1   # Maintenance of fertility in the face of meiotic drive

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1

16   **Abstract**

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18   Selfish genetic elements that gain a transmission advantage through the destruction of

19   sperm have grave implications for drive male fertility. In the X-linked SR meiotic drive

20   system of a stalk-eyed fly, we found that drive males have greatly enlarged testes and

21   maintain high fertility despite the destruction of half their sperm, even when challenged

22   with fertilising large numbers of females. Conversely, we observed reduced allocation of

23   resources to the accessory glands that probably explains the lower mating frequency of SR

24   males. Body size and eyespan were also reduced, which are likely to impair viability and pre-

25   copulatory success. We discuss the potential evolutionary causes of these differences

26   between drive and standard males.

27 **Introduction**

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29 Meiotic drive genes gain a transmission advantage through manipulation of meiosis or

30 gametogenesis and are likely to have profound ecological and evolutionary consequences,

31 ranging from the evolution of sex determination systems and changes in karyotype, to

32 impacts on population persistence and sexual selection (Hurst and Werren 2001; Jaenike

33 2001; Werren 2011; Lindholm et al. 2016). Drivers have been uncovered in a wide range of

34 taxa, with a preponderance of linkage to the sex chromosomes in the heterogametic sex

35 (Hurst and Pomiankowski 1991; Jaenike 2001; Taylor and Ingvarsson 2003). When meiotic

36 drive occurs in males, it severely disrupts the maturation and fertilisation capacity of non-

37 carrier sperm, imposing a fertility disadvantage to organismal fitness (Price and Wedell

38 2008) which is exaggerated under conditions of sperm competition (Taylor et al. 1999;

39 Angelard et al. 2008; Price et al. 2008*a*) and typically has pleiotropic viability costs in both

40 sexes (Burt and Trivers 2006).

41

42 The extent to which these and other detrimental effects of sperm-killer drive promote

43 adaptive responses in the host species has received limited attention. There is an extensive

44 literature on genetic elements that interfere and suppress the action of drive. For example,

45 in *Drosophila* species, suppressors of X-linked drive have been found on the Y chromosome

46 (Carvalho et al. 1997; Cazemajor et al. 1997; Branco et al. 2013) and throughout the rest of

47 the genome (Carvalho and Klaczko 1993; Atlan et al. 2003; Tao et al. 2007). A more recent

48 suggestion is that drive may promote the evolution of female polyandry in order to dilute

49 the ejaculates of drive males (Haig and Bergstrom 1995; Zeh and Zeh 1997; Wedell 2013).

50 There is some evidence for this from experimental evolution studies using populations

51   exposed to meiotic drive in *D. pseudoobscura* (Price et al. 2008*b*) and *Mus musculus*

52   (Manser et al. 2017), and from natural populations in which the rate of multiple mating

53   correlates negatively with the frequency of drive in *D. pseudoobscura* (Price et al. 2014) and

54   *D. neotestacea* (Pinzone and Dyer 2013). Female mate choice may additionally evolve in

55   response to drive. In stalk-eyed flies, meiotic drive has been linked to small eyespan, which

56   may allow females to avoid mating with carrier males through assessing eyespan (Wilkinson

57   et al. 1998*b*; Cotton et al. 2014). Female house mice could avoid mating with drive males

58   through detecting unique major histocompatibility alleles linked to the driving *t* complex

59   (Silver 1985; Lindholm et al. 2013), although evidence remains unclear (Lindholm and Price

60   2016).

61

62   Another, as yet unexplored, route by which males could adapt to drive is by increasing the

63   allocation of resources to sperm production, to offset the destructive effect of drive on

64   gametogenesis. Sperm number is positively correlated with testis size in many intra-specific

65   studies (Gage 1994; Fry 2006; Hettyey and Roberts 2006) and increased testis size is a well

66   characterised evolutionary response to heightened sperm competition favouring greater

67   sperm production (Hosken and Ward 2001; Pitnick et al. 2001; Simmons and García-

68   González 2008; Gay et al. 2009). The loss of sperm in drive males could be compensated for

69   by increased investment in testis. Meiotic drive elements are typically found within

70   inversions or other areas of low recombination that keep drive and insensitive responder

71   loci together (Palopoli and Wu 1996; Johns et al. 2005; Dyer et al. 2007), facilitate the

72   spread of modifiers that enhance transmission distortion (Hartl 1975; Larracuente and

73   Presgraves 2012) and are predicted to be enriched for male beneficial sexually-antagonistic

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74   alleles (Rydzewski et al. 2016). For similar reasons, alleles that enable compensatory

75   investment in testes could become associated with the drive haplotype.

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77   We test this idea using the Malaysian stalk-eyed fly species *Teleopsis dalmanni*. This species

78   harbours SR, an X-linked driver, which produces strongly female-biased broods due to the

79   destruction of Y-bearing sperm (Presgraves et al. 1997; Wilkinson and Sanchez 2001).

80   Meiotic drive arose around 2-3.5 Mya in the *Teleopsis* clade, and the X^{SR} drive chromosome

81   in *T. dalmanni* is estimated to have diverged from a non-driving ancestor (X^{ST}) around 1 Mya

82   (Swallow et al. 2005; Paczolt et al. 2017), and is characterised by a large inversion(s)

83   covering most of the X chromosome (Johns et al. 2005; Paczolt et al. 2017). X^{SR} is found at

84   appreciable frequencies (10 – 30%) across populations and generations (Wilkinson et al.

85   2003; Cotton et al. 2014) but appears to lack genetic suppressors (Reinhold et al. 1999;

86   Wolfenbarger and Wilkinson 2001; Paczolt et al. 2017). This means that there has been

87   ample time and opportunity for adaptive responses to selection to evolve in male carriers of

88   the drive chromosome.

89

90   We determined whether SR and standard (ST) males differed in their reproductive (testis

91   and accessory gland size) and morphological traits (eyespan and body size). Testis size

92   predicts the amount of sperm found within female storage (Fry 2006). Accessory glands

93   produce all non-sperm components of the ejaculate, and accessory gland size is positively

94   associated with male mating frequency (Baker et al. 2003; Rogers et al. 2005*a*, 2005*b*).

95   Body size and eyespan are also important predictors of male mating frequency (Wilkinson et

96   al. 1998*a*; Small et al. 2009; Cotton et al. 2010). We determined SR and ST sperm production

97   by mating them to low or high numbers of females over a 10-hour period, counting the

98   number of fertilized eggs produced. Males were also exposed to females over a short time

99   period (30-minutes) to compare the copulation rate of SR and ST males.

100

101   **Methods**

102

103   Details of stock collection and day-to-day upkeep can be found in the Appendix.

104   Experimental males were taken from the SR-stock population in which males are a ~50:50

105   mix of X^{SR} and X^{ST} genotypes. Experimental females were taken from the ST-stock

106   population, which lacks meiotic drive. Single non-virgin males were allowed to mate freely

107   with either one or five virgin ST-stock females, over a period of 10 hours. Mated females

108   were allowed to lay eggs for 14 days, by which time most females had stopped laying fertile

109   eggs. Fecundity was recorded through egg counts, and egg hatch was used as an estimate

110   for fertility. On the following day, experimental males, and a similar number of unmated

111   males, were anaesthetised on ice and their testes and accessory glands were removed (fig.

112   1A) and photographed under differential interference contrast microscopy. Organ area was

113   measured at x50 magnification by tracing the outline. Male eyespan (Hingle et al. 2001) and

114   a proxy for body size, thorax length, (Rogers et al. 2008) were measured.

115

116   In a second experiment, SR-stock males were introduced to two ST-stock non-virgin females

117   at artificial dawn. All copulations were counted during 30 minutes. To minimise any effects

118   on mating frequency due to female choice, the experimental males were standardised to

119   have a narrow range of eyespan (7.5 – 8.5 mm).

120

121   Males from both experiments were genotyped using either two X-linked INDEL markers,

122   *comp162710* and *cnv395*, or a microsatellite marker, *ms395*. Allele size of these markers

123   reliably indicates the SR genotype of the males in our laboratory stocks (Meade et al. 2018).

124

125   *Statistical analysis*

126

127   We tested if male genotypes differed in their morphological (body size and eyespan; linear

128   models) and reproductive traits (testis size and accessory gland size; linear mixed effects

129   models). Differences in relative trait sizes between genotypes, as well as in absolute trait

130   sizes (models where body size is excluded) are reported. The total number of fertile eggs

131   (Poisson generalised linear mixed effects model (GLMM)) and proportion fertility (fertile

132   eggs, non-fertile eggs; binomial GLMM) of females are compared when mated to SR (i.e.

133   XSR/Y genotype) or ST (i.e. XST/Y genotype) males. We also tested if male reproductive

134   traits, and their interaction with male genotype, were important predictors of fertility.

135   Lastly, we tested whether SR and ST males differed in their mating frequency over 30-

136   minutes by comparing the likelihood that SR and ST males mate at all (binomial GLMM), as

137   well as the total number of copulations among males that mated at least once (Poisson

138   GLMM).

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140   To avoid collinearity of male morphological and reproductive traits with body size, models

141   used residual values (Dormann et al. 2013). Where appropriate, experimental batch was

142   included as a random effect. Further details and model effect sizes can be found in the

143   Appendix.

144

145 **Results**

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147 *SR trait size*

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149 SR males had small body size (mean ± s.e. 2.290 ± 0.013 mm) compared to ST males (2.336 ±

150 0.009 mm; $F_{1,357}$ = 8.745, P = 0.003; fig. 1B). SR males also had small absolute (SR: 8.048 ±

151 0.046mm; ST: 8.402 ± 0.031mm; $F_{1,357}$ = 42.631, P < 0.001; fig. 1B) and relative eyespan

152 ($F_{1,355}$ = 0.713, P = 0.016), especially when body size was small (body size by genotype

153 interaction $F_{1,355}$ = 4.175, P = 0.042).

154

155 Despite their small body size, SR testis size was large (1.940 ± 0.050 mm^2) compared to ST

156 males (1.54 ± 0.028 mm^2; $F_{1,280.16}$ = 73.796, P < 0.001; fig. 1C). SR males also had large

157 relative testis size ($F_{1,282.78}$ = 99.982, P < 0.001). In contrast, SR males had small absolute (SR:

158 0.306 ± 0.011 mm^2; ST: 0.348 ± 0.010 mm^2; $F_{1,335.36}$ = 16.353, P < 0.001; fig. 1D) and relative

159 accessory gland size ($F_{1,334.03}$ = 7.801, P = 0.006). Taking relative values for each genotype,

160 eyespan ($F_{1,286}$ = 19.892, P <0.001) and accessory gland size ($F_{1,274.418}$ = 26.008, P <0.001)

161 increased with testes size, but the rate was reduced in SR males (interaction eyespan: $F_{1,286}$

162 = 5.261, P = 0.023, fig. A1; interaction accessory glands: $F_{1,268}$ = 8.375, P = 0.004, fig. A2).

163

164 *SR fertility*

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166 SR males did not differ from ST males in total (mean ± s.e. SR: 112.047 ± 8.290, ST: 107.053

167 ± 5.597; χ^2_1 = 2.416, P = 0.120, N = 215; fig. 2A, 2B) or proportion fertility (SR: 0.833 ± 0.025,

168 ST: 0.762 ± 0.019; χ^2_1 = 2.469, P = 0.116, N = 215) when kept with females over an extended

8

169   10-hour period. Males mating with five females achieved higher total fertility (one female:

170   79.231 ± 5.090, five females: 138.123 ± 6.653; χ^2_1 = 43.698, P < 0.001, N = 215) but a lower

171   proportion fertility (one female: 0.804 ± 0.024, five females: 0.763 ± 0.199; χ^2_1 = 6.021, P =

172   0.014, N = 215) than those mating with a single female. The interaction between mating

173   group (one or five females) and genotype did not influence total (χ^2_1 = 0.591, P = 0.442, N =

174   215) or proportion fertility (χ^2_1 = 1.377, P = 0.241, N = 215).

175

176   Male testis size was an important predictor of fertility. Both total (χ^2_1 = 5.897, P = 0.015, N =

177   165; fig. 2C, 2D) and proportion fertility (χ^2_1 = 18.837, P < 0.001, N = 165) were greater

178   amongst males with larger testis size, even when accounting for male body size (total: χ^2_1 =

179   6.216, P = 0.013, N = 165; proportion: χ^2_1 = 16.646, P < 0.001, N = 165). The addition of testis

180   size did not alter the relationship between genotype and total (χ^2_1 = 0.018, P = 0.895, N =

181   173) or proportion fertility (χ^2_1 = 0.260, P = 0.610, N = 173). There was no interaction

182   between testis size and genotype predicting total (χ^2_1 = 0.164, P = 0.686, N = 173) or

183   proportion fertility (χ^2_1 = 0.617, P = 0.432, N = 173). Accessory gland size did not predict

184   total (χ^2_1 = 0.032, P = 0.858, N = 165) or proportion fertility (χ^2_1 = 0.160, P = 0.689, N = 165).

185

186   *SR mating frequency*

187

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

189   SR males (mean ± s.e. 2.750 ± 0.175, N = 81) copulated fewer times on average than ST

190   males (3.550 ± 0.186, N = 76; χ^2_1 = 6.304, P = 0.012; fig. 1E), but were not less likely to mate

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

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Discussion

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194

195 One of the main features of drive in males is reduced sperm production due to the

196 dysfunction of non-carrier sperm. This has been reported to cause a loss in fertility in a

197 variety of species including *Drosophila* (Hartl et al. 1967; Jaenike 1996; Angelard et al. 2008;

198 Price et al. 2012; Pinzone and Dyer 2013), house mice (Carroll et al. 2004), and *Silene alba*

199 (Taylor et al. 1999). Here, we present evidence that SR males in *T. dalmanni* overcome this

200 deficit by having greatly enlarged testes. SR males carry an extreme form of the X^{SR} drive

201 chromosome, siring female-only broods due to the dysfunction of Y-bearing gametes.

202 Despite gamete loss, SR males achieve fertility at a level equivalent to that of ST males, both

203 when exposed to a single female or 5 females over a 10-hour period (fig. 2). Our results

204 contradict a previous study which found an SR fertility deficit using a similar design

205 (Wilkinson et al. 2006). But this study measured fertility as the number of adults that

206 eclosed, compounding fertility with egg-to-adult survival. Recent work shows larval survival

207 is reduced in drive heterozygous females (Finnegan et al. 2019), which could account for the

208 drop in SR male fertility. The patterns in *T. dalmanni* are in contrast to other insect species

209 with X-linked meiotic drive which generally show a deficiency in fertility of drive males

210 either after a single or multiple matings (Jaenike 1996; Atlan et al. 2004; Angelard et al.

211 2008; Price et al. 2012; Pinzone and Dyer 2013).

212

213 These experiments were designed to test whether daily sperm reserves differ between SR

214 and ST males, not to replicate normal levels of mating observed under natural conditions

215 which occur at far lower rates (Cotton et al. 2015). On dissection, we discovered that SR

216 males have greatly enlarged testes (fig. 1C), about 26% larger than ST males. This difference

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217   remained after controlling for body size (fig. 1C). Our interpretation is that the increase in

218   testis size allowed SR males to compensate for the loss of sperm due to the action of

219   meiotic drive. This is supported by the finding that fertility increased with increasing testis

220   size, both for absolute and relative testis size, in both SR and ST males (fig. 2). Our

221   interpretation also aligns with previous findings that SR male ejaculates deliver similar

222   numbers of sperm as ST males, after single and multiple matings (Meade et al. 2018).

223   Despite the destruction of half their sperm, the increased investment in SR testis size (i.e.

224   sperm production) allows them to deliver sufficient sperm to achieve similar fertility as ST

225   males. To further understand the extent of this compensation, we need to assess SR male

226   success under sperm competition, which is the norm in *T. dalmanni* (Wilkinson et al. 1998*a*;

227   Baker et al. 2001; Corley et al. 2006). Previous work suggests that SR males perform poorly

228   under sperm competition (Wilkinson et al. 2006) but this assessment again does not take

229   account of the lower egg-to-adult viability of X^{SR} carriers (Finnegan et al. 2019) which could

230   simulate an advantage of ST males in sperm competition. In our experimental design,

231   autosomal background was standardised across SR and ST males. So, it seems likely that

232   control of testis size is linked to alleles that are located in the X^{SR} chromosomal inversion

233   and that such alleles arose as an adaptive response to sperm dysfunction caused by drive,

234   but further investigation is needed to establish this view.

235

236   We found morphological trait divergence in accessory gland size, which are small in SR

237   males, even after controlling for body size (fig. 1D). Previous work in *T. dalmanni* shows that

238   accessory gland size is linked with the mating rate (Baker et al. 2003; Rogers et al. 2005*a*).

239   This might explain why the mating frequency of SR males was low, being about 75% of the

240   rate for ST males over a 30-minute period (fig. 1E). In addition, SR males have small body

241   size and small eyespan for their body size (fig. 1), traits likely to reduce male mating success,

242   both in male-male agonistic interactions (Panhuis and Wilkinson 1999; Small et al. 2009) and

243   in attracting and mating with females (Wilkinson and Reillo 1994; Hingle et al. 2001; Cotton

244   et al. 2010). The increased allocation of resources to testes in SR males potentially causes a

245   reduction in the resources available for investment in accessory glands, as both traits

246   develop over several weeks post-eclosion (Baker et al. 2003; Rogers et al. 2008). Resource

247   competition with testes is not an obvious reason for reduced body size and eyespan which

248   are determined during larval development. However, expression of these traits might be

249   connected via juvenile hormone which has been shown to mediate a trade-off between

250   eyespan and testes in stalk-eyed flies (Fry 2006).

251

252   Small body size and eyespan are also likely to arise from the low genetic condition of drive

253   males. The *T. dalmanni* SR inversion(s) covers nearly all of the X chromosome, capturing one

254   third of the stalk-eyed fly genome (Johns et al. 2005; Paczolt et al. 2017). X^{SR} alleles will be

255   subject to weak natural selection due to reduced recombination and liable to accumulate

256   deleterious mutational effects (Kirkpatrick 2010). Consistent with a lack of recombination,

257   there are 955 fixed sequence differences between transcripts linked to X^{SR} and X^{ST}

258   (Reinhardt et al. 2014). Such mutations are expected to have a negative effect on costly,

259   condition-dependent traits, such as body size and eyespan, whose expression is affected by

260   multiple loci distributed throughout the genome (David et al. 2000; Cotton et al. 2004;

261   Bellamy et al. 2013). Given SR males have small eyespan, they will be unattractive and gain

262   fewer mating opportunities. Consequently, investment in accessory glands which enable

263   higher mating rates will give lower returns than the diversion of resources into larger testes

264   which allow SR males to produce ejaculates of equivalent size to those of ST males, and be

265   able to compete under the conditions of high sperm competition seen in stalk-eyed flies.

266   These ideas about linking resource allocation, condition and mating rates need further

267   investigation, in particular under the mating conditions that occur in the wild.

268

269   Here we demonstrate for the first time that through investment in testis, drive males can

270   maintain fertility, despite sperm destruction. Other responses to drive, such as genetic

271   suppression, polyandry and female choice, reduce the transmission advantage gained by

272   drive, and lead to reductions in the equilibrium frequency of drive (Hartl 1975; Taylor and

273   Jaenike 2002; Holman et al. 2015). In sharp contrast, increased investment in sperm

274   production intensifies the transmission of drive, because the fertility gain to the individual

275   male is also beneficial to the drive element itself. Such an association with meiotic drive has

276   neither been theoretically modelled nor empirically studied previously, but has implications

277   for the spread and equilibrium frequency of drive in natural populations.

278 **Authors' contributions**

279 All authors contributed to conceiving the project and methodology; LM and RK collected

280 data on fertility and morphology; SF collected data on mating frequency; LM analysed the

281 data; LM, KF and AP led the writing of the manuscript. All authors contributed critically to

282 the drafts and gave final approval for publication.

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288 **Data accessibility**

289 Data will be uploaded to the Dryad Digital Repository

290

291 **Ethical statement**

292 No ethical approval was required for this research

293 **Literature cited**

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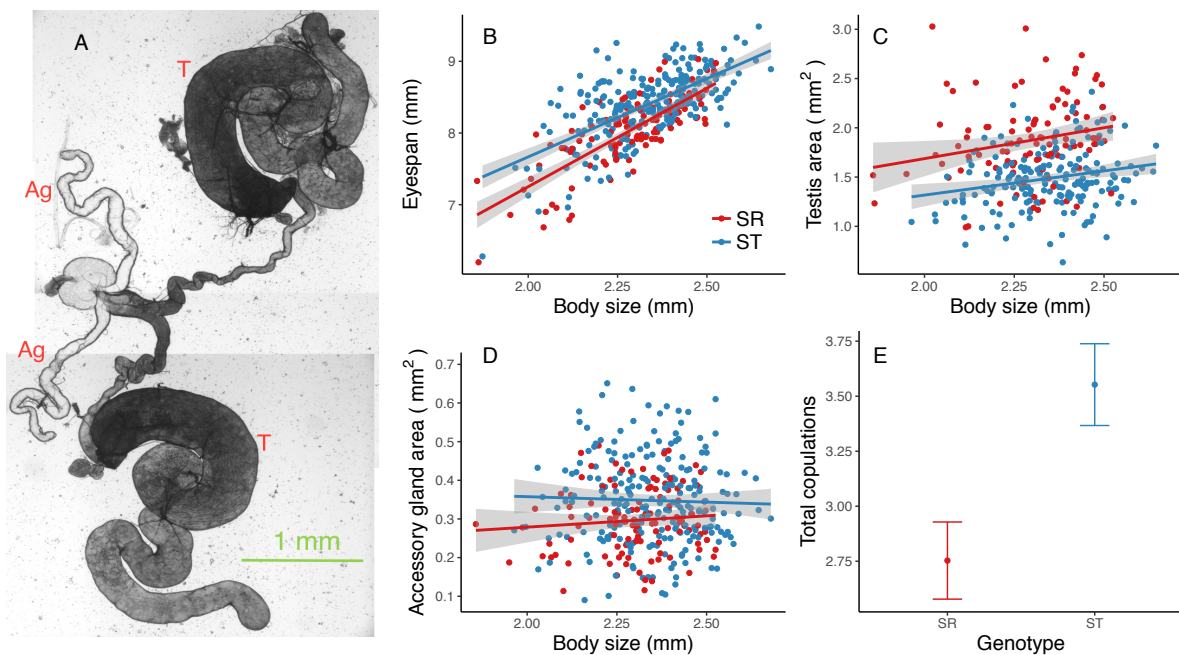
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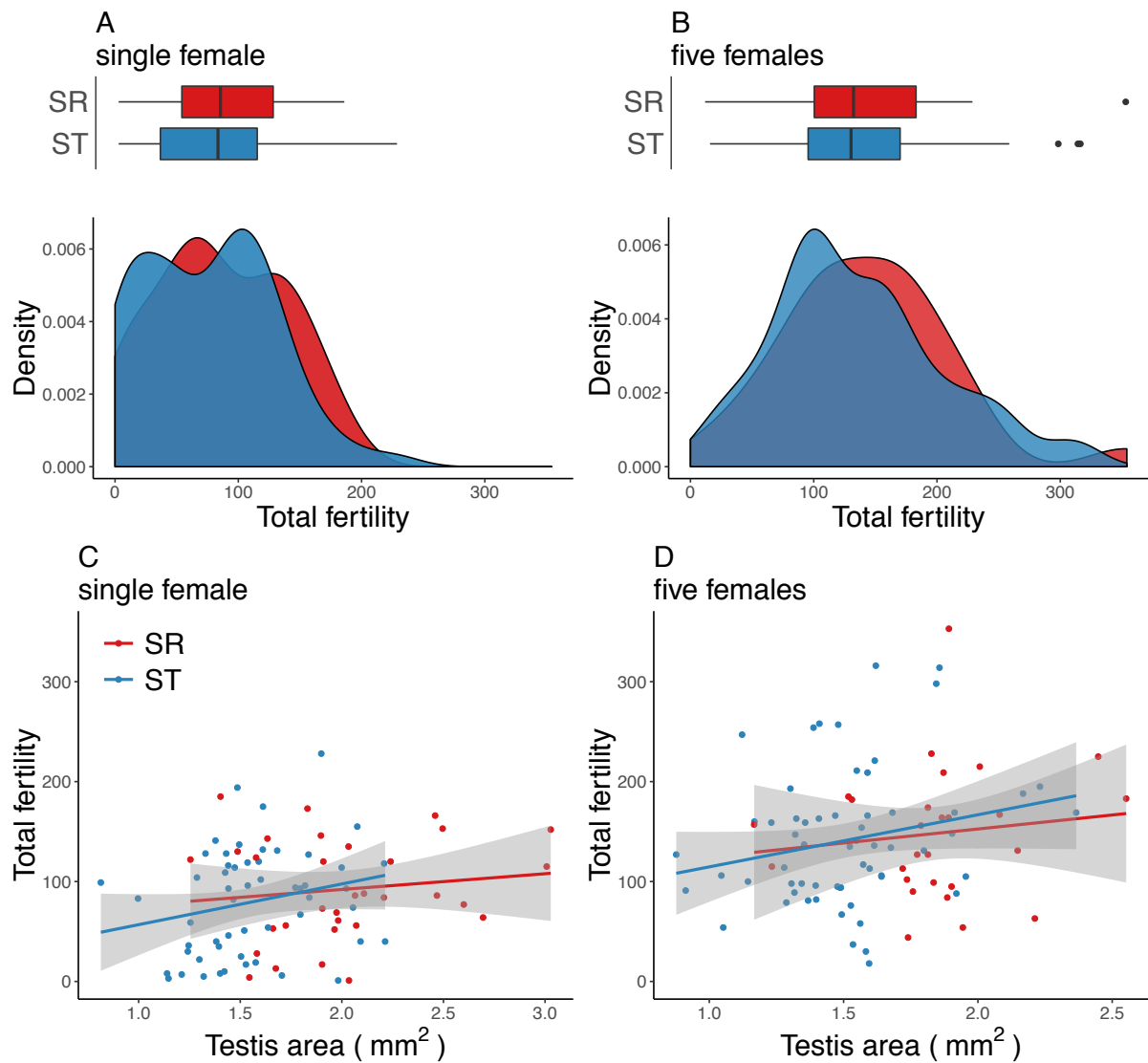

Figure 1

A, testes (T) and accessory glands (Ag) after dissection. *B–D*, male morphological and reproductive trait size for SR (red) and ST (blue) males, plotted against male body size. *B*, male eyespan. *C*, male testis area. *D*, male accessory gland area. SR males have smaller body size, eyespan and accessory gland size, but larger testis size. Grey shading shows ± s.e. *E*, mating frequency, measured as total number of copulations (mean ± s.e.) observed over 30 minutes.

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. Across both mating regimes, SR and ST males did not differ in the number of eggs fertilised. *C–D,* absolute testis area plotted against total fertility. *C,* mated to a single female. *D*, mated to five females. Across both mating regimes, total fertility increased with testis area in SR and ST males. Grey shading shows ± s.e.