Year: 2016

# Mate choice and the operational sex ratio: an experimental test with robotic crabs

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Abstract: The operational sex ratio (OSR: sexually active males: receptive females) predicts the intensity of competition for mates. It is less clear, however, under what circumstances, the OSR predicts the strength of sexual selection – that is, the extent to which variation in mating success is attributable to traits that increase the bearer's attractiveness and/or fighting ability. To establish causality, experiments that manipulate the OSR are required. Furthermore, if it is possible to control for any OSR-dependent changes in the chosen sex (e.g. changes in male courtship), we can directly test whether the OSR affects the behaviour of the choosing sex (e.g. female choice decisions). We conducted female mate choice experiments in the field using robotic models of male fiddler crabs (Uca mjoebergi). We used a novel design with two females tested sequentially per trial. As in nature, the choice of the first female to mate therefore affected the mates available to the next female. In general, we detected significant sexual selection due to female choice for 'males' with larger claws. Importantly, the strength of sexual selection did not vary across five different OSR/density treatments. However, as the OSR decreased (hence the number of available males declined), females chose the 'males' with the largest claws available significantly more often than expected by chance. Possible reasons for this mismatch between the expected and observed effects of the OSR on the strength of sexual selection are discussed.

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11   # test with robotic crabs

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37   **Title:** Mate choice and the operational sex ratio: an experimental test

38   **Abbreviated title:** Mate choice and the OSR

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42    predicts the intensity of competition for mates. It is less clear, however, under what

43    circumstances the OSR predicts the strength of sexual selection – that is, the extent to

44    which variation in mating success is attributable to traits that increase the bearer's

45    attractiveness and/or fighting ability. To establish causality experiments are required

46    that manipulate OSR. Furthermore, if it is possible to control for any OSR-dependent

47    changes in the chosen sex (e.g. changes in male courtship), we can directly test

48    whether the OSR affects the behaviour of the choosing sex (e.g. female choice

49    decisions). We conducted female mate choice experiments in the field using robotic

50    models of male fiddler crabs (*Uca mjoebergi*). We used a novel design with two

51    females tested sequentially per trial. As in nature, the choice of the first female to

52    mate therefore affected the mates available to the next female. In general we detected

53    significant sexual selection due to female choice for 'males' with larger claws.

54    Importantly, the strength of sexual selection did not vary across five different

55    OSR/density treatments. However, as the OSR decreased (hence the number of

56    available males declined), females chose the 'males' with the largest claws available

57    significantly more often than expected by chance. Possible reasons for this mismatch

58    between the expected and observed effects of the OSR on the strength of sexual

59    selection are discussed.

60

61    **Keywords:** density, female choice, mate sampling, mating preferences

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63

64 **INTRODUCTION**

65

66 It is well established that sexual selection has led to the evolution of costly male traits

67 that are advantageous during male-male contests, or when competing to attract

68 females (Andersson, 1994). It is less clear what demographic or ecological factors

69 generate variation among species or populations in these secondary sexual traits

70 (Cornwallis & Uller, 2010). One parameter traditionally used to predict the strength of

71 sexual selection is the operational sex ratio (OSR: ratio of sexually active males to

72 receptive females) (Emlen & Oring, 1977; for a review of sexual selection indices see

73 Henshaw *et al*., 2016). By definition the intensity of competition for mates is stronger

74 when the OSR is more biased. Greater mating competition need not, however, equate

75 to stronger sexual selection. This is because sexual selection is measured as the

76 *response* to competition (i.e. the extent to which variation in mating success among

77 individuals is due to differences in the expression of sexual traits) (Shuster, 2009;

78 Klug *et al*., 2010; Jennions *et al*., 2012). In some cases, it is more profitable to invest

79 in other fitness-enhancing traits when the intensity of mating competition increases

80 (e.g. parental care; Kokko & Jennions, 2008). As the OSR becomes increasingly

81 male-biased, variation in male mating success attributed to individual differences in

82 sexually selected traits might therefore even decrease.

83

84 There are several reasons why the relationship between the OSR and the strength of

85 sexual selection on focal traits cannot be derived from first principles. First, there is

86 an imperfect correlation between the intensity of sexual competition (i.e. OSR) and a

87 sexually selected response (e.g. greater investment in ornaments or weaponry) if there

88 are also ways to increase fitness via naturally selected traits (e.g. parental care,

89  defence against parasitism) (reviews: Kokko & Jennions, 2008; Jones, 2009). Second,

90  proximate factors that affect the intensity of sexual selection on different traits can

91  change with the OSR (Shuster, 2009; Klug *et al*., 2010). For example, controlling for

92  density, when the OSR is more male-biased the encounter rates between males will

93  increase. Numerous aggressive interactions might make harem defence less

94  economically viable in some species (Emlen & Oring, 1977; Klemme *et al*., 2007).

95  This could reduce sexual selection on weapons, but might increase sexual selection on

96  other sexually selected traits (e.g. testes size if females are then more inclined to mate

97  multiply). Third, although it is counter-intuitive, all else being equal, female mate

98  sampling tactics that involve assessment of a random subset of males yield the same

99  strength of sexual selection on preferred male traits regardless of the OSR (Klug *et*

100  *al*., 2010). Fourth, all else is not necessarily equal, however, as greater choice can

101  reduce the ability of choosy individuals to identify preferred items, including mates

102  (e.g. consumers/shoppers: Lenton & Francesconi, 2011; but see Scheibehenne *et al*.,

103  2010; mate-searching animals: Hutchinson, 2005; Alem *et al*., 2015). The density of

104  one sex and the OSR are related, and they covary perfectly if the population density is

105  constant. Consequently, with a more biased OSR, there are often more mates

106  available for the rarer sex to choose from at any moment.

107

108  There is no formal theoretical link between the OSR and the strength of sexual

109  selection on focal male traits, so the true relationship has to be determined empirically

110  (Klug *et al*., 2010). Comparative analyses suggest that male-biased sex ratios are

111  generally associated with greater male ornamentation and/or weaponry, but the OSR

112  covaries with other factors, notably the adult sex ratio, so it is problematic to assign a

113  causal role to the OSR (Fromhage & Jennions, submitted). Experimental studies that

114   manipulate the OSR to determine its causal effects yield contrasting results. For

115   example, as expected, sexual selection on male body size increased as the OSR was

116   made more male-biased in two-spotted gobbies, *Gobiusculus flavescens* (Wacker *et*

117   *al.*, 2013). There was, however, no relationship between the OSR and the strength of

118   sexual selection on attractive male traits in guppies, *Poecilia reticulata* (Head *et al.*,

119   2008) or mosquitofish, *Gambusia holbrooki* (Head et al. submitted); and there was

120   *weaker* sexual selection on male body size in bank voles *Clethrionomys glareolus*

121   (Klemme *et al.*, 2007) and common lizards *Lacerta vivipara* (Fitze & Le Galliard,

122   2008) when the OSR was made more male-biased.

123

124   Experiments manipulating the OSR are needed to determine whether general trends

125   can be identified.  For example, is the OSR a better predictor of sexual selection in

126   some types of mating systems and/or taxa than others? Such OSR manipulation

127   experiments, while straightforward, are surprisingly few in number. Even more rare

128   are studies where OSR-dependent behavioural changes in one sex are controlled for,

129   so that the direct effect of the OSR on the other sex can be calculated. For example, if

130   female choice based on male courtship varies with the OSR, is this due to OSR-

131   dependent changes in how males court, or in how females evaluate males? To tease

132   sex-specific effects apart necessitates experiments that use artificial stimuli, such as

133   acoustic playbacks or robots, so that 'male' behaviour is unaffected by the OSR.

134

135   Here, we examine how the OSR affects female choice for male claw size in fiddler

136   crabs. We tested females with custom-built robots 'males' that we have successfully

137   used in many previous studies (e.g. Reaney, 2009; Kahn *et al.*, 2013). To avoid any

138   confusion about our non-standard experimental design (two females per trial, see

139    *Methods*), we note three points. First, the OSR changes constantly but, to be a useful

140    predictive tool, it should be measured at a biologically appropriate scale. For example,

141    100 calling male frogs and 20 gravid females might be at a pond over a night. Most

142    researchers would report this as a 5:1 OSR (e.g. Ryan, 1981). Strictly speaking,

143    however, for the first female the OSR was 100:1 and for the last female it was 81:1

144    (male frogs rarely return to the mating pool on the night that they mate). In general,

145    most researchers are interested in this type of 'population level' OSR. Unless

146    otherwise stated we follow this convention in our study. Specifically, we refer to the

147    OSR at the start of an experimental trial rather than that experienced by successive

148    females within a trial. It is less common to refer to the OSR experienced by an

149    individual female. This is probably because the 'female-level' OSR is synonymous

150    with how many sexually receptive males a female encounters. In such case, rather

151    than refer to the OSR, we simply refer to the number of males available as mates.

152

153    Second, most mate choice experiments test a single female per scenario so that each

154    female chooses from the same set of stimuli.  The disadvantage, hinted at in the frog

155    scenario, is that this design ignores changes in mate availability that will arise when

156    females sequentially arrive to mate. Biologically realistic mate choice experiments

157    could include the possibility that several females chose from the same set of males but

158    that earlier choices constrain future options. Third, as noted earlier, the population

159    level OSR and the total number of available males ('male density') tend to be

160    correlated in the field. Although density and the population level OSR can be teased

161    apart in experiments (Kokko & Rankin, 2006; Wacker *et al*., 2013), this does not

162    negate the fact that a more male-biased population level OSR can often equally well

163    be described as a case where females choose among a greater number of males.

164 Researchers interchangeably describe this as the effect of either greater choice of

165 mates (standard terminology for those interested in proximate sensory mechanisms) or

166 of the OSR (standard terminology for those interested in how sex ratios affect

167 selection gradients) on female mating preferences.

168

169 **METHODS**

170

171 We studied *Uca mjoebergi* at East Point Reserve in Darwin, Australia in October-

172 December 2010. Both sexes defend burrows on inter-tidal mudflats. During the five-

173 day mating period that occurs each semi-lunar tidal cycle, mate-searching females

174 leave their burrow, move across the mudflat and sequentially encounter clusters of 2-

175 10 males. This is the main level at which mate choice decisions occur. The population

176 level OSR is always male-biased. Males court females by vigorously waving their

177 enlarged major claw. In the field, females more often approach larger-clawed males

178 (Reaney & Backwell, 2007) and males that wave earlier (i.e. produce 'leading'

179 waves) (Reaney *et al.*, 2008). After choosing a male the female enters his burrow to

180 inspect it. Burrow features then influence whether she stays and breeds, or continues

181 mate searching (Reaney & Backwell, 2007). Sexual selection presumably favours

182 males with large claws that increase the likelihood that a female inspects his burrow.

183 Male claw size in the population at the time of our study ranged from 4.6 to 25.4 mm

184 (mean ± SD: 14.97±4.29 mm; *n* = 222; based on transects). This is consistent with

185 three recent estimates from the same study area [14.84 mm (*n* = 82), 14.95 mm (*n* =

186 199) and 14.9 mm (*n* =177); Clark & Backwell 2015). Even though males within this

187 size range all produce courtship waves, it is possible that the smaller males have

188 burrows that are too narrow for females. A better estimate of the size range of males

189    available as mates is obtained by restricting the dataset to males whose burrows are

190    inspected by females. Three recent estimates from the field at the same site for the

191    mean ± SD claw size of these males are 14.84 ± 1.63 ($n = 37$), 16.05 ± 2.29 ($n = 135$)

192    and 16.60 ± 2.92 mm ($n = 57$) (data from Clark & Backwell 2015).

193

194    We ran mate choice experiments where we sequentially presented two test females

195    with 3, 5 or 7 robotic 'males' (i.e. the population level OSR over the trial was 7:2, 5:2

196    or 3:2, and the OSR for individual females ranged from 7:1 to 2:1). Each robot

197    consists of a painted cast of a claw attached to a motorised metal arm that mimics

198    courtship waving (details in Reaney *et al.*, 2008). Robots waved in synchrony so that

199    leadership did not affect female choice. The test arena was an area of mudflat from

200    which we removed all resident crabs. We used mate-sampling females caught *in situ*

201    who were measured (carapace width ±0.1 mm) and held in individual containers until

202    tested (<30 mins). We placed the first female under a clear plastic cup at a point

203    equidistant from all robots (20 cm). After the female settled we raised the cup and

204    scored a choice decision if she moved to <2 cm of a 'male'. A female was discarded

205    and replaced with another if she ran immediately after release, or did not choose

206    within 3 minutes ($n = 47$). This criterion for choice produces highly repeatable results

207    in the many studies we have conducted (e.g. Reaney, 2009; Kahn *et al.*, 2013). The

208    results from female choice trials using robotic crabs have largely been corroborated

209    by field studies of correlates of male mating success (e.g. Reaney & Backwell, 2007;

210    Clark & Backwell 2015). Given the large numbers of mate-searching females, and

211    that females mate on the day that they begin to search (Clark & Backwell 2015), it is

212    highly improbable that we tested a female more than once. The first female saw all the

213    'males' wave before she was released. Her chosen 'male' was then removed and the

214  second female chose from the remaining 'males'. This mimics the natural situation. In

215  thousands of hours of field observations we have almost never seen two females

216  simultaneously approach a cluster of males – rather, females sequentially approach

217  sets of males. Mate choice by an earlier female removes the chosen male from the set

218  of potential mates of the next female because a male remains underground once a

219  female has chosen to stay in his burrow. Ideally we would have collected data blind to

220  the OSR and male claw size, but this was not possible (see Holman *et al.* 2015).

221  Female choice of a given 'male' is, however, clear-cut based on her behaviour, and

222  we had no *a priori* expectations as to how the OSR would affect female choice.

223

224  We used three high-density treatments where the spacing between 'males' was

225  constant (5 cm) to test if the OSR affects the strength of sexual selection: 7:2, 5:2 and

226  3:2 (Fig 1a). Our design reflects the clusters of waving males encountered by a mate-

227  searching female. The smallest, median and largest claw sizes we used were always

228  the same (Table 1). The mean 'male' claw size (18.2 mm) was larger than the mean

229  for males whose burrows are inspected (16.0 mm; Clark & Backwell, 2015): 10 of

230  the 15 'males' had claws greater than 16.0 mm. This makes tests for a directional

231  mating preference conservative, although only moderately so given that naturally 7 of

232  15 males are larger than average (assuming the median and the mean are the same).

233  The chosen size range was necessary to ensure the tested claws spanned the natural

234  size range, but were evenly distributed in size while keeping the mean value constant.

235  The position of robots with different sized claws was randomised. To test for an

236  effect of male density independent of the OSR (e.g. Head *et al.*, 2008, Wacker *et al.*,

237  2013) we re-ran the 5:2 and 3:2 OSR treatments at lower densities, adjusting the

238  spacing between robots to cover the same area as the 7:2 treatment (Fig. 1b). We ran

239    40 trials (= 80 females) per treatment ($n = 400$ choice trials). Each female was used in

240    one trial/treatment. We randomised the order of the treatments during each day of

241    testing.

242

243    To quantify sexual selection we calculated the selection differential ($s$) as the mean

244    size of the chosen claws minus the original mean claw size. Unless otherwise stated

245    this is based on the mean value of the two females. We ran $t$-tests to determine

246    whether $s$ differed from zero, and an ANOVA to test whether $s$ differed among the

247    OSR treatments with even 'male' spacing. We used a general linear model to test

248    whether density (high/low) and its interaction with the OSR (3:2 or 5:2) affected $s$.

249    There was no effect of day of cycle (relative to spring tide) on $s$ (or the difference

250    between the observed and maximum possible value of $s$), nor did day of cycle interact

251    with OSR treatment (all $p > 0.254$).

252

253    When the OSR is more male-biased, the mean size of the two largest males is greater

254    (Table 1). To determine whether female choice is more error prone when the OSR is

255    more male-biased it is tempting to test whether the difference between the mean size

256    of the chosen males and the maximum mean possible increases with the OSR. This is,

257    however, a problematic approach as the probability that by chance alone the two

258    largest males are chosen is higher when the OSR is less male biased (i.e. there are

259    fewer males). The null prediction is that, by chance, the two largest males are chosen

260    1 in 3 times when the OSR is 3:2, 1 in 10 times when the OSR is 5:2 and 1 in 21 times

261    when the OSR is 7:2. We therefore tested whether the observed number of times the

262    two largest 'males' were chosen was significantly greater than expected using

263    separate one-tailed binomial tests. To indirectly compare female error rates among the

264    high density OSR treatments we ran an ANOVA to test whether the claw size chosen

265    by the first female per trial differed (this is equivalent to asking whether s differed

266    because the mean claw size is identical for all three OSRs).

267

268    Finally, we compared *s* between the first and second female (*s* for the second female

269    was based on the mean size of the remaining available claws) with a paired *t*-test. We

270    tested whether there was an effect of the OSR treatment on the difference in *s*

271    between the first and second female using separate ANOVAs for high and low density

272    tests. We also calculated the correlation between female size and chosen claw size for

273    each treatment. We used the first female per trial to ensure comparable male

274    availability. All tests were two-tailed ($\alpha = 0.05$) and run in SPSS 19.0.

275

276    **RESULTS**

277

278    In the high-density treatments the selection differential was significantly greater than

279    zero at all three OSRs. Females prefer larger claws: 7:2 ($t_{39} = 2.968$, $p = 0.005$), 5:2

280    ($t_{39} = 2.507$, $p = 0.016$) and 3:2 ($t_{39} = 3.798$, $p < 0.001$) (Table 1). The strength of

281    sexual selection (*s*) did not depend on the OSR ($F_{2,117} = 0.242$, $p = 0.785$; 7:2 =

282    1.187mm; 5:2 = 0.98mm ; 3:2 = 1.35mm). Females in the 3:2 treatment more often

283    chose the two largest available claws than did females in the 5:2 or 7:2 treatments.

284    The frequency of trials in which the two largest males were chosen was significantly

285    greater than expected by chance for the 3:2 treatment (24 of 40 trials, $p = 0.001$), but

286    not for the 5:2 (4 of 40 trials) or 7:2 treatments (2 of 40 trials) (both $p > 0.50$). There

287    was, however, no evidence that the first female more often selected the largest claw at

288    a less male-biased OSR as the mean claw size in first trials did not differ across

289  treatments ($F_{2,117} = 0.482$, $p = 0.62$; mean claw size: 3:2 = 18.35mm,; 5:2 = 19.08mm,

290  7:2 = 19.19 mm). The greater than expected number of choices of the two largest

291  males in the 3:2 treatment must therefore be driven by the choice of the second

292  female.

293

294  In the low-density treatments, although females still tended to choose larger claws,

295  sexual selection for larger claws was not significant at either a 3:2 ($t_{39} = 1.312$, $p =$

296  0.197) or 5:2 OSR ($t_{39} = 1.936$, $p = 0.06$). The frequency of trials in which the two

297  largest males were chosen was, however, not significantly greater than expected by

298  chance in either the 3:2 treatment (18 of 40 trials, $p = 0.083$), or the 5:2 treatment (7

299  of 40 trials, $p = 0.10$). There was no evidence that the first female more often selected

300  the largest claw at a less male-biased OSR, as the mean claw did not differ ($F_{1,78} =$

301  0.860, $p = 0.357$; 3:2 = 19.55mm,; 5:2 = 18.55mm).

302

303  When analysing results from the two densities together, there was no significant effect

304  of density on the strength of sexual selection ($s$) ($F_{1,156} = 2.076$, $p = 0.152$), nor was

305  there a density by OSR interaction ($F_{1,156} = 0.548$, $p = 0.460$) or a main effect of OSR

306  ($F_{1,156} = 0.063$, $p = 0.802$) (Mean s was 1.35 and 0.525 mm at high and low densities

307  for the 3:2 OSR, and 0.975 and 0.710 mm at high and low densities for the 5:2 OSR).

308

309  Combining all available trials there was no evidence that the selection differential of

310  the second female was stronger than that of the first female ($t_{199} = 1.533$, $p = 0.127$;

311  first = 0.756 mm, second = 1.389 mm). There was no effect of the OSR on the

312  magnitude of the difference in the selection differential between the first and second

313  female (low-density: $F_{2,117} = 1.779$, $p = 0.173$; high-density: $F_{1,78} = 1.653$, $p = 0.202$;

314   the direction of the effect was first $s$ < second $s$ at both densities).

315

316   Finally, female size was uncorrelated with chosen claw size in all five treatments ($r =$

317   0.173, -0.058, 0.157, 0.053, -0.027, all $p$ > 0.285). The mean correlation was $r =$

318   0.060 ($t_4 = 1.275$, $p = 0.271$). There was no evidence that larger claws additionally

319   enhance male fitness by preferentially attracting larger, more fecund, females.

320

321   **DISCUSSION**

322

323   Female *Uca mjoebergi* preferred to visit larger-clawed robotic 'males' in three of our

324   five OSR/density treatments, and there was a marginally non-significant preference in

325   one treatment ($p = 0.06$). These results corroborate findings from comparable mate

326   choice experiments with robotic crabs (e.g. Reaney, 2009; Kahn *et al*., 2013). They

327   also agree with our estimates of male mating success in the field (Reaney &

328   Backwell, 2007). Our mate choice experiments were conservative with respect to

329   female choice for larger males as the mean test claw size (18.2mm) was larger than

330   the population mean (14.9 mm). The disparity was smaller when using the estimated

331   mean of 16.0mm based on males naturally visited by females (from Clark & Backwell

332   2015). Ten of the 15 'males' available across the three OSR treatments were larger

333   than this mean. The strong directional female mating preference for larger claws that

334   we still observed can therefore partly explain why, like all fiddler crabs, male *U.*

335   *mjoebergi* have a greatly enlarged major claw.

336

337   Although directional selection on claw size was not statistically significant in all

338   treatments, the strength of sexual selection due to female choice did not depend on the

339    OSR. More specifically, sexual selection was not stronger when the OSR was more

340    male-biased. In general, this positive trend is widely predicted because the mean size

341    of the two largest claws was *bigger* when the OSR was more male biased (Table 1). If

342    each female chose the largest available claw with the same propensity irrespective of

343    the OSR, the strength of sexual selection should have *increased* with a more male-

344    biased OSR. (There is no confounding effect of mate-sampling tactics affecting the

345    mean size of available males [for examples see Klug *et al*., 2010] because females had

346    simultaneous access to all available 'males'). Instead, we found that the females'

347    propensity to choose the two largest claws available decreased as the OSR became

348    more male-biased. Specifically, females chose the two largest claws significantly

349    more often than expected by chance only in the least male-biased OSR (3:2, at high

350    density). The absence of a relationship between the OSR and strength of sexual

351    selection in *U. mjoebergi* is a reminder that the OSR is an imperfect predictor (Jones,

352    2009; Shuster, 2009). Indeed, a recent simulation study of various mating systems that

353    tested a range of proposed indices of sexual selection, showed that the OSR tended to

354    be a consistently poor predictor of sexual selection on a focal trait (Henshaw *et al*.,

355    2016).

356

357    There are several possible reasons why the OSR did not predict the strength of sexual

358    selection driven by female choice for larger claws in *U. mjoebergi.*  To start though,

359    we can eliminate any role of OSR-dependent changes in male behaviour (review:

360    Weir *et al*., 2011) that might affect the attractiveness of a larger claw to females. This

361    is because we tested females using robotic 'males' with constant wave rates. An OSR-

362    mediated change in non-focal traits is potentially important in the field because the

363    correlation between claw size and wave rate, which are both traits affecting male

364  attractiveness, varies over the mating period in *U. mjoebergi* (Kahn *et al.,* 2013).

365

366  We suggest that the OSR affected the strength of sexual selection due to psycho-

367  sensory processes that altered a female's ability to discriminate between males and

368  chose the largest available. The greater potential for stronger sexual selection with a

369  more male-biased OSR (because the two largest available claws have a greater mean

370  size) is then negated by increased mistakes by females so that there is no net effect of

371  the OSR on the strength of sexual selection. There are two factors likely to increase

372  female error rates in identifying the largest claw. First, the size difference between

373  adjacent sized 'males' declined from 6 mm to 2 mm as the OSR went from 3:2 to 7:2

374  (Table 1). This is not an experimental flaw: it is inevitable that the size difference

375  between adjacent sized 'males' decreases when more are present. Discrimination

376  between similar sized objects is always more difficult (citations in Abbas *et al*., 2013).

377  It is worth noting that the mean claw size chosen by the first female did not differ

378  among the OSR treatments. However, the proportion of tests in which the two largest

379  males were selected was greater than expected by chance was only significantly for

380  the 3:2 OSR treatment. This suggests that it is the choice of the second female that

381  drives this result, implying that when there are only two males to chose between in the

382  3:2 OSR treatment females are more likely to select the largest available male than

383  when there are four or six available males as in the 5:2 and 7:2 OSR treatments.

384

385  Second, the number of 'males' present increased with the OSR, again reflecting the

386  situation in the field. There is some evidence that choice of a preferred item becomes

387  more difficult when there are more items to choose from (review: Hutchinson, 2005).

388  Together this implies that the number of 'males' and/or size-differences between

389 'males' that both covaried with the OSR increased the error rate of choosy females.

390 Similar relationships seem likely to occur in many taxa where the biologically

391 relevant OSR largely depends on the number of males present on a breeding/mating

392 site while individual females are sampling males. Arguing against a role for the

393 number of males and/or size-differences between males is that there was no

394 significant increase in the selection differential of the second female relative to that of

395 the first female. This occurred even though the second female saw one fewer 'male',

396 and, on average, there was a greater size difference between the largest and next

397 largest 'male'. It is worth noting, however, that we might have reported a significant

398 effect if we had used a different distribution of claw sizes so that there were fewer

399 'males' with above average sized claws (relative to the natural mean). In such a case,

400 the choice of a large 'male' by the first female would have increased the proportion of

401 below average 'males' so that females might have become less choosy. More

402 convincingly, in previous two-choice experiments the only treatment in which

403 females did *not* choose the bigger claw was when they differed in size by just 2 mm

404 (Reaney 2009).

405

406 To test whether the number of 'males' *per se* has a direct effect on female choice it is

407 necessary first to control for the size differences between 'males', *and* vary the

408 number of 'males' (e.g. double the number of 'males' in the 3:2 OSR treatment and

409 compare the 3:2 and 6:2 treatments). It should be noted, however, that in the

410 proposed, as well as the current, experiment the variance in male claw size is greater

411 when the OSR is less male biased. This is a constraint of the design given the decision

412 to have the same range in claw size. In a natural setting the number of males that an

413 individual female encounters might not show the same relationship with variation in

414    male claw size. Finally, it is possible that the OSR directly changes female mating

415    preferences. Females might have a weaker preference for larger males when, say,

416    more males are present although there is no obvious adaptive advantage to such a shift

417    in mating preference.

418

419    In conclusion, the proximate mechanisms underlying consistent sexual selection for

420    larger claw size independent of the OSR in *U. mjoebergi* are unknown. This does not,

421    however, negate our key finding sexual selection theory. Despite the OSR covarying

422    with the opportunity for sexual selection (i.e. maximum selection differential) sexual

423    selection on male claw size did not increase as the OSR became more male-biased.

424

425    **DATA ACCESSIBILITY**

426    Data for this study is available from the DRYAD repository doi:10.5061/dryad.5qb78

427

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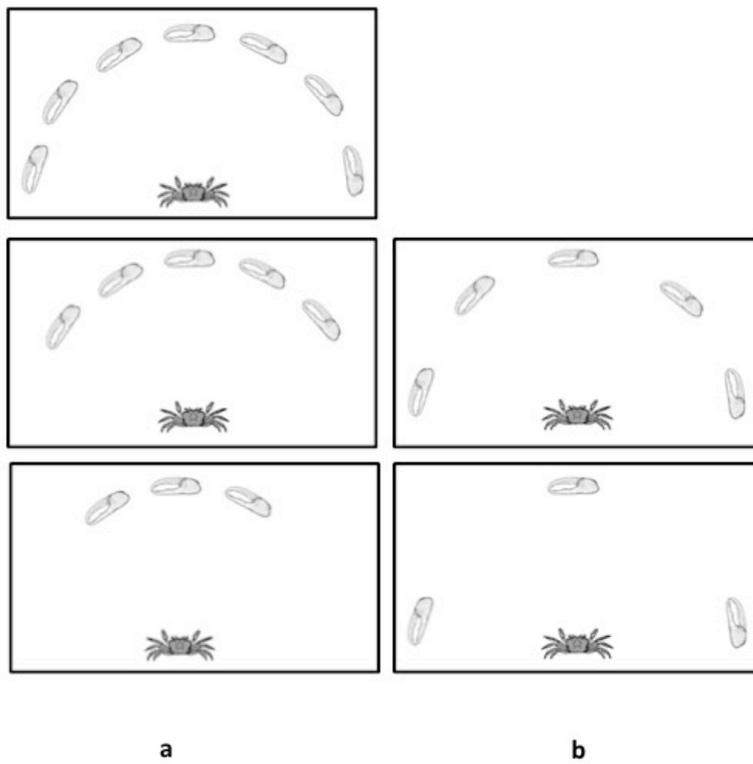
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499      **Table 1.** Available, observed mean of chosen males, and maximum possible mean

500      claw length (i.e. largest two males) (in mm) for the five OSR-density treatments.

501

| Treatment | | | Chosen mean ± SD (all $n$=40 trials) | | Maximum possible mean |
|---|---|---|---|---|---|
| OSR | Male claw length | Mean | High density | Low density | |
| 7:2 | 12.2, 14.1, 16.2, 18.2, 20.2, 22.1, 24.2 | 18.2 | 19.34 ± 2.53 | | 23.15 |
| 5:2 | 12.2, 15.2, 18.2, 21.1, 24.2 | 18.2 | 19.16 ± 2.46 | 18.89 ± 2.32 | 22.65 |
| 3:2 | 12.2, 18.2, 24.2 | 18.2 | 19.55 ± 2.25 | 18.73 ± 2.53 | 21.2 |

502

503 **Figure 1.** Diagram of the three OSR treatments: (a) even male spacing (high density)

504 and (b) spacing such that the same area is covered in all three OSR treatments. Claws:

505 robotic 'males'; crab: female release point.

506



a                                    b

507