Journal of Ethology

Operational sex ratio does not influence the evolution of male mate choice in the Indian meal moth
--Manuscript Draft--

Manuscript Number:	
Full Title:	Operational sex ratio does not influence the evolution of male mate choice in the Indian meal moth
Article Type:	Article
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Abstract:	The costs and benefits of mate choice vary according to a range of social and demographic factors, including the operational sex ratio (OSR). The OSR influences the number of mating opportunities available to each sex, and can therefore strongly influences the cost of being choosy. Accordingly, many animals show short-term plasticity in choosiness in relation to changes in the OSR. However, whether such change persists over evolutionary time has rarely been tested experimentally. We examined male pre-copulatory mate choice in relation to female body size, in the Indian meal moth Plodia interpunctella. We used populations that have been reared at different adult sex ratios (and therefore OSRs) for 130+ generations. We found that small females were more likely to mate compared to large females, however this effect could not be attributed exclusively to male mate choice. There was no difference in male mate choice or mating latency between populations exposed to different sex ratio regimes. We would suggest that male post-copulatory choice is potentially more important than pre-copulatory choice in this species.

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Dougherty et al., 2019

1   For *Journal of Ethology*

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9   the Indian meal moth

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49   Introduction

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51   Mate choice involves the acceptance of some partners as mates and the rejection of others,

52   and is virtually ubiquitous across the animal kingdom (Andersson, 1994; Bonduriansky,

53   2001; Rosenthal, 2017). Mate choice is favoured when potential partners vary in quality in

54   some way, so that choosers can gain direct or indirect (genetic) benefits from mating

55   preferentially with high-quality individuals (Andersson, 1994; Kokko et al., 2003). However,

56   there are also potential costs associated with mate choice, including time and energy costs

57   associated with searching for and assessing potential partners (Milinski & Bakker, 1992;

58   Jennions & Petrie, 1997), and the risk of remaining unmated if acceptable partners are not

59   encountered (Barry & Kokko, 2010; Greenway et al., 2015).

60

61   Importantly, the potential costs and benefits of mate choice are not constant, but can vary

62   depending on the social or ecological environment (Jennions & Petrie, 1997). This means

63   that the optimal strength of choice for a given trait (or "choosiness" following the

64   terminology of Reinhold & Schielzeth, 2014) may depend strongly on the environment.

65   Because of this, animals frequently exhibit plasticity in choosiness in relation to a range of

66   factors that can influence the costs and benefits of choice (Jennions & Petrie, 1997;

67   Dougherty & Shuker, 2015; Ah-King & Gowaty, 2016). For example, the number of available

68   mating opportunities is an important factor for determining the economics of choice, as it

69   influences the risk of remaining unmated if suitable partners are not encountered (Jennions

70   & Petrie, 1997; Barry & Kokko, 2010). The number of available mating opportunities is often

71   strongly dependent on the adult sex ratio, or, more correctly, the operational sex ratio

72   (OSR). The OSR represents the ratio of sexually active members of each sex in the

73   population (Kvarnemo & Ahnesjo, 1996), and represents the degree of competition for

74   access to mates: intrasexual competition is stronger in whichever sex is more common.

75   Accordingly, experimental manipulation of the OSR in several species has shown that mate

76   choice is stronger in whichever sex becomes more common (Berglund, 1994; Jirotkul, 1999),

77   and in extreme cases, changes in the OSR can lead to a complete reversal in which sex is

78   choosy (Gwynne & Simmons, 1990; Forsgren et al., 2004).

79

80   As well as examining how behaviours change in response to social and ecological conditions,

81   we can also use an experimental evolution approach to examine how such behaviours

82   change over evolutionary time (Kawecki et al., 2012). Experimental evolution is a powerful

83   technique because it allows us to test whether changes in the environment lead to

84   evolutionary responses, or whether there are other selection pressures which prevent this.

85   Experimental evolution studies have shown that mating preferences can evolve following

86   changes in larval diet (Dodd, 1989; Rundle et al., 2005) and artificial selection on ornaments

87   (Brooks & Couldridge, 1999). They have also shown that other aspects of mating behaviour

88   can evolve in response to changes in the population sex ratio (Snook et al., 2005; Ingleby et

89   al., 2010). However, we know of only one study that has examined changes in mate choice

90   following experimental manipulation of the population sex ratio. Sales et al. (2018)

91   manipulated adult sex ratio in the flour beetle *Tribolium castaneum* for 82-106 generations,

92   and then examined the frequency of male-male mating attempts, which may arise due to

93   failures in mate recognition or a reduction in choosiness in relation to partner sex. They

94   show that males from female-biased lines attempted to mate more frequently with males

95   compared to females, possibly because there is reduced selection on males in these lines to

96   discriminate between the sexes. More widely however, it remains unclear whether changes

97   in the social environment can drive changes in the strength of mate choice over multiple

98   generations, and how rapidly these changes can occur.

99

100   In this study we test whether the strength of male mate choice has evolved in response to

101   experimental manipulation of the adult sex ratio in the Indian meal moth *Plodia*

102   *interpunctella*. We used populations of *P. interpunctella* that have been maintained under a

103   male-biased (MB; 3 males: 1 female) or a female-biased (FB; 1 male: 3 females) sex ratio for

104   over 130 generations (differences in development time mean the generation number differs

105   between populations). These populations differ in the number of available mating

106   opportunities available to each sex, in addition to mating rate: males gain more matings in

107   the female-biased regime than the male-biased regime, and vice-versa (Ingleby et al., 2010).

108   Further, these populations have diverged in a number of traits related to mating and

109   reproduction, including male ejaculate investment (Ingleby et al., 2010) and male and

110   female immune activity (McNamara et al., 2013). We here compare the strength of mate

111   choice for female body size between lines maintained at three different adult sex ratios,

112   including general lab populations maintained at an equal (1:1) sex ratio. Males in this

113   species have been shown to allocate sperm strategically in response to female body size

114   (Gage, 1998; but see Ingleby et al. 2010), relatedness (Lewis & Wedell, 2009), and age and

115   mating history (Cook & Gage, 1995). However, male pre-copulatory choice is yet to be

116   examined in this species.

117

118   We have two main questions. First, do males exhibit a mating preference based on female

119   body size? Second, has the strength of this preference evolved in response to changes in the

120   population sex ratio? We have two main predictions. First, we predict that males should

121 prefer to mate with larger females, as female size is positively correlated with fecundity in

122 this species (Gage, 1998; Lewis et al., 2011). Second, we predict that male mate choice

123 should be stronger in female-biased populations, as males here have both more potential

124 mating opportunities and so should be more selective with regards to mate quality.

125

Methods

127

128 We tested for male mating preferences using laboratory-reared males from seven

129 populations of *P. interpunctella*: three male-biased populations (MB1, MB2, MB3), three

130 female-biased populations (FB1, FB2, FB3) and a stock population maintained at equal sex

131 ratio. The stock population originated from individuals collected in Perth, Australia in 2001.

132 Larvae were reared on a standard diet of bran, yeast, organic honey and glycerol,

133 maintained at 28°c with a 16:8 h light:dark cycle (Cook & Gage, 1995). The selection lines

134 have been maintained in the laboratory for over ten years (for full methodology see Ingleby

135 et al., 2010). Briefly, the adult sex ratio is manipulated by introducing adults (120 adults per

136 generation) into mating pots at the correct ratio (stock populations maintained at 1:1 sex

137 ratio). Adults are then allowed to mate for 72 hours, and eggs are collected into a pot below

138 separated by gauze. After 72 hours, 400 eggs are randomly sampled to establish the next

139 generation. Therefore larval density and sex ratio is the same for all populations. Due to

140 divergence in the average development time the exact generation number varies across

141 populations. However, males from each population were taken from a single generation

142 only. We tested males from generations 133 (FB3), 134 (FB1, FB2, MB3), 135 (MB2) & 143

143 (MB1).

144

145   In order to control for female effects, all males were tested using females from the stock

146   population. In order to try to increase the variation in female body size for males to choose

147   between, we manipulated larval food for stock females. We created two food treatments,

148   each having the same larval density (60 eggs per pot), but with either high or low food

149   (High: 20g of food per pot, 333mg per larvae; Low: 4g of food per pot, 66mg per larvae). All

150   experimental males were reared at the same density as the high food treatment. Larvae

151   were sexed and separated into individual tubes containing a small amount of food (to help

152   pupation) after 14 days to ensure virginity. These tubes were checked daily for the presence

153   of adults in order to accurately determine age.

154

155   To assess male mating preference we used a dichotomous choice design, with two females

156   introduced into a universal tube with a male, with one female from each food treatment. In

157   order to distinguish between the females a small portion of the wing was cut from one of

158   the females the day before a trial under CO_2 anaesthesia. We randomly allocated females so

159   that half of the cut females were from the low food treatment and half from the high food

160   treatment. The females that were not cut were also anaesthetised as a sham control. During

161   trials the observer was blind to the female treatment. In each tube the females were

162   matched for age, and only receptive females were used in trials (observed in the 'calling'

163   position either on the previous day or on the morning of the trial). All individuals were

164   virgins aged between 1 and 4 days post adult eclosion. All trials were performed in the first

165   three hours of the dark phase (10am-1pm), under ambient light and at room temperature.

166   We recorded the mating latency and choice made by males. Trials were ended after 30

167   minutes if the male failed to mate (N= 25). Mating pairs were allowed to finish copulation

168   naturally (this takes between 60 and 120 minutes), and then all mated and unmated

169   individuals were euthanised. We performed 270 mating trials in total.

170

171   After mating trials we measured the size of one of the forewings for all individuals, as a

172   proxy for body size (Lewis et al., 2011). Wings were removed, and dipped briefly in HCl and

173   then bleach to remove scales. Wings were then placed on a microscope slide and

174   photographed using a mobile phone attached to a dissecting microscope. In the majority of

175   cases we removed the left forewing (428 out of 486, 88.1%), however there was no

176   significant difference in length between the left and right forewings (T test, t= -1.009, N=

177   486, P= 0.316). Wing lengths were measured using the software package ImageJ (Schneider

178   et al., 2012). We measured a sample of wings twice, and found that measurements were

179   highly repeatable (N= 36, R= 0.983). We obtained morphological data for 242 mated males

180   (25-32 males from each selection population and 68 from the stock population) and 486

181   females (243 trials).

182

183   We tested for the presence of male mate choice in two ways. First, we ran a generalised

184   linear mixed model, with mate choice across trials as a binary response variable, either in

185   relation to female food treatment (high or low food) or female size (large or small). Sex ratio

186   regime, male size, and male and female age (in days) were included as fixed factors, and

187   male line was included as a random factor. Second, we analysed female mating success,

188   with female individual (not trial) as the unit of analysis, using a generalised linear mixed

189   model with mating success as a binary response variable, and tube (243 trials) as a random

190   effect. We included sex ratio regime, female cutting treatment, female size, and the

191   interaction between female size and sex ratio regime, as fixed factors. We also present the

192 results of a simplified model after excluding two fixed factors (sex ratio regime and the

193 interaction effect) which had low explanatory power. In order to visualise the (potentially

194 non-linear) shape of sexual selection on female size, we produced fitness surfaces using

195 nonparametric cubic splines (Schluter, 1988). Curves were calculated using general additive

196 models using the package mgcv v1.8 (Wood, 2011), including female wing length as the

197 single predictor variable and the smoothing parameter obtained by minimising the GCV

198 score.

199

200 We also tested for differences in mating latency (irrespective of which female was chosen),

201 using a linear mixed model, with male line as a random factor, and sex ratio regime, male

202 size, and male and female age (in days) as fixed factors. Latency data was log-transformed

203 prior to analysis. All statistical analyses were performed in R version 3.5 (R Development

204 Core Team 2018).

205

206 Results

207

208 245 out of 270 mating trials resulted in the male making a choice. Males were not more

209 likely to choose high-food females versus low-food females (males mated with high-food

210 females in 128 out of 245 trials; Chi-square test, $\chi^2 = 0.49$, $df = 1$, $P = 0.48$). Further, the

211 proportion of males mating with high-food females did not differ across the three sex ratio

212 treatments ($\chi^2 = 0.39$, $df = 2$, $P = 0.82$). However, wing measurements revealed that there was

213 no difference in size between high-food and low-food females (T-test, $t = -1.047$, $N = 486$, $P = $

214 0.29).

215

216   We next tested whether males were more likely to mate with the larger of the two females

217   present during the trial. Males were in fact more likely to mate with the smaller of the two

218   females, however this effect was marginally non-significant (males mated with the smaller

219   female in 136 out of 243 trials; χ^2= 3.46, df= 1, P= 0.062; **Figure 1**). The likelihood of males

220   mating with the larger of the two females (N= 243 trials) was not significantly influenced by

221   sex ratio regime (χ^2_2= 3.75, P= 0.15; **Figure 1**), male size (χ^2_1= 0.38, P= 0.54), male age (χ^2_1=

222   0.07, P= 0.8) or female age (χ^2_1= 0.55, P= 0.46).

223

224   We next examined predictors of female mating success, this time using all females (N= 486

225   females from 243 trials). Again smaller females were more likely to mate (**Figure 2**), and this

226   relationship was significant for the minimal model, but not for the full model (**Table 1**).

227   However this relationship was not influenced by sex ratio regime (**Table 1**: interaction

228   between female size and male sex ratio). Further, female mating success was also

229   influenced by wing cutting, with cut females more likely to mate (**Table 1**; 243 mated

230   females, 145 cut and 98 uncut). This effect is not due to a size difference between cut and

231   uncut females (T-test, t= 1.3, N= 486, P= 0.19).

232

233   We also tested for differences in male mating latency, irrespective of which female was

234   chosen in a trial. Log-transformed male mating latency (N= 242 trials) was not significantly

235   influenced by sex ratio regime (χ^2_2= 2.9, P= 0.24; **Figure 3**), male size (χ^2_1= 1.8, P= 0.18), male

236   age (χ^2_1= 2.4, P= 0.12) or female age (χ^2_1= 2.61, P= 0.11).

237

238   Discussion

239

240   This study tested two main questions in relation to male mate choice in the Indian meal

241   moth *Plodia interpunctella*. First, is there male mate choice in relation to female body size in

242   this species? Second, has the strength of male choice evolved in response to changes in the

243   adult sex ratio over 140+ generations? We found that smaller females were more likely to

244   mate across all populations, suggesting that males may prefer to mate with smaller females.

245   This effect was detected even though our food treatments did not significantly influence

246   adult female body size. However, this relationship was not influenced by the sex ratio

247   experienced by males, despite large differences in the number of available mating

248   opportunities across experimental regimes. We also found no difference in overall male

249   mating latency in relation to male sex ratio regime.

250

251   We found evidence for pre-copulatory choice across our experimental populations: smaller

252   females were more likely to mate following dichotomous choice tests. This is opposite to

253   our prediction that larger females should be preferred by males, given that they have a

254   higher reproductive potential (Gage, 1998; Lewis et al., 2011), and that males allocate more

255   sperm to larger females during mating (Gage, 1998). Why would smaller females be more

256   likely to mate in this species? One problem with interpreting our results is that our

257   experimental design does not test male mate choice directly; because males and females

258   were able to fully interact before mating we cannot rule out the possibility that females also

259   contribute to mating decisions. Female mate choice has not been studied in this species, but

260   this pattern could arise if smaller females were more likely to accept matings than large

261   females, perhaps because the latter have higher fecundity and so may be choosier.

262   Courtship involves short-range male pheromone (Grant, 1974) and ultrasound production

263   (Trematerra & Gavan, 1995), but the actual behavioural interactions between males and

264 females are very brief. However, successful coupling does require female cooperation, and

265 females are able to reject matings (Grant, 1974; Lewis et al., 2013). Alternatively, the

266 observed pattern could also be due to smaller females being less able to resist forced

267 matings, as seen in other species (Arnqvist & Rowe, 2005). There is little evidence for active

268 mating struggles in this species. However, the last stage of courtship involves the male

269 attempting to grab the end of the female abdomen with his while both are in a face-to-face

270 position (Grant & Brady, 1974), a manoeuvre which would likely be easier if the female was

271 small and so closer to his size.

272

273 We did not detect any changes in male mating latency or choosiness due to the differing

274 population sex ratios. This is surprising given that males gain four times as many mating on

275 average in female-biased lines compared to male-biased lines (Ingleby et al., 2010).

276 Importantly, *P. interpunctella* do not feed as larvae; all spermatogenesis occurs during larval

277 development (Cook & Gage, 1995), so that males have a limited sperm supply that cannot

278 be replenished during adulthood. This life-history strategy, as well as the potential

279 reproductive benefits associated with mating with larger females (Gage, 1998; Lewis et al.,

280 2011), both support the idea that mate choice would benefit males in this species. However,

281 male mate choice appears weak, with males choosing the smaller female only 56% of the

282 time during dichotomous choice tests. One reason for this could be that male mating

283 success more strongly depends on a high mating rate or a high level of polygyny. Males have

284 been recorded as mating up to eight times in eight days in staged mating trials (Ryne et al.,

285 2001). However, in our experimental populations the male mating rate is more modest,

286 averaging only three matings in the female-biased lines (Ingleby et al., 2010). Alternatively,

287 in wild populations it may be that long-range male attraction to pheromone-emitting

288 females is more important than short-range mating interactions (Zhu et al., 2001). However,

289 such long-range signalling is probably not important in our lab populations, given the high

290 densities and small pots used for maintenance (1045 cm^3).

291

292 Another possibility is that in this species males may have historically been selected to accept

293 essentially all matings, and then strategically allocate sperm during mating. This is

294 supported by the fact that male post-copulatory choice appears much stronger than pre-

295 copulatory choice, with males being much more prudent when it comes to sperm allocation

296 in relation to female size, age, mating history and relatedness (Cook & Gage, 1995; Gage,

297 1998; Lewis & Wedell, 2009). However, we must note that male mate choice in relation to

298 the latter three traits has not been tested in this species. This difference between pre- and

299 post-copulatory choice could arise for example if males are unable to assess female state

300 before mating.

301

302 This study provides a rare examination of whether mate choice evolves in response to

303 consistent changes in the economics of choice over multiple generations. We detected no

304 clear effect of the population sex ratio on male mate choice or mating latency. This is

305 surprising given the importance of the OSR for driving short-term changes in choosiness

306 (Jennions & Petrie, 1997; Ah-King & Gowaty, 2016), as well as being a key determinant of

307 the strength of sexual selection acting on each sex (Janicke & Morrow, 2018). Nevertheless,

308 we suggest that the evolvability of mate choice should be investigated further, both in

309 relation to the operational sex ratio and to other factors that influence the economics of

310 mate choice, such as predation risk or population density (Jennions & Petrie, 1997; Ah-King

311 & Gowaty, 2016). Such work is needed in order to bridge the gap between the many studies

312 showing short-term plasticity in choosiness, and long-term population-level changes. Doing

313 so will help us to better elucidate the conditions favouring the origin and maintenance of

314 mating preferences over evolutionary time.

315

Acknowledgments

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Figures and tables

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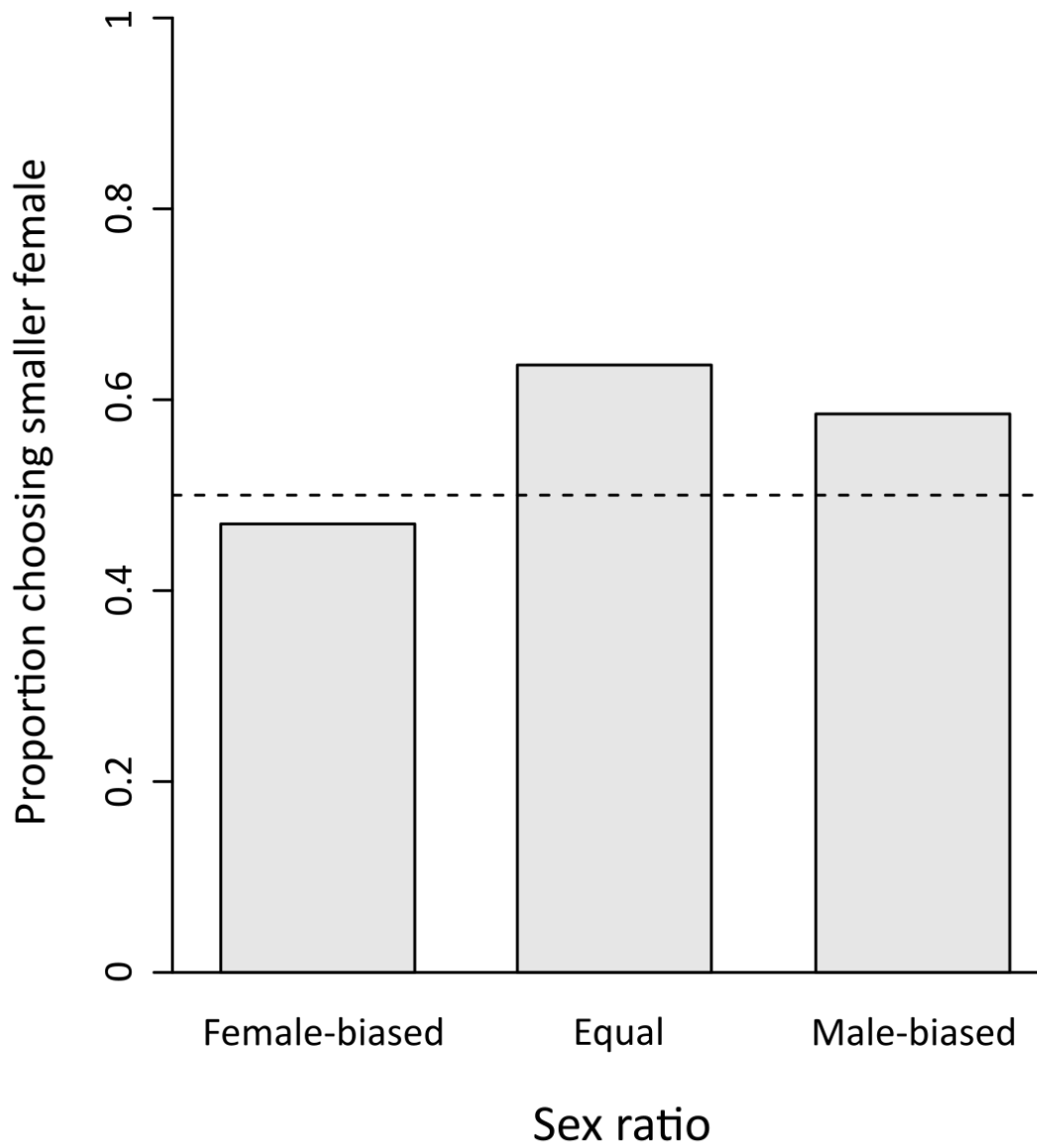53
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Figure 1. The proportion of males mating with the largest female during dichotomous choice trials, separated according to the three male sex ratio regimes (N= 83 female-biased, 66 equal, 94 male-biased). All females originated from an equal sex ratio population.

Table 1. Results of generalised linear mixed-models testing for determinants of female

mating success across all trials (N= 486 females in both cases). In both models mating

success was a binary response variable (mated or unmated) and trial was included as a

random effect. The full model (left) includes all relevant predictors and interactions. The

minimal model (right) shows the remaining predictors are removing non-significant

interactions and the main effect of sex ratio.

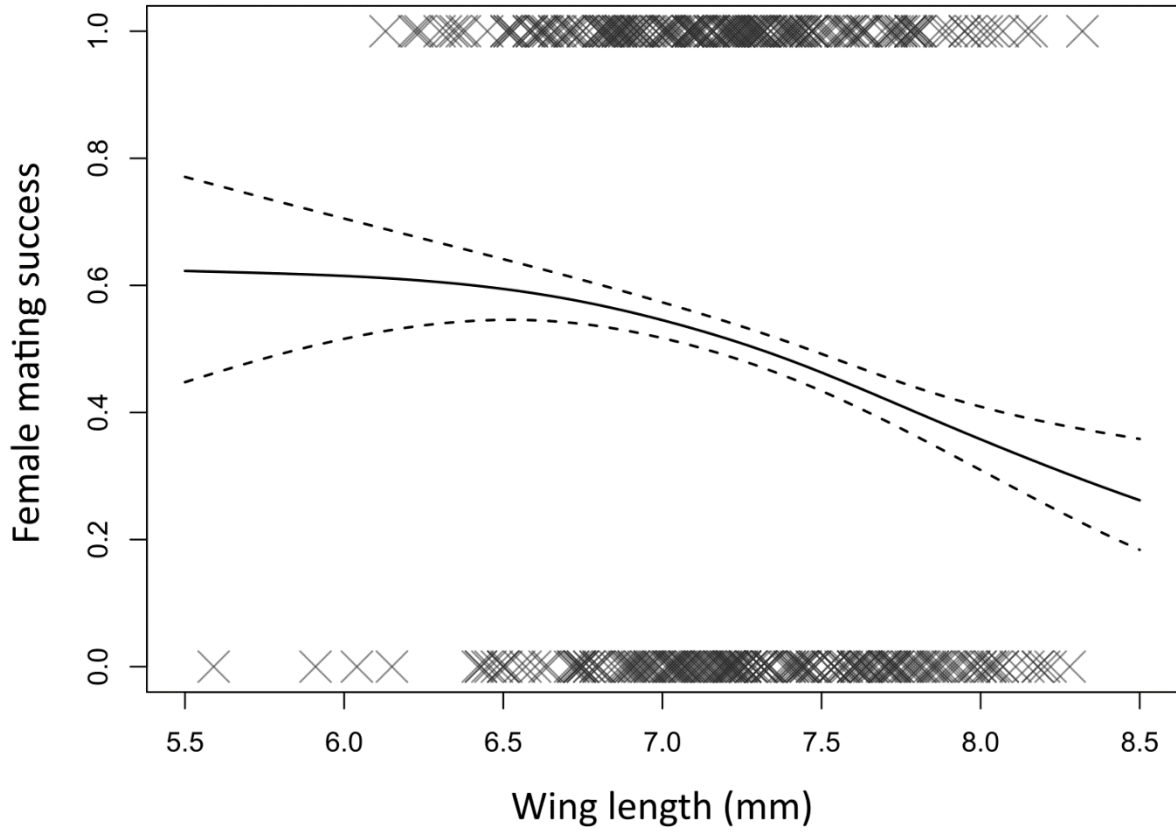|	Full model				Minimal model		
Factor	χ^2	df	P	*Factor*	χ^2	df	P
(Intercept)	0.4	1	0.53	(Intercept)	12.55	1	<0.001
Female size	0.24	1	0.62	Female size	10.96	1	<0.001
Sex ratio	2.35	2	0.31	Female cut	19.85	1	<0.001
Female cut	20.05	1	<0.001				
Female size * sex ratio	2.38	2	0.3				

Figure 2. Relationship between female wing length and mating success (0= unmated, 1= mated) for stock females mating with males from all sex ratio regimes combined (N= 486 females). Fitted curve is a cubic spline (dotted lines shows ±1 standard error) fitted using a general additive model, see text for details.
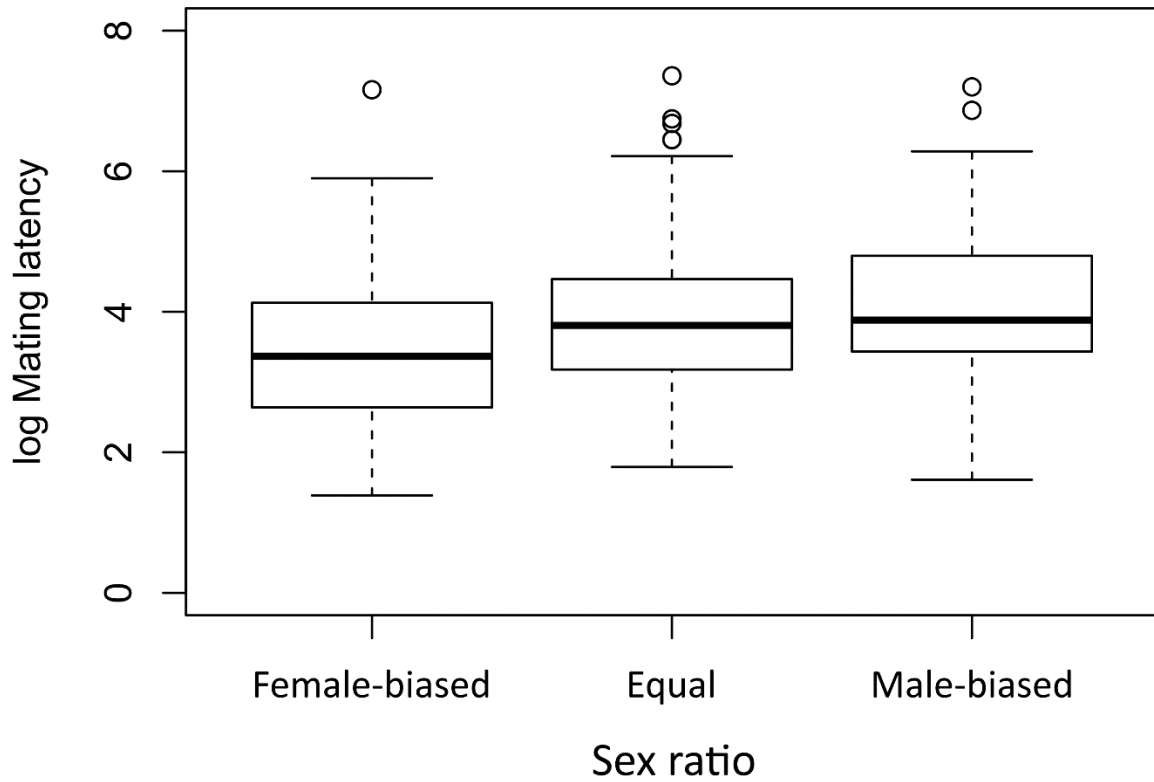
Figure 3. Mating latency (natural log, in seconds) in relation to male sex ratio regime, for

mated males (N= 83 female-biased, 68 equal, 94 male-biased). All females originated from

an equal sex ratio population.