1   Do female association preferences predict the likelihood of reproduction?

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15

25 **Abstract**

26 Sexual selection acting on male traits through female mate choice is commonly

27 inferred from female association preferences in dichotomous mate choice

28 experiments. However, there are surprisingly few empirical demonstrations that such

29 association preferences predict the likelihood of females reproducing with a particular

30 male. This information is essential to confirm association preferences as good

31 predictors of mate choice. We used green swordtails (*Xiphophorus helleri*) to test

32 whether association preferences predict the likelihood of a female reproducing with a

33 male. Females were tested for a preference for long- or short-sworded males in a

34 standard dichotomous choice experiment and then allowed free access to either their

35 preferred or non-preferred male. If females subsequently failed to produce fry, they

36 were provided a second unfamiliar male with similar sword length to the first male.

37 Females were more likely to reproduce with preferred than non-preferred males, but

38 for those that reproduced, neither the status (preferred/non-preferred) nor the sword

39 length (long/short) of the male had an effect on brood size or relative investment in

40 growth by the female. There was no overall preference based on sword length in this

41 study, but male sword length did affect likelihood of reproduction, with females more

42 likely to reproduce with long- than short-sworded males (independent of preference

43 for such males in earlier choice tests). These results suggest that female association

44 preferences are good indicators of female mate choice, but that ornament

45 characteristics of the male are also important.

46 Female pre-copulatory mating preferences have been implicated in the evolution of

47 the exaggeration of male traits in a wide range of species (Andersson 1994). This

48 process requires that female mating preferences influence the mating success of

49 males. One of the major problems in demonstrating the importance of female

50 preferences is in separating their effects on male reproductive success from the effects

51 of other processes such as male-male competition (Jennions & Petrie 1997). This is

52 particularly problematic in natural populations and, as a result, the most accurate way

53 of assessing female mating preferences is the use of experimental setups in laboratory

54 conditions (Rutstein et al. 2007).

55   There are a number of laboratory based experimental methodologies for

56 assessing female mating preferences (Houde 1997; Shackleton et al. 2005; Rutstein et

57 al. 2007) which vary in their efficacy at removing the effect of male-male competition

58 (Houde 1997). One of the most commonly used is the dichotomous mate choice or

59 two-stimulus design (Shackleton et al. 2005; Rutstein et al. 2007). Here, females are

60 allowed to associate with one of two males usually differing in their expression of a

61 single trait. In order to remove any effects of male-male competition males are usually

62 prevented from interacting with each other through the use of barriers or other

63 methods for restraining males. Often experiments are set up such that the female can

64 interact with both males, but the males are unaware of each other's presence. Female

65 preference is then assessed based on the relative amount of time the female spends

66 associating with the two males. While this protocol removes the effect of male-male

67 competition, it has been criticised for preventing physical interaction, removing cues

68 that females may use under natural conditions (such as chemical and mechanical),

69 reducing the search costs to females to essentially zero and allowing males to be

70 compared side-by-side rather than sequentially (which may be more common in

71   nature), making it difficult to know whether association preferences represent sexual

72   preferences (Houde 1997; Wagner 1998; Gabor 1999; Shackleton et al. 2005).

73   Surprisingly, given these criticisms, there are relatively few experiments that

74   test whether or not the males that are preferred by females in such tests are also those

75   that would obtain matings if the sexes were able to mix freely (but see Houde 1988;

76   Kodric-Brown 1989; Clayton 1990; Aspbury & Basolo 2002; Cummings &

77   Mollaghan 2006; Lehtonen & Lindström 2008). One of the reasons for this is the

78   difficulty of allowing males to reproduce with females without reintroducing the

79   effect of male-male competition (Houde 1988; Shackleton et al. 2005). However, if

80   females have some control over mating, it is possible to allow single males of

81   differing status access to females and to compare some measure of mating success

82   between preferred and non-preferred males. Such experiments are described in the

83   literature as no choice tests (Shackleton et al. 2005). Measures of mating success

84   usually involve scoring the time until the first copulation, the number of times a

85   female copulates with a male, or the number of sexual responses from a female

86   (Pilastro et al. 2002; Shackleton et al. 2005). However in species where it is unclear

87   which copulations lead to successful fertilization (e.g. some poeciliid fish), another

88   option is to allow females unrestricted access to males and then monitor their

89   subsequent reproductive output. This set-up allows a test of whether or not female

90   mating preferences, as assessed from association times in dichotomous choice

91   experiments, translate into differences in the reproductive success of a male when

92   allowed free access to females, but in the absence of male-male competition.

93   It has been demonstrated that females mated with preferred males can allocate

94   more resources into the production of offspring than those mated with non-preferred

95   males (differential allocation) (Burley 1988; Rintamäki et al. 1998; Sheldon 2000).

96   Females can do this, for example, by adjusting clutch size, egg size, or egg

97   composition (Sheldon 2000). However, the majority of this work has been based on

98   the manipulation of male traits known to be important from other studies, or separate

99   experiments within the same study (Gil et al. 1999; Cunningham and Russell 2000;

100 Kolm 2001; Rutstein et al. 2004; Gorman et al. 2005). Very few studies have allowed

101 females to choose between males and then provided them with the actual male that

102 was preferred or non-preferred (but see Drickamer et al. 2000). Furthermore, the

103 generality of differential allocation remains unclear, since the great majority of studies

104 have been performed on birds (Sheldon 2000). In particular, the occurrence of

105 differential allocation in fish has rarely been tested (Sheldon 2000; but see Kolm

106 2001).

107   The green swordtail (*Xiphophorus helleri*) is a small species of neotropical

108 livebearing fish. Males almost cease body growth after sexual maturation, and instead,

109 invest in the growth of an extension of the caudal fin known as the "sword" that

110 develops during sexual maturation (Basolo 1998a). Females have been shown to

111 associate preferentially with males with larger swords in dichotomous mate choice

112 experiments (Basolo 1990, 1998b), but no experiment has directly tested whether this

113 translates into greater reproductive success for such males (but see Ryan & Wagner

114 1987; Cummings & Mollaghan 2006 for indirect support). We tested the hypothesis

115 that males that were preferred in association tests were also the males that obtained

116 matings with these same females when allowed free access. We also used this data to

117 test the hypothesis that females differentially allocate resources to reproduction with

118 preferred males. Females in this study varied in their preference, with some females

119 preferring long- and some short-sworded males. This allowed an examination of

120 whether or not male sword length influenced the likelihood of females reproducing

121 with preferred or non-preferred males.

122

123 **Materials and methods**

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125 Experimental animals

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127　　　　Females used in this experiment were second or third generation offspring of

128 wild-caught females from Belize, Central America. Juveniles were reared in small

129 mixed sex groups (2-5 individuals) and checked daily for sexual maturation, defined

130 as the initiation of the development of the gonopodium for males and the development

131 of the gravid spot for females (Walling et al. 2007, 2008). Males were removed from

132 these tanks as soon as development of the gonopodium was detected. Females were

133 mate choice tested 4-6 weeks after the gravid spot was first detected to ensure they

134 were sexually mature. The individuals used in this experiment were derived from a

135 larger study investigating the effect of social environment on rates of maturation and

136 the development of female mating preferences (Walling et al. 2007, 2008). However,

137 this variation in early social environment had no effect on any of the measures

138 analysed in the present study (analyses not shown) and so data were pooled for the

139 analyses presented here. All experiments took place during the period August 2003 to

140 June 2005, with behavioural trials being conducted between 0900 and 1600 h. Fish

141 were maintained on a 12:12 hour light:dark cycle at 23 ^{o}C and fed ad lib twice daily

142 with commercial fish pellet (Hikari tropical micro-pellets; Kyorin, Japan). All

143 experiments were conducted under full spectrum lights and all tanks contained gravel

144 substrate, corner filters and numerous artificial plants. Home tanks contained a mesh

145 cylinder as a refuge for fry and were visually isolated from any males but not females.

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148 Dichotomous mate choice tests

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150 A detailed description of the dichotomous mate choice design is given elsewhere

151 (Walling et al. 2008), so it is only described briefly here. Females were given the

152 choice between two males, size matched for standard length (±3mm) but differing in

153 sword length (sword length of long-sworded male, mean±SE = 22.05±0.39mm; short-

154 sworded male = 7.87±0.31mm). Males were presented at the front of the tank, with

155 the two males visually isolated from each other by opaque partitions. The female

156 could interact visually with both males through a transparent partition that was

157 siliconed in place to prevent water flow between compartments and thus the transfer

158 of mechanical and chemical signals. All females were assigned a preference based on

159 the relative amount of time during a 30 minute trial spent associating with each male

160 (i.e. within a 5cm preference zone in front of the male's compartment). Preference for

161 a particular male was defined simply as spending more time with one male than the

162 other, without considering the magnitude of the difference in time.

163   Sixty eight females that had been mate choice tested in this way were used in

164 this experiment. There was no population level preference for long-sworded males in

165 this study (time spent with long - time spent with short-sworded male (s); mean±SE =

166 -63.01±39.36s, one-sample t-test for significant deviation from the expectation of 0; t

167 = -1.601, df = 67, p = 0.11) (Walling et al. 2008); 39 females had a preference (as

168 defined above) for the short-sworded male and 29 for the long-sworded male. Of

169 females that preferred the short-sworded male, 20 were given access (see below) to

170 their preferred male and 19 to their non-preferred male, whereas of the females that

171 preferred the long-sworded male, 15 were given access to their preferred male and 14

172 to their non-preferred male. Thus approximately half of all females were allowed

173 access to their preferred male (35/68) and half (33/68) to their non-preferred male.

174

175 No choice protocol

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177 Pairing took place two weeks after mate choice assessment. Single females were

178 placed into large, visually-isolated mating tanks ($60 \times 30 \times 30$ cm (lwh), filled to a

179 depth of 22 cm) and allowed free access to their allocated male for two weeks.

180 Females were then removed and placed singly in 6 litre home tanks and checked daily

181 for fry. They were given a minimum of 6 weeks (mean for the whole experiment = 9.1

182 weeks) after being removed from the mating tank to give birth. Given the published

183 gestation period of 5 weeks for this species (Chong et al. 2004) this was assumed to

184 be enough time for them to produce a brood.

185   If females did not give birth during this time (N = 45 females, 20/45 with

186 preference for long-sworded male, 25/45 with preference for short-sworded male;

187 22/45 mated to non-preferred male, 23/45 mated to preferred male), they were

188 returned to the mating tanks and paired with a new male of the same phenotype as the

189 first male (i.e. long- or short-sworded). This new male was always previously

190 unfamiliar to the female. Again fish were allowed the opportunity of mating for two

191 weeks before the female was removed to her home tank and left for at least 6 weeks in

192 order to give birth. If females still failed to give birth after this period they were

193 scored as not reproducing for the analysis of the likelihood of reproduction and

194 removed from all other analyses. Because of time limitations, 7 females that failed to

195 give birth were not given access to a second male and were therefore scored as not

196 reproducing after access to a single male. Exclusion of these females did not influence

197 the results presented below and so all analyses are based on data including these

198 females.

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200 Monitoring female growth

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202 Females were measured on the day of pairing with the first male, and then every four

203 weeks after this for a period of 12 weeks. From 12 weeks onwards, weights and

204 measurements were only taken every eight weeks. Females were also measured the

205 day after giving birth. Measures were taken by first anaesthetising fish (licensed under

206 the UK Home Office Animal Scientific Procedures Act, licence number 60/3625) in

207 an aerated water bath using benzocaine in 95% alcohol at a concentration of 8ml/l.

208 Once anaesthetised, they were measured (to 0.1mm) for standard length (sl – from the

209 tip of the mouth to the caudal peduncle), total length (tl - from the tip of the mouth to

210 the end of the caudal fin), and body depth (maximum distance between the dorsal and

211 ventral surfaces), and were weighed (to 0.001g). Throughout this experiment female

212 size was defined as standard length cubed. This measure was used because mass was

213 greatly influenced by whether or not the female was close to parturition at the time of

214 weighing and there was a strong positive linear relationship between standard length

215 cubed and mass for newly matured females (linear regression, N = 105, Adjusted R-

216 squared = 0.98, $F_{1,103}$ = 4108, p < 0.001). Fish recovered from the anaesthetic within

217 seconds and this procedure had no obvious short or long term effects.

218

219   Brood measures

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221   Of the 68 females allowed access to a male in this experiment, 36 subsequently

222   reproduced. When fry were found in a home tank, the female was left for one day to

223   ensure the female had finished giving birth. Females were never observed chasing or

224   consuming fry within their home tank. The female was then removed for measuring,

225   and the total number of fry (live and dead) counted. On the same day, live fry were

226   transferred to a small tank to be weighed. Fry were weighed in a small plastic cup

227   (4.5cm diameter, 8cm height), which had the base removed and replaced with fine

228   mesh. This cup was wetted, dried with blotting paper, and weighed on an electronic

229   balance. This was repeated five times to give a mean value of the mass of the empty

230   cup. Fry were then transferred to the cup and the procedure for weighing the cup was

231   repeated, again five times (the average variance of the 5 measures = 0.5milligrams);

232   the mean mass of the empty cup was then subtracted from that of the cup plus fry, to

233   give the brood mass. This was then divided by the number of fry in the brood to give

234   an estimate of mass of individual fry. Only broods of 5 live fry or larger were weighed

235   in this way as the method was not accurate enough for smaller broods; measurements

236   were therefore only obtained from 25 of the 36 broods that were produced in total. Fry

237   were subsequently placed into six litre tanks in groups of 10 or less. Fry showed no ill

238   effects from the process of being weighed and mortality rates of broods that were

239   weighed were similar to those of unweighed broods (C. A. Walling unpubl. data).

240

241   Estimating reproductive investment

242

243   There is no evidence that female green swordtails can further invest in offspring

244   following fertilisation of the egg, being lecithotrophic as opposed to matrotrophic

245   (Houde 1997; Kruger et al. 2001). Consequently, measures that reflect the total

246   number of eggs a female allocates to be fertilised by a particular male should be a

247   more accurate representation of female investment in reproduction than measures of

248   the number/size of offspring surviving birth. Given the lack of correlation between the

249   average mass of a fry in a brood and either brood size (Spearman's rank correlation, N

250   = 25 broods, r = 0.227, P = 0.3), or female size (Spearman's rank correlation, N = 25,

251   r = 0.307, P = 0.14) and the strong correlation between brood size and brood mass

252   (Spearman's rank correlation, N = 25, r = 0.871, P < 0.001), only the total number of

253   fry in a brood was used as a measure of female investment in reproduction.

254   As a measure of female investment in future versus current reproduction, we

255   calculated female growth rates between pairing with a male and parturition. Growth

256   rates were calculated by dividing the change in female size (sl^3 at parturition - sl^3 at

257   mating) by the number of days between pairing and parturition. Growth was

258   calculated as a linear change with time as there was no evidence of an asymptotic

259   relationship (regression of growth rate on natural log transformed time: R-squared =

260   0.02857, $F_{1, 34}$ = 2.029, P = 0.16).

261

262   Data analysis

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264   This experiment generated three measures of female investment in reproduction: 1.

265   whether or not a female gave birth (likelihood of reproduction), 2. the number of fry

266   in a brood (brood size) and 3. female growth rate between mating and giving birth

267   (growth). Each of these three measures of female investment was independently

268   related to the factors; mate's status (whether or not a female was mated to a preferred

269   or non-preferred male), mate's sword type (whether a female was allocated a long- or

270   short-sworded male), male number (the number (1 or 2) of males a female had been

271   given access to before she reproduced), female age (days) and female size (sl^3). We

272   also included brood size in the analysis of female growth to test for a negative

273   relationship between the number of fry produced and the amount females grew. To

274   determine the significance of the factors in each of the 3 models, we constructed a

275   maximal model (containing the above factors and their two-way interactions) and then

276   compared models following sequential removal of the least significant terms (starting

277   with the highest order terms) until we reached a minimal model containing only

278   significant factors or interactions (Crawley 2005). Models were compared using

279   ANOVA (F tests) or penalised log likelihood scores (AIC) (Crawley 2005). Minimal

280   models were checked by adding all terms and their interactions back in, one by one,

281   and examining model AIC scores for possible improvement in model fit.

282   Data on whether or not females gave birth were analysed using a generalised

283   linear model (GLM) with binomial error structure. Data on brood sizes and female

284   growth were analysed using standard linear models. All data were assessed for

285   normality and homogeneity of variance and treated accordingly. Analyses were

286   performed in either R release 2.7.2 (R Development Core Team 2008) or SPSS 15.0

287   for windows.

288

289   **Results**

290

291   Likelihood of reproduction

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293   Following model simplification, the only factors to remain in the model were mate's

294   status (preferred or non-preferred) and mate's sword type (long or short). Females

295   were more likely to reproduce when paired with a preferred than a non-preferred male

296   (GLM with binomial error structure, $Z_{65} = 2.49$, $P = 0.01$ (Fig. 1)) and when paired

297   with a long- than a short-sworded male (GLM with binomial error structure, $Z_{65} =$

298   2.31, $P = 0.02$ (Fig. 1)). Illustrating this another way, there was a significant

299   interaction between a female's preference (i.e. for a long- or a short-sworded male)

300   and her likelihood of reproducing when given access to a preferred or non-preferred

301   male (GLM with binomial error structure, $Z_{64} = -2.31$, $P = 0.02$). Females that

302   preferred long-sworded males were more likely to reproduce with preferred males

303   (Fig. 1 column 1 compared to Fig. 1 column 4) whereas females that preferred short-

304   sworded males were equally likely to reproduce with preferred and non-preferred

305   males (Fig. 1 column 2 compared to Fig. 1 column 3).

306

307   Brood size

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309   None of the factors measured in this study or their interactions had a significant effect

310   on the size of the brood females produced. Model simplification resulted in the

311   removal of all terms from the model.

312

313   Growth

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315   The minimal model of female growth contained the factor male number. Females'

316   growth rate between pairing and parturition was slower when reproducing after access

317   to 2 males than when reproducing after access to 1 male (GLM, $t_{34} = -2.41$, $P = 0.02$,

318   Fig. 2). This significant effect of male number on growth rate was still significant

319   when including female size in the model to control for the possibility that females

320   mated to 2 males may be larger and therefore grow at a different rate than females

321   mated to a single male (GLM, effect of male number: t_{33} = -2.22, P = 0.03, effect of

322   female size: t_{33} = 0.064, p = 0.9).

323

324   **Discussion**

325

326   The results of this experiment demonstrate that female association preferences from

327   dichotomous choice experiments predict the likelihood of a female reproducing with a

328   male in the green swordtail. When given unrestricted access to a male, females were

329   more likely to reproduce if they had previously spent more time associating with that

330   male (or another male with similar sword length) in a dichotomous mate choice

331   experiment. However, the likelihood of reproduction was also influenced by the

332   sword length of the male to which a female was allowed access. Females were more

333   likely to reproduce when allowed access to a long- rather than a short-sworded male,

334   suggesting long-sworded males are either better able to solicit matings from females

335   or are more fertile. There was no strong evidence for differential allocation by

336   females: the number of fry produced by a female was not related to any measure of

337   female preference or any other factors measured in this study. A female's subsequent

338   growth rate after having mated appeared to vary depending on the number of males

339   she had access to prior to reproducing. Females that reproduced after access to two

340   males had a slower growth rate than those that reproduced after access to one male.

341   Despite the importance placed on female mating preferences in the evolution

342   of the exaggeration of male secondary sexual traits (Andersson 1994; Kokko et al.

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343   2003) and the number of studies using association preferences to infer female mating

344   preferences (e.g. Basolo 1990; Hill 1994; Blount et al. 2003; Fernandez & Morris

345   2008), this study is one of very few to test the relationship between association

346   preferences and actual reproduction. Even in the guppy (*Poecilia reticulata*), one of

347   the best studied systems in sexual selection, such studies are infrequent and either

348   include the effect of male-male competition (Bischoff et al. 1985) or use female

349   sexual responses to male displays rather than just association time as a measure of

350   female preference (Houde 1988; Pitcher et al. 2003). In general, experiments

351   addressing the link between female mating preferences based on association times and

352   male mating success tend either to confound the effect of mating preference and male-

353   male competition when assessing male mating success (Bischoff et al. 1985; Clayton

354   1990; Lehtonen & Lindström 2008) or to produce unclear results (Gabor 1999;

355   Aspbury & Basolo 2002; Rutstein et al. 2007).

356   Within the *Xiphophorus* genus particularly, confirmation of the relationship

357   between female association preferences and male mating success is indirect. In

358   *Xiphophorus nigrensis*, association preferences match the greater mating success of

359   larger males in nature (Ryan & Wagner 1987; Ryan et al. 1990) and there is a

360   correlation between association time and female gliding behaviour, which occurred

361   prior to putative copulation events (contact between the male gonopodium and the

362   female gonopore) in open access trials (Cummings & Mollaghan 2006). The results

363   presented here therefore represent the first direct test of the correlation between

364   female association preferences and the likelihood of reproduction in the *Xiphophorus*

365   genus. The lack of significant effect of the number of males a female had access to on

366   the likelihood of reproducing indicates that female preferences were generalised: if a

367   long-sworded male was preferred in the mate choice test and females did not

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368   reproduce after access to the first male, they were more likely to reproduce if

369   subsequent males were of that same, preferred sword-length.

370   Although our results generally support the use of association preferences as a

371   proxy for mating preferences, they also suggest that other factors may be important;

372   for instance, the sword length of the male a female was mated with also had a

373   significant effect on the likelihood of reproduction. Females that were paired with

374   long-sworded males were more likely to reproduce than females that were given

375   short-sworded males. Put another way, females were only more likely to reproduce

376   with a preferred male if their preference was for long- rather than short-sworded

377   males.

378   One possible explanation is that long-sworded males may be better able to

379   fertilize females than short-sworded males. This could be the result of differences

380   between long- and short-sworded males in fertility (sperm and ejaculate quality and/or

381   quantity) or the ability to coerce females into mating (female poeciliids are not in

382   complete control of mating (Houde 1997; Bisazza et al. 2001)). Although controlling

383   for male size should help to control for variation in sperm and ejaculate quantity, it is

384   possible that males with longer swords are in better condition and thus produce

385   ejaculates of better quality (for example sperm with less damage). For example, in

386   guppies, it has been demonstrated that more colourful males sire more offspring than

387   duller males when equal amounts of sperm from both were artificially inseminated

388   into females (Evans et al. 2003). This suggests that males with greater expression of a

389   sexually selected trait may produce more successful ejaculates, and this may interfere

390   with female mate preferences if females prefer males with lower expression of

391   sexually selected traits (this study; Wong & Rosenthal 2006).

392   Natural variation in sword length was used to produce the long- and short-

393   sworded males used in this study. Given that swords are grown throughout a males

394   sexually mature life (Basolo 1990), it is therefore likely that long-sworded males were

395   also older than short-sworded males. Age differences between long- and short-

396   sworded males would explain our results if older males were either better able to

397   solicit matings from females or produce more successful ejaculates. A relationship

398   between age, degree of sexual signal and testes size has been shown in the sedge

399   warbler (*Acrocephalus schoenobaenus*) (Birkhead et al. 1997), suggesting that older

400   males may produce larger and therefore more successful ejaculates. Artificial

401   manipulation of sword lengths would allow future studies to control for the effects of

402   male age, and close observation of pairs in mating tanks may allow quantification of

403   differences in males' ability to coerce a female into mating.

404   A limitation of the results presented here is that although females were

405   initially given a visual choice between two males, they were only given access to

406   solitary males and therefore were not given a choice at the time of actual mating. In

407   wild populations, although it has been suggested that simultaneous choice may be

408   uncommon (Wagner 1998), females may mate with multiple males (Luo et al. 2005).

409   Recent studies have shown that females can bias fertilisations towards males with

410   preferred phenotypes in some systems (Pizzari & Birkhead 2000; Evans et al. 2003;

411   Lewis & Austad 2004). However, in this experiment although preferred males were

412   more likely to gain reproductive success, there was still an effect of the sword length

413   of the male (a sexually selected trait (Basolo 1990; Rosenthal & Evans 1998)) on

414   likelihood of reproduction. It will therefore be interesting to investigate in this and

415   other study systems the relative influence of female preference and male sexually

416   selected trait expression on the reproductive success of males when females have

417   access to multiple males as may be more common in natural situations.

418   Finally, our measures of female investment in reproduction suggest that

419   females do not differentially allocate resources to offspring production depending on

420   their perception of the attractiveness of a male. Neither brood size nor female growth

421   rate depended on whether or not a female was mated to a preferred or non-preferred

422   male, with brood size unrelated to any factors measures in this study and growth rate

423   dependent only on the number of males a female had access to before she reproduced.

424   This may indicate that females are investing more in reproduction (as opposed to

425   growth) having rejected (or for some reason not reproduced with) one male than when

426   they reproduce with the first male they are given access to. It may also indicate that

427   females that failed to reproduce with the first male were in worse condition than those

428   that did reproduce with the first male and thus grow slower between mating and

429   parturition. However, the current data do not allow us to distinguish between these

430   hypotheses.

431   In conclusion, the results of this experiment support the use of female

432   association preferences as a proxy for female mating preferences. Such support is

433   generally rare and particularly so in the *Xiphophorus* genus of livebearing fish, a

434   common system for research into sexual selection. However, the sword-length of the

435   mate was also important, independent of preference, in predicting the likelihood of

436   reproduction. Further research is required to fully understand why this is the case and

437   to attempt to disentangle the effects of changes in female preferences from differences

438   between long- and short-sworded males in their ability to fertilize females.

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444

445   **References**

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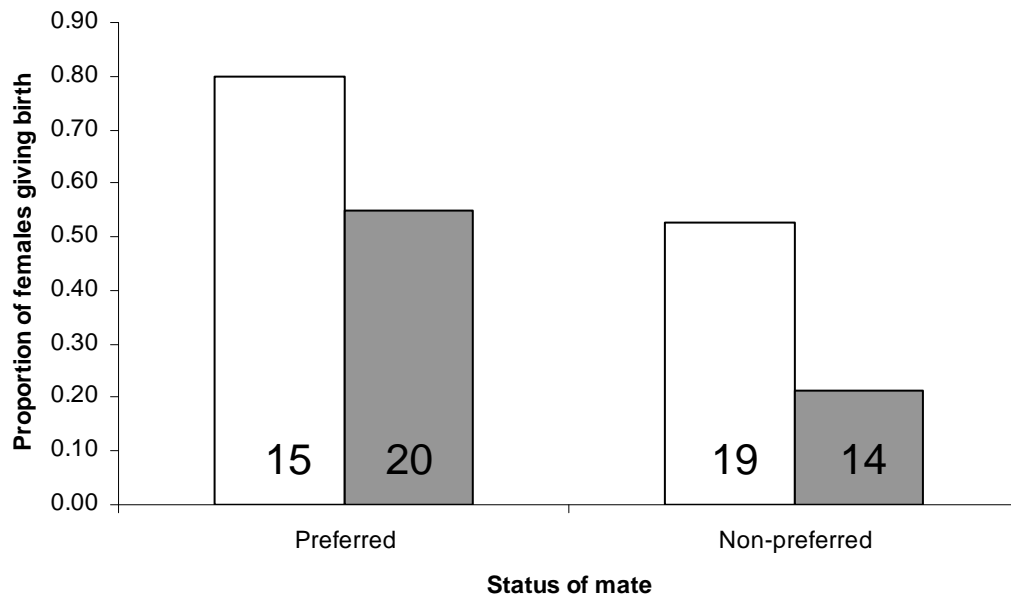
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550 **Fig 1** The effect of the male's status and sword length on the proportion of females

551 that gave birth (the total number of females in each group are given in the associated

552 bars). Females were more likely to give birth after access to a preferred male than a

553 non-preferred male and after access to a long- (clear bars) than a short-sworded (dark
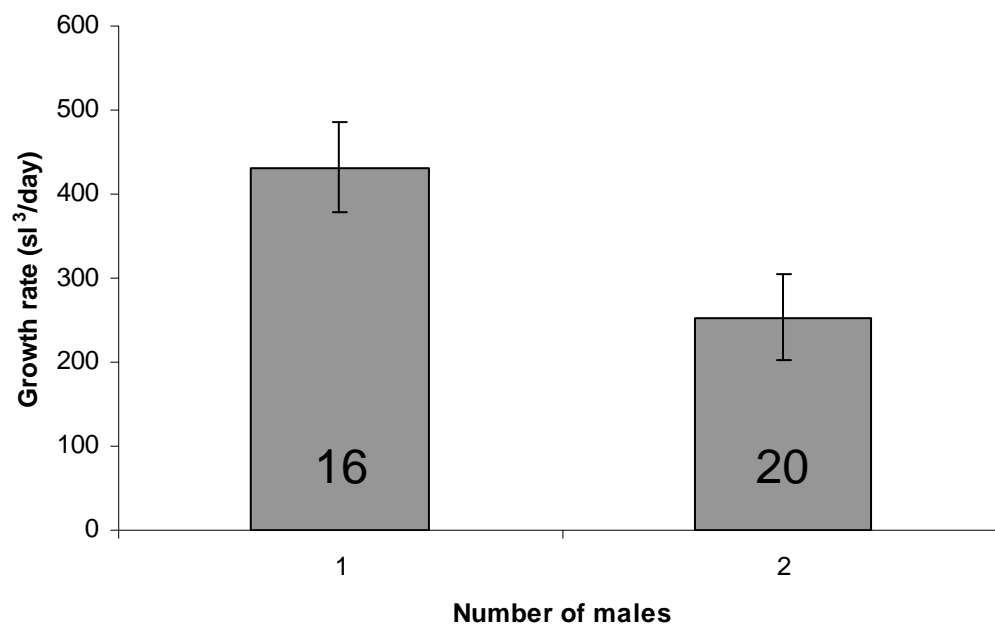
554 bars) male.

555

556 **Fig 2** The effect of the number of males a female had been given access to before

557 reproducing on her growth rate between pairing and parturition. Females grew slower

558 between pairing and parturition when reproducing after access to 2 males than after

559 access to 1 male (sample sizes are given in the associated bars).

560   **Fig 1**

561

562   **Fig 2**

563