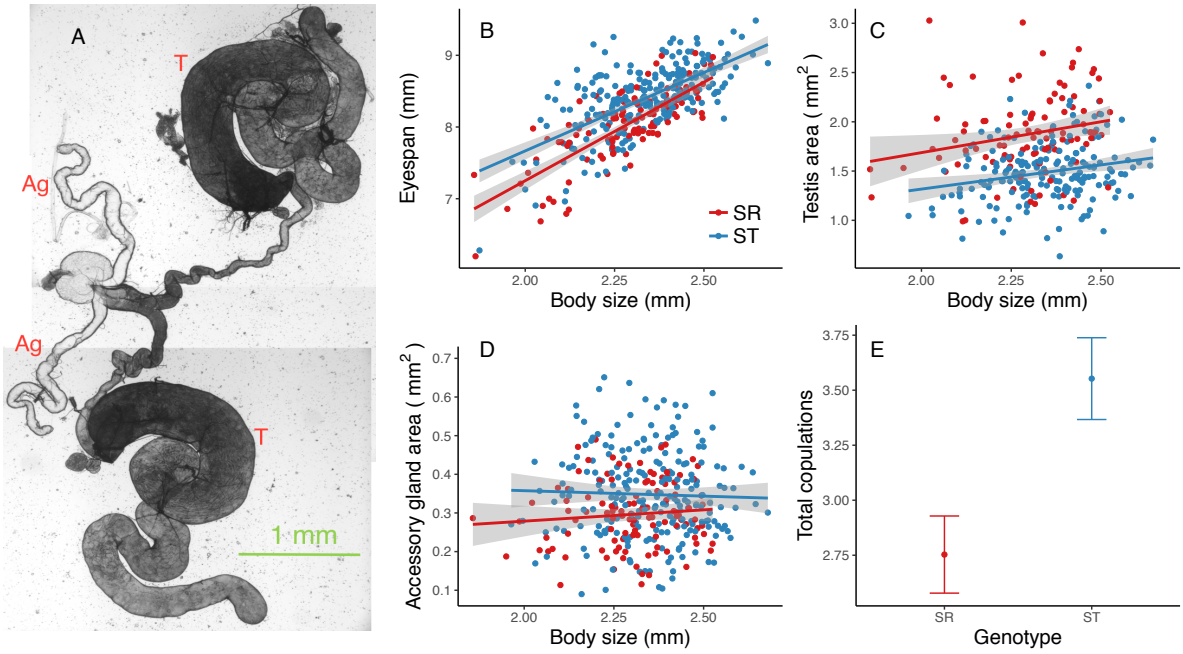
Wilkinson, G. S., J. G. Swallow, S. J. Christianson, and K. Madden. 2003. Phylogeography of sex ratio and multiple mating in stalk-eyed flies from southeast Asia. Genetica 117:37–46.

Wolfenbarger, L. L., and G. S. Wilkinson. 2001. Sex-linked expression of a sexually selected trait in the stalk-eyed fly, *Cyrtodiopsis dalmanni*. Evolution 55:103–110.

Zeh, J. A., and D. W. Zeh. 1997. The evolution of polyandry II: Post-copulatory defenses against genetic incompatibility. Proceedings of the Royal Society B: Biological Sciences 264:69–75.

500 **Figures**

501



502

503 **Figure 1**

504 *A*, *T. dalmanni* testes (T) and accessory glands (Ag) after dissection of males. *B–D*, male

505 morphological and reproductive trait size for SR (red) and ST (blue) males, plotted against

506 male body size. *B,* male eyespan. *C*, male testis area. *D*, male accessory gland area. SR males

507 had smaller body size, eyespan and accessory gland size than ST males, but larger testis size.

508 Grey shading shows ± s.e. *E*, Mating frequency, measured as total number of copulations

509 (mean ± s.e.) observed over 30 minutes, for SR (red) and ST (blue) males.

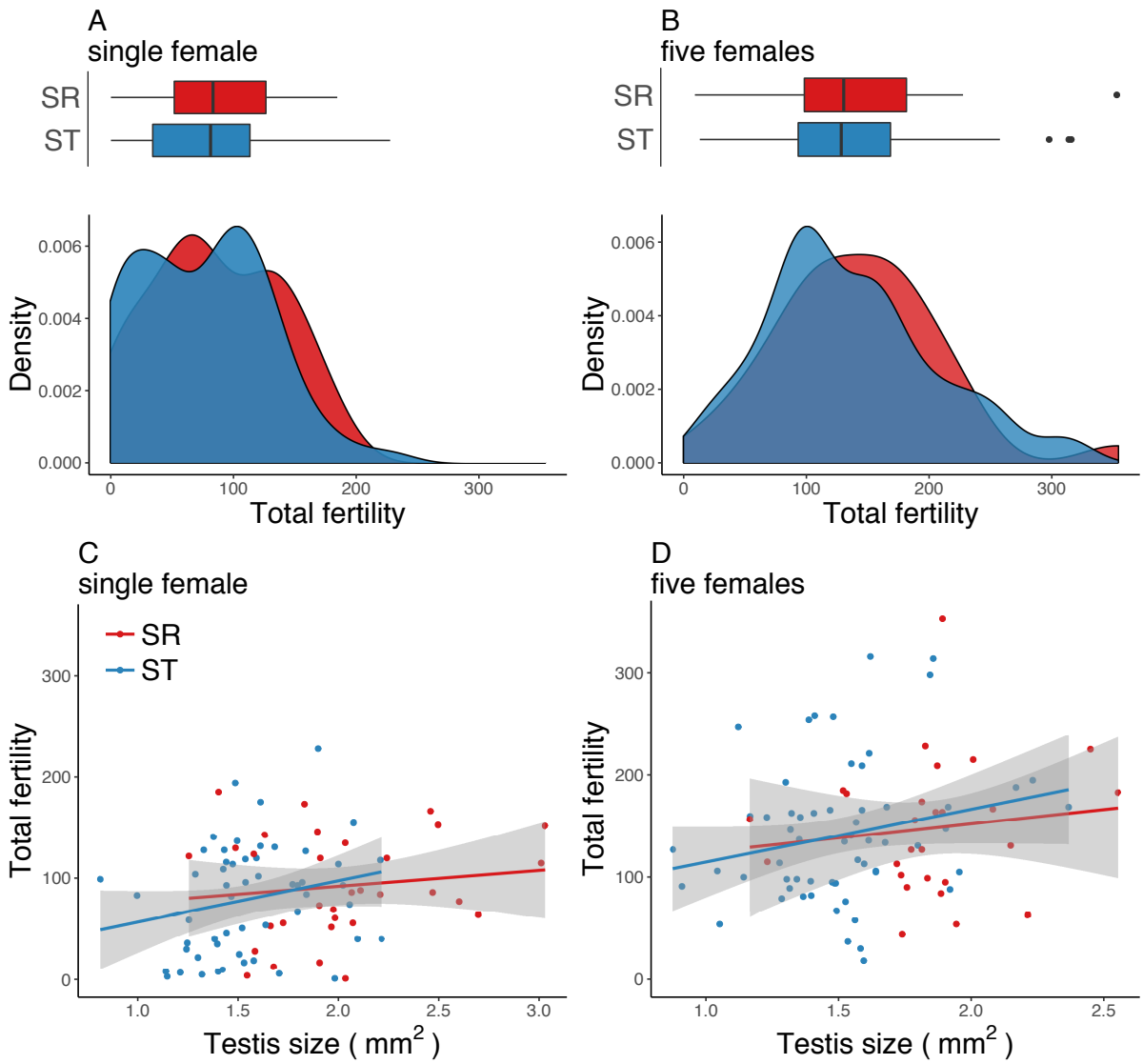


Figure 2

A–B, upper: box plots (median and interquartile range) and *lower:* Kernel probability density of measures of total fertility of SR (red) and ST (blue) males. *A,* mated to a single female. *B,* mated to five females. SR and ST males did not differ in the number of eggs that they fertilised. *C–D,* male testis area plotted against total fertility. *C*, mated to a single female. *D*, mated to five females. Total fertility increased with testis size, for SR and ST males. Grey shading shows ± s.e.