Behavioral estimates of mating success corroborate genetic evidence for pre-copulatory sexual selection in male *Anolis sagrei* lizards

Rachana S. Bhave[1], Heidi A. Seears[1], Aaron M. Reedy[1,2], Tyler N. Wittman[1], Christopher D. Robinson[1], and Robert M. Cox[1]

[1] University of Virginia, Charlottesville, VA, USA

[2] DataClassroom Inc, Charlottesville, VA, USA

Corresponding Author:

Rachana S. Bhave

Department of Biology, University of Virginia, 485 McCormick Road, PO Box 400328,

Charlottesville, VA 22904, USA

Email: rachanabhave@gmail.com ; rsb7bz@virginia.edu

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1 **Abstract**

2   In promiscuous species, fitness estimates obtained from genetic parentage may often

3   reflect both pre- and post-copulatory components of sexual selection. Directly observing

4   copulations can help isolate the role of pre-copulatory selection, but such behavioral data are

5   difficult to obtain in the wild and may also overlook post-copulatory factors that alter the

6   relationship between mating success and reproductive success. To overcome these limitations,

7   we combined genetic parentage analysis with behavioral estimates of size-specific mating in a

8   wild population of brown anole lizards (*Anolis sagrei*). Males of this species are twice as large as

9   females and multiple mating among females is common, suggesting the scope for both pre- and

10 post-copulatory processes to shape sexual selection on male body size. Our genetic estimates of

11 reproductive success revealed strong positive directional selection for male size, which was also

12 strongly associated with the number of mates inferred from parentage. In contrast, a male's size

13 was not associated with the fecundity of his mates or his competitive fertilization success. By

14 simultaneously tracking copulations in the wild via the transfer of colored powder to females by

15 males from different size quartiles, we independently confirmed that large males were more

16 likely than small males to mate. We conclude that body size is primarily under pre-copulatory

17 sexual selection in brown anoles, and that post-copulatory processes do not substantially alter

18 this pre-copulatory selection. Our study also illustrates the utility of combining both behavioral

19 and genetic methods to estimate mating success to disentangle pre- and post-copulatory

20 processes in promiscuous species.

21

22

Introduction

In species where females mate promiscuously with multiple partners, sexual selection on male traits can continue to occur after copulation, through sperm competition and female cryptic choice. These post-copulatory processes can alter the siring success of males and thereby modify the strength of sexual selection on traits linked to mating success (Parker 1970; Kvarnemo and Simmons 2013; Simmons et al. 2017; Glaudas et al. 2020). For example, larger males may mate with more females, but this may not translate into strong sexual selection if they are poor sperm competitors. In addition to pre- and post-copulatory sexual selection, the net reproductive fitness of a male is also influenced by the fecundity of his mating partners (Wong and Candolin 2005; Venner et al. 2010; Pincheira-Donoso and Hunt 2017). For any given trait, total selection due to variance in reproductive success can thus be partitioned into selection acting through variance in pre-copulatory mating success, post-copulatory fertilization success, and female fecundity (Arnold and Wade 1984; Koenig et al. 1991; Collet et al. 2012; Pélissié et al. 2014). Furthermore, selection mediated through any one of these components of fitness may be reinforced or weakened by selection acting through other components (Arnold and Wade 1984; Shuster et al. 2013). Therefore, a complete understanding of selection on a given trait requires estimating phenotypic selection as a function of total reproductive success as well as its underlying components (Arnold and Wade 1984). However, our ability to partition sexual selection in wild populations is hindered by both the cryptic nature of post-copulatory processes and the difficulty of independently measuring mating success, mate fecundity, and fertilization success (Droge-Young et al. 2012; Oneal and Knowles 2015; Marie-Orleach et al. 2016).

Studies of sexual selection in wild populations have typically measured fitness using either genetic estimates of parentage or behavioral observations of mating success. However,

46   either of these approaches can provide an incomplete picture of sexual selection when considered

47   alone (Thompson et al. 2011; Marie-Orleach et al. 2016; Olsson et al. 2019), making it difficult

48   to disentangle pre- and post-copulatory selection (Danielsson 2001; Mobley and Jones 2013;

49   Kamath and Losos 2018; Cramer et al. 2020). Genetic parentage analysis can identify mating

50   pairs from shared parentage, thereby providing minimum estimates of the number of mating

51   partners for both males and females (Flanagan and Jones 2019). Such data can be used to

52   estimate both pre-copulatory mating success (minimum number of known mates per male) and

53   post-copulatory fertilization success (proportion of offspring sired with females who have

54   multiple known mates) (Rose et al. 2013; Evans and Garcia-Gonzalez 2016). However, many

55   copulations may go undetected if females mate with many partners but produce relatively few

56   offspring, potentially leading to mis-estimation of selection via mating success. (Pemberton et al.

57   1992; Flanagan and Jones 2019; Olsson et al. 2019; Baird and York 2021). Direct observations

58   of copulations avoid this problem, but it is usually impossible to comprehensively track all

59   copulations in wild populations. For example, animals may copulate in obscure or sheltered

60   locations, the duration of mating may be short, the population density may be too low, or the

61   population size may be too high for comprehensive observations (Candolin 1998; Dunn et al.

62   2012; Johnson et al. 2014; Cramer et al. 2020). Therefore, reliance on either genetic or

63   behavioral methods alone to measure fitness may lead to misestimation of the strength of pre-

64   and post-copulatory sexual selection (Pischedda and Rice 2012; Evans and Garcia-Gonzalez

65   2016; Baird and York 2021). Consequently, there is increasing emphasis on approaches that

66   measure fitness and its components using a combination of both behavioral observations and

67   genetic parentage analyses to help partition pre- and post-copulatory dimensions of sexual

68   selection (Collet et al. 2012; Pischedda and Rice 2012; Evans and Garcia-Gonzalez 2016;

69   McDonald et al. 2017; Simmons et al. 2017; Olsson et al. 2019).

70   We studied the sexually dimorphic brown anole lizard, *Anolis sagrei*, to determine which

71   components of male reproductive success (i.e., mating success, average mate fecundity, or

72   competitive fertilization success) generate selection for larger body size in this species. Adult

73   male brown anoles are, on average, two to three times larger than adult females in body mass

74   (Cox and Calsbeek 2010a). Larger males are more likely to succeed in competitive interactions

75   that lead to female encounters and to sire more offspring (Tokarz 1985; Kamath and Losos

76   2018). However, female brown anoles produce offspring with multiple sires during the breeding

77   season and can store sperm for several months (Calsbeek et al. 2007; Calsbeek and Bonneaud

78   2008; Duryea et al. 2016; Kamath and Losos 2018; Kahrl et al. 2021). Females may also bias

79   their offspring sex ratio based on the body size or condition of the males with which they mate,

80   suggesting that post-copulatory processes can also shape selection on male body size (Calsbeek

81   and Bonneaud 2008; Cox and Calsbeek 2010b; Cox et al. 2011). Although several studies have

82   detected selection for larger body size in male brown anoles (Cox and Calsbeek 2010a; Duryea

83   et al. 2016; Kamath and Losos 2018), no study to date has assessed the extent to which the

84   higher reproductive success of larger males is due to higher mating success, higher average mate

85   fecundity, higher fertilization success, or a combination of these components of reproductive

86   success (Friesen et al. 2020).

87   Given the scope for both pre- and post-copulatory selection to act on male body size in

88   brown anoles (Calsbeek et al. 2007; Kahrl et al. 2016), we combined genetic parentage and

89   behavioral observations of mating to estimate fitness components in a wild population of this

90   species. Based on the established role of body size in mediating aggressive interactions among

91   males, we hypothesized that body size is primarily subject to pre-copulatory selection (Tokarz

92   1985; Duryea et al. 2016; Kamath and Losos 2018)

93   Specifically, we predicted that body size would be positively associated with both total

94   reproductive success (number of offspring sired) and mating success (number of mates identified

95   via genetic parentage). Although anoles only lay one egg at a time, larger females produce more

96   offspring compared to smaller females and tend to be more fecund (Andrews & Rand, 1974; Cox

97   & Calsbeek, 2011; Duryea et al., 2016; Warner & Lovern, 2014). Thus, we also explored

98   whether large males preferentially mate with larger and more fecund females. Since post-

99   copulatory selection could weaken or reinforce pre-copulatory selection (Danielsson 2001;

100   Hosken et al. 2008; Kvarnemo and Simmons 2013; Parker et al. 2013; Turnell and Shaw 2015),

101   we also tested whether competitive fertilization success (i.e., the proportion of offspring sired

102   with females who also produced offspring with other males) differed as a function of male body

103   size. To corroborate our inferences based on genetic parentage with behavioral estimates of

104   mating success, we quantified size-specific mating rates in the wild by tracking the copulatory

105   transfer of fluorescent powders from males to females, with different colors of powder

106   corresponding to different quartiles for male body size. We then tested whether larger males

107   obtained a greater number of copulations, whether larger males mated with larger females, and

108   whether female body size and fecundity were positively correlated. Our study design thus

109   allowed us to separate the contributions of pre-copulatory mating success, female fecundity, and

110   post-copulatory fertilization success.

111

112   ### *Methods*

113   *Field site and sampling*

114   We studied an island population of brown anole lizards (*Anolis sagrei*) in the Guano

115   Tolomato Matanzas Natural Estuarine Research Reserve in northern Florida (29°37′53″ N,

116   81°12′ 46″ W). Adults begin mating around March (Lee et al. 1989) and females typically lay

117   one egg every 7-14 days from April through October. Juveniles emerge between late May and

118   November, and most do not enter the breeding population as adults until the subsequent year. To

119   assay the reproductive success of males in the wild, we sampled all adults and juveniles of the

120   population at four different times during the breeding season (March, May, July, and October) in

121   2019. We marked each new individual with a unique toe clip and preserved a small (1-2 cm) tail

122   clip in 100% ethanol at -20˚C for genotyping. We measured snout-vent length (SVL, nearest 1

123   mm) and body mass (nearest 0.01g) of all individuals prior to releasing them at their site of

124   capture the following day. We captured and measured a total of 920 adults (hatched prior to

125   2019) and 905 juveniles (hatched in 2019) on the island. Most of the adults were first captured as

126   hatchlings in their year of birth and genotyped during previous sampling censuses.

127

128   *Genotyping and parentage assignment*

129   We extracted DNA by adding 3-5 mg of tail tissue to 150 µl of 10% Chelex^R resin (Bio-

130   Rad, Inc.) with 1.4 µl of Proteinase K (20 mg/ml, Qiagen, Chatsworth, CA), incubating at 55˚C

131   for 180 min, and denaturing at 99˚C for 10 min. If the DNA concentration was not within the

132   desired range of 10-25 ng/µl, we repeated extractions and modified the above protocol by

133   incubating new tail samples in 40 µl of 10% Chelex^R resin with 1.5 µl of Proteinase K. After

134   centrifugation at 2250 *g* at 4˚C for 15 minutes, we collected 3 µl of supernatant from these

135   extractions to genotype individuals using the Genotyping-in-Thousands by sequencing (GT-seq)

136   protocol (Campbell et al. 2015) with a custom panel of primers for 215 biallelic SNP loci that

137   were previously identified from RAD-seq data (HA Seears, unpublished). For all extractions

138   with an average DNA concentration of <10 ng/µl (n = 282 of 1319 samples), we carried out an

139   additional purification step on the supernatant using 1.8x volume of AMPure XP beads

140   (Beckman Coulter, Brea, CA, USA) and eluted samples in 20 µl 1x TE (Fisher Bioreagents, Fair

141   Lawns, NJ, USA) to concentrate the DNA to >10 ng/µl. After extraction, we shipped DNA

142   samples to GTseek LLC (Twin Falls, ID, USA) for library preparation, sequencing, and data

143   processing to obtain genotypes. Briefly, all 215 loci were simultaneously amplified and tagged

144   with Illumina priming sequences in a multiplexed polymerase chain reaction (PCR). Each

145   sample was then tagged with well-specific and plate-specific indices in a second PCR. The PCR

146   products were then standardized to similar concentrations, pooled, cleaned, and then sequenced

147   on an Illumina NextSeq 550 with 1 × 75 bp reads. The raw Illumina reads were checked for

148   quality using FastQC and then de-multiplexed and assigned genotypes following Campbell et al.

149   (2015).

150   We used SNPPIT 2.0 (Anderson 2012) to assign genetic parentage. We included all

151   offspring known to have hatched in 2019 that were successfully genotyped at a minimum of 128

152   loci ($< 40\%$ missing loci; n = 885 successfully genotyped of 905 total offspring). We included

153   adults as potential parents if they were successfully genotyped at a minimum of 165 loci ($< 23\%$

154   missing loci). Since individuals that were present but were not captured in 2019 may have also

155   produced offspring in that year, we included all successfully genotyped individuals captured on

156   the island between 2015 and 2018 as potential parents (n = 7042 individuals genotyped in

157   previous studies). Of these putative parents, 870 individuals were captured as adults in 2019. We

158   used a significance threshold of $P < 0.05$ after correcting for the false discovery rate (FDR) to

159   assign parentage. We successfully assigned 736 offspring (83.2% of 885 genotyped offspring) to

160   a total of 610 parents ($n = 357$ females, 253 males). Of these 610 parents, 479 (78.5%) were

161   among the 870 successfully genotyped adults that we captured in 2019 ($n = 276$ females, 203

162   males) and 131 (21.4% of 610) were only captured in previous sampling years ($n = 81$ females,

163   50 males). Because we did not measure body size for this subset of 50 adult males in 2019, we

164   excluded them from our calculations of relative fitness and our analyses of sexual selection.

165   Among the 870 successfully genotyped adults that we captured in 2019, a total of 391

166   individuals ($n = 213$ females, 178 males, 44.9%) were found to have zero reproductive success,

167   since they were included in the SNPPIT analysis but were not assigned offspring.

168

169   *Partitioning reproductive success and measuring selection*

170   All statistical analyses were performed in R v. 4.2.1 (R Core Team 2022) using the

171   RStudio interface (RStudio Team 2022). We conducted univariate selection analyses to test

172   whether body size of males was a predictor of reproductive success (total number of offspring

173   sired in 2019) and its components (i.e., mating success, average mate fecundity, and competitive

174   fertilization success), as estimated by genetic parentage. We measured mating success as the

175   total number of unique females with which a male sired offspring. We measured average mate

176   fecundity as the mean number of offspring produced across all female partners of a male,

177   including offspring sired by other males. We measured competitive fertilization success by

178   calculating the mean proportion of offspring sired by a male with each of his partners. To detect

179   competing males from parentage data, a female must produce at least two offspring that are

180   assigned to at least two mates. Thus, our measure of competitive fertilization success excluded

181   all situations in which females produced either a single offspring or multiple offspring sired by a

182   single male (following Devigili et al. 2015). To account for the fact that the null expectation for

183   proportional fertilization success decreases with the number of additional males with which a

184   female has mated, we used the following formula (Devigili et al. 2015):

185    $$\text{Competitive fertilization success} = \sum_{i=1}^{i=k} \left(\frac{PS_i \cdot (n_i - 1)}{PS_i \cdot (n_i - 2) + 1} \right) / k,$$

186   where PS_i is the proportion of offspring sired for each ith female with which that male mated,

187   k is the total number of females with which that male mated that had more than two mates, and

188   n_i is the total number of mates of the ith female. Thus, a male that sired 33.3% of the offspring

189   from a female that had three total mates would have a competitive fertilization success score of

190   0.5, which would be the same as a male that sired 50% of the offspring from a female that had

191   only two mates.

192   We estimated univariate linear (*s*) and non-linear (*c*) selection differentials following

193   Lande and Arnold (1983). We standardized body mass to a mean of 0 and a standard deviation of

194   1. We calculated relative fitness by dividing total reproductive success and each of its

195   components (see above) by the mean value of that fitness component across all males in the

196   population that were included in the analysis. We used ordinary least-squares regressions of each

197   measure of relative fitness on standardized body mass to estimate univariate linear selection

198   differentials, with separate regressions for each fitness component. To estimate *s*, we included

199   only the linear term for body mass, and to estimate *c*, we included both the linear and quadratic

200   terms (i.e., $0.5 \times \text{body mass}^2$) (Lande and Arnold 1983; Stinchcombe et al. 2008). We used

201   generalized linear models to test the significance of selection estimates. We used a negative

202   binomial distribution for all components of fitness except competitive fertilization success,

203   which had a Gaussian distribution. Non-linear selection differentials were not significant for any

204   fitness component, so we only present visualizations of linear selection differentials. We

205   considered individuals with zero reproductive success to have zero mating success, whereas the

206   remaining fitness components were considered inestimable. This approach assumes that failure

207   to reproduce is due to failure to mate when it could, in principle, also reflect low mate fecundity

208   and/or poor competitive fertilization success. To confirm that including these zero values did not

209   bias our partitioning of selection among components of reproductive success, we repeated the

210   above analyses using only the subset of males that had at least one offspring (Fig. S1).

211

212   *Assessing size-specific mating success with fluorescent powders*

213   We directly assessed the relationship between body size and copulation rates at two

214   points in the middle of the breeding season: May 12-16 and July 26-Aug 3, 2019. In the first two

215   days of each sampling period, we captured as many adult males on the island as possible and

216   distributed them into size quartiles based on their body mass (May: $n = 153$; July: $n = 128$).

217   Before releasing each male to its site of capture the following day, we powdered males on their

218   venters with one of four colors of fluorescent powder corresponding to their size quartiles (A/AX

219   Series, DayGlo Color Corp., Ohio). The four colors (orange, yellow, pink and green) were

220   selected after pilot studies confirmed that different colors of powder transferred during

221   successive copulations could be clearly distinguished in the event of multiple mating across

222   different size quartiles. These powders are non-toxic, easily differentiated under ultraviolet (UV)

223   light, and wear off after a few days without negatively affecting the fitness of animals (Holbrook

224   et al. 1970; Rojas-Araya et al. 2020). We switched the colors assigned to each size quartile

225   between May and July to ensure that any observed mating patterns were not due to underlying

226   differences in our ability to detect each color. We were not blind to the size quartile associated

227   with the colors during the study. Subsequent studies in the same population using a double-blind

228   study design have not uncovered significant biases in estimation of copulation rates (RS Bhave,

229   unpublished). Two days after males were released, we captured as many adult females on the

230   island as possible in a single day in May (n = 132) and across 5 days in July (n = 312; 50% of

231   these captures occurred on the first day). We noted the color of any powder on or near the cloaca

232   under UV light. Presence of color found on any other part of the body was uncommon and

233   treated as a non-copulation contact. We tested whether observed copulations within each size

234   quartile (as determined by the color of transferred powder) significantly differed from null

235   expectation using a chi-square test with 3 degrees of freedom. The expected number of

236   copulations for each size quartile was a product of the proportion of powdered males that were

237   assigned to each quartile and the total number of copulations detected across all females.

238   We also used data on transfer of fluorescent powder to test whether large males mated

239   more frequently with large females, potentially benefiting males through the increased fecundity

240   of larger females. To test this prediction, we conducted an ordinary least square regression of

241   female body mass (continuous dependent variable) on male size quartile (ordinal independent

242   variable) and estimated significance with a type II ANOVA using the *car* package (Fox et al.

243   2019) in R. We tested the underlying assumption that female fecundity is positively correlated

244   with body size by regressing the total number of offspring assigned to a female using genetic

245   parentage (continuous response variable) on female body mass (continuous independent

246   variable) using generalized linear models with a negative binomial error distribution and a logit

247   link function. Because female body mass can vary depending on the presence or absence of

248   oviductal eggs, we also repeated the above analyses by considering SVL as an alternate measure

249   of female body size. In all cases, we conducted two separate analyses using data from May and

250   July, followed by a third analysis on data combined across May and July.

251

252   *Comparing behavioral and genetic approaches*

253   To compare behavioral and genetic approaches, we assessed whether males belonging to

254   larger size quartiles in our powdering experiment (behavioral) also differed in their fitness

255   components as measured by parentage (genetic). We conducted separate generalized linear

256   regressions for males captured in May versus July, with reproductive success (negative

257   binomial), mating success (negative binomial), average mate fecundity (negative binomial) and

258   competitive fertilization success (Gaussian) as response variables. We treated the size quartile

259   that males belonged to in each month as an ordinal predictor variable. In each analysis, we only

260   considered males that were powdered in that month and successfully genotyped. To test whether

261   associations between size quartile and fitness components varied across months, we repeated the

262   above analyses on data combined across May and July while also including an effect of month

263   and its interaction with size quartile. A subset of successfully genotyped males that were

264   captured and powdered in May were also captured and powdered in July ($n = 37$), so these

265   individuals were included twice in our combined analysis. Given that these males constituted

266   only 15% of all individuals that were powdered, and that model results were similar with or

267   without inclusion of these repeated measures across both months, we did not include individual

268   ID as a random effect to simplify the model fit. We obtained effect sizes of all main effects in

269   these models from a type II ANOVA unless the interaction of size quartile × month was

270   significant, in which case we conducted a type III ANOVA.

271   We carried out a chi-square test with 3 degrees of freedom to test whether the number of

272   copulations in each size quartile, as determined by powdering (observed), corresponded to the

273   number of copulations predicted from genetic parentage (expected). To calculate the expected

274   proportion of copulations in each size quartile, we first estimated the number of unique dam-sire

275   pairs from genetic parentage for sires. We assumed that each parental pair indicates at least one

276   copulation with a male belonging to a particular size quartile, then divided the total copulations

277   assigned in each size quartile by the total number of copulations attributable to all males that

278   were measured and powdered in either May or in July. The expected number of copulations was

279   calculated by multiplying this proportion by the total number of copulations observed from the

280   transfer of fluorescent powder in the respective months.

281

282   *Results*

283   *Partitioning pre- and post-copulatory selection on body size*

284   We found significant directional selection favoring large male body mass when using

285   total reproductive success as a measure of fitness ($s = 0.40 \pm 0.08$, $\chi^2 = 22.43$, $P < 0.001$, Fig.

286   1A), and we found similarly strong selection when using only its pre-copulatory component of

287   mating success ($s = 0.33 \pm 0.07$, $\chi^2 = 19.36$, $P < 0.001$, Fig. 1B). Directional selection favoring

288   large size persisted when we excluded males who did not sire any progeny from our analyses

289   using reproductive success and mating success (Fig. S1). However, neither average mate

290   fecundity ($s = -0.03 \pm 0.04$, $\chi^2 = 0.15$, $P = 0.70$, Fig. 1C) nor competitive fertilization success

291   (adjusted for number of competing males) generated significant selection on male body mass ($s =$

292   0.02 ± 0.02, $F_{1,115} = 2.18$, $P = 0.17$, Fig. 1D). There was no significant quadratic (non-linear)

293   selection on male body mass with respect to total reproductive success ($c = 0.30 \pm 0.12$, $\chi^2 =$

294   2.51, $P = 0.11$), mating success ($c = 0.30 \pm 0.12$, $\chi^2 = 2.33$, $P = 0.13$), average mate fecundity (c

295   $= 0.19 \pm 0.09$, $\chi^2 = 1.21$, $P = 0.27$) or competitive fertilization success ($c = -0.0009 \pm 0.02$, $F_{1,115}$

296   $= 0.001$, $P = 0.97$).

297

298   *Behavioral estimates of size-specific mating success*

299   We powdered a total of 241 males across May and July to test whether actual copulation

300   rates differed across male size quartiles (Fig. 2A-C). Based on detection of transferred powder

301   (Fig. 2D), we found that 38 of 132 (28.8%) females in May and 151 of 312 (48.4%) females in

302   July mated within the five-day collection period, with most of these copulations occurring within

303   three days of the release of powdered males. We also found that 1 of 38 (2.6%) females in May

304   and 10 of 151 (6.6%) females in July mated with males from more than one size quartile during

305   that period. We omitted 2 of 39 and 5 of 161 total copulations in May and July respectively,

306   since we could not accurately resolve the color of fluorescent powder. Omitting these instances

307   from the analyses did not bias the number of copulations for any size quartile. Within each

308   month, observed copulations differed significantly from our null expectation of an equal number

309   of matings across size quartiles (May: $\chi^2 = 8.03$, df = 3, $P = 0.045$, $n = 37$ copulations, July: $\chi^2 =$

310   8.33, df = 3, $P = 0.039$, $n = 156$ copulations; Fig. 2E-F). This difference was primarily

311   attributable to the smallest size quartile having consistently fewer copulations than expected in

312   each month. We saw a similar relationship between male size quartile and mating success after

313   pooling data from both months ($\chi^2 = 11.64$, df = 3, $P = 0.009$, $n = 193$ copulations).

314   Although there was a weak trend towards positive size-assortative mating, female body

315   mass did not differ significantly across male size quartiles in either May ($F_{3,33} = 2.40$, $P = 0.085$,

316   Fig. 3A) or July ($F_{1,152} = 2.12$, $P = 0.10$, Fig. 3B). However, when considering data combined

317   across both months, we found a weak but significant positive correlation between female body

318   mass and the size quartiles of males with which they mated (Size Quartile: $F_{3,185} = 3.24$, $P =$

319   0.023, Month: $F_{1,185} = 3.71$, $P = 0.056$, Size Quartile x Month: $F_{3,185} = 0.75$, $P = 0.52$). Female

320   SVL did not differ significantly across male size quartiles in May ($F_{3,33} = 1.48$, $P = 0.22$, Fig.

321 3C), July ($F_{1,152} = 2.26$, $P = 0.083$, Fig. 3D), or when combining both months (Size Quartile:

322 $F_{3,185} = 1.48$, $P = 0.22$, Month: $F_{1,185} = 1.23$, $P = 0.27$, Size Quartile x Month: $F_{3,185} = 1.43$, $P =$

323 0.24). The total number of offspring produced by a female in a year tended to increase with her

324 body mass, but this weak relationship was not significant in May ($\chi^2 = 3.50$, $P = 0.061$) or July

325 ($\chi^2 = 2.54$, $P = 0.11$). However, when considering SVL as a measure of female size, total number

326 of offspring had a strong positive association with female body size in May ($\chi^2 = 9.91$, $P =$

327 0.002), though not in July ($\chi^2 = 0.11$, $P = 0.74$).

328

329 *Comparing behavioral and genetic approaches*

330   Of the 365 males that we successfully genotyped, measured, and included in genetic

331 parentage analysis, 225 were also powdered in either May or July. Total reproductive success

332 estimated from genetic data increased with male size quartile in May ($\chi^2 = 39.83$, $P < 0.001$, Fig.

333 4A), although this positive relationship was weaker and not significant in July ($\chi^2 = 7.21$, $P =$

334 0.065, Fig. 4B). Combining data across both months confirmed a weak overall relationship

335 between size and reproductive success (Size Quartile: $\chi^2 = 6.93$, $P = 0.074$), a large effect of

336 month on reproductive success (Month: $\chi^2 = 19.30$, $P < 0.001$), and a significant difference

337 between months in the relationship between size and reproductive success (Size Quartile x

338 Month: $\chi^2 = 14.16$, $P = 0.002$, Table 1). Likewise, we found that male mating success increased

339 with size quartile in May ($\chi^2 = 32.05$, $P < 0.001$, Fig. 4C), but this relationship was weaker and

340 not significant in July ($\chi^2 = 5.43$, $P = 0.14$, Fig. 4D). Pooling data confirmed a significant

341 difference between months in the relationship between size and mating success (Size Quartile: χ^2

342 $= 5.31$, $P = 0.15$, Month: $\chi^2 = 14.12$, $P = 0.002$, Size Quartile x Month: $\chi^2 = 10.52$, $P = 0.014$,

343 Table 1). Average mate fecundity was unrelated to male size quartile in May ($\chi^2 = 4.01$, $P =$

344   0.26, Fig. 4E) or July (χ^2 = 6.92, P = 0.075, Fig. 4F), and pooling data across months revealed a

345   weak but significant tendency for average mate fecundity to decrease with male size (Size

346   Quartile: χ^2 = 9.39, P = 0.025, Month: χ^2 = 0.19, P = 0.66, Size Quartile x Month: χ^2 = 1.10, P =

347   0.78, Table 1). Competitive fertilization success was unrelated to male size in May ($F_{3,62}$ = 1.34,

348   P = 0.27, Fig. 4G), in July ($F_{3,65}$ = 2.17, P = 0.099, Fig. 4H), and when pooling data across

349   months (Size Quartile: $F_{3,127}$ = 1.73, P = 0.17, Month: $F_{1,127}$ = 1.87, P = 0.35, Size Quartile x

350   Month: $F_{3,127}$ = 1.82, P = 0.15, Table 1).

351   The size distribution of copulation rates inferred from the transfer of fluorescent powder

352   was significantly different from the size distribution of copulation rates estimated from genetic

353   parentage in May (χ^2 = 8.35, P = 0.039, Fig. 5A). In particular, males in the second size quartile

354   had more observed copulations than expected from genetic parentage, whereas males in the

355   smallest and largest size quartiles had fewer copulations than expected (Fig. 5A). However, our

356   analyses for May are based on substantially fewer observed copulations (n = 37) than our

357   analyses for July (n = 156), in which size-specific mating rates observed in the wild did not

358   significantly differ from rates estimated from parentage (χ^2 = 1.41, df = 3, P = 0.70, Fig. 5B).

359

360   **Discussion**

361   Pre-copulatory and post-copulatory components of sexual selection can be difficult to

362   disentangle in wild populations, especially for promiscuous species that lack parental care or

363   stable mating pairs. In brown anoles, which lack both, genetic parentage data revealed that 65%

364   of females that produced 2 or more offspring (i.e., females for which multiple paternity could be

365   detected) did so with more than one mate (mean = 1.92, range = 1-4 mates), suggesting the

366   potential for post-copulatory processes to modulate pre-copulatory sexual selection. We detected

367 strong positive directional selection on male body size using estimates of total reproductive

368 success from genetic parentage. Partitioning male reproductive success into its components

369 revealed that the higher reproductive success of larger males was primarily mediated by an

370 increase in their mating success. This result was corroborated by our behavioral assay involving

371 the transfer of fluorescent powder from males to females during copulation, which allowed us to

372 track copulations in the wild and revealed that larger males indeed mated more frequently. By

373 contrast, neither average mate fecundity nor male competitive fertilization success covaried

374 positively with male body size, suggesting that pre-copulatory sexual selection is largely

375 responsible for the strong association between reproductive success and body size in male brown

376 anoles. This was further confirmed by our finding that both behavioral and genetic parentage

377 estimates of mating success were similarly distributed across different male size quartiles. Thus,

378 despite multiple mating by females, post-copulatory processes did not significantly modify pre-

379 copulatory sexual selection for large male body size.

380

381 *Body size and mating success*

382   We found that larger body mass is directly associated with greater mating success in the

383 wild (Figs. 1B, 2E-F). This pattern is corroborated by both behavioral and genetic estimates of

384 mating success (Figs. 2E-F, 4C-D). Consequently, larger males sired a greater number of

385 offspring than average throughout the breeding season (Figs. 1A, 4A-B). Our findings are in line

386 with the general consensus that there is strong pre-copulatory sexual selection on male body size

387 in species with extreme male-biased size dimorphism (Stamps et al. 1997; Kingsolver and

388 Pfennig 2004; Fairbairn et al. 2007; Kingsolver and Diamond 2011).

389   The observed pattern of pre-copulatory sexual selection for large body size is likely due

390   to success in male-male competition (Andersson and Iwasa 1996; Eberhard 1996; Cox et al.

391   2003; Roff and Fairbairn 2007; Janicke and Fromonteil 2021). Previous studies have shown that

392   larger male anoles are more active (Jenssen et al., 2005; Tokarz, 1985), move across larger areas

393   (Stamps et al. 1997; Kamath and Losos 2018), and are more likely to win in aggressive

394   interactions with other males, resulting in more frequent encounters with females (Steffen and

395   Guyer 2014). This is the case in many other species with male-biased size dimorphism or contest

396   competition (Cox et al. 2003; Fairbairn et al. 2007; Emlen 2008; Janicke et al. 2016; Horne et al.

397   2020). Although examples of sexual selection via female choice are relatively rare in reptiles

398   (Olsson and Madsen 1995; Tokarz 1995; Cox and Kahrl 2014; Ord et al. 2015; Rosenthal 2017),

399   our study cannot eliminate the role of female choice for large males (Wong and Candolin 2005;

400   Fitze et al. 2008; Karsten et al. 2009; Debelle et al. 2016). Selection due to female choice may

401   occur directly for body size or indirectly through correlated traits such as territory quality,

402   display behaviors, activity levels, and ornaments which signal male aggression and quality

403   (Cooper and Vitt 1993; Censky 1997; Hamilton and Sullivan 2005; Swierk and Langkilde 2013;

404   Flanagan and Bevier 2014; Ord et al. 2015).

405   Although genetic estimates of reproductive success and mating success were strongly

406   correlated with body size or size quartiles measured early in the breeding season (May, Figs. 1A-

407   B; 4A, 4C), they were not strongly correlated with size quartiles measured later in the breeding

408   season (July, Figs. 4B, 4D; Table 1). Estimates of mating success from both behavioral and

409   genetic measures were similarly high for males beyond the first size quartile in July (Figs. 2F;

410   4D). This may indicate that, beyond a certain threshold, the advantage of large size in agonistic

411   interactions with other males can saturate (Cox and Calsbeek 2010a; Reedy et al. 2017).

412

Body size and average mate fecundity

414   Male body mass was mostly unrelated to, or sometimes even negatively correlated with,

the average fecundity of female partners (Figs. 1C, 4E-F; Table 1). This may reflect the fact that

the relationship between female body mass and male size quartile was weak and nonsignificant

within each month (Fig. 3A-B), and female mass itself was unrelated to fecundity. Although an

alternative measure of female size (SVL) was significantly related to fecundity, consistent with

previous work showing that larger female anoles may achieve a higher reproductive output

(Warner and Lovern 2014; Duryea et al. 2016) by laying eggs more frequently (Cox and

Calsbeek 2011), we did not find any association between male size quartile and female SVL

(Fig. 3C-D). Thus, neither body mass nor SVL of females provided a strong intermediate linking

male size to female fecundity via size-assortative mating. These findings are consistent with the

general observation that size-assortative mating is rare, particularly in species with male-biased

sexual size dimorphism, such as anoles (Shine et al. 2001; Hofmann and Henle 2006; Harrison

2013; Rios Moura et al. 2021). When mate choice has been detected in anoles, males appear to

prefer novel females rather than larger females (Tokarz 1992; Orrell and Jenssen 2002). This

would be expected if males are primarily under selection to mate with a greater number of

females, rather than more fecund females. In contrast, larger males often mate with larger and/or

more fecund females in species with female-biased sexual size dimorphism (Verrell 1989;

Olsson 1993; Whiting and Bateman 1999; Cox et al. 2005; John-Alder et al. 2009; Jiang et al.

2013).

433

Body size and competitive fertilization success

435   Consistent with previous findings in brown anoles, we found that over 50% of the

436   females having at least two genotyped offspring produced these offspring with more than one

437   mate (Calsbeek et al. 2007; Duryea et al. 2016; Kahrl et al. 2021). Moreover, at least 6% of the

438   females in our powdering studies mated with multiple partners within a short 2-5 day span. The

439   actual frequency of multiple mating is likely to be much higher because our powdering method

440   cannot detect instances of multiple mating within size quartiles, and because our ability to detect

441   multiple paternity is limited by the relatively low number of offspring produced by females

442   (mean = 2.06, range = 1-9 offspring). Although multiple mating by females was common, in

443   situations where females produced offspring with multiple males, male size was unrelated to

444   fertilization success (Figs. 1D, 4G-H, Table 1).

445   Post-copulatory processes can oppose pre-copulatory selection on a given trait if

446   investment in corresponding fitness components is drawn from the same limited resource, or if

447   the genetic covariance among fitness components is negative (Roff and Fairbairn 2007; Parker et

448   al. 2013). Accordingly, inter- and intraspecific comparisons across several lineages, including

449   reptiles, have shown that traits typically subjected to pre-copulatory selection trade-off with

450   those under post-copulatory selection (Moczek and Nijhout 2004; Fitzpatrick et al. 2012; Dines

451   et al. 2015; Kahrl et al. 2016; Somjee et al. 2018). On the other hand, when there is high mean

452   and variance in resource acquisition, this association is likely to be positive since any increase in

453   resource availability allows for more investment in both pre- and post-copulatory competition,

454   (Saeki et al. 2014; Simmons et al. 2017). Consistent with this idea, several intra-specific studies

455   have reported a positive correlation between targets of pre-copulatory sexual selection and

456   ejaculate traits (*reviewed in* Mautz et al. 2013; Supriya et al. 2019). Although some studies report

457   positive associations between standardized fertilization success and traits such as body size,

458   singing effort and/or weapon size (Preston et al. 2001; Hosken et al. 2008; Turnell and Shaw

459   2015; House et al. 2016), others report negative associations (Danielsson 2001; Evans et al.

460   2003; Kelly and Jennions 2011). However, our findings are consistent with those studies in

461   which male fertilization success is unrelated to body size or ornament size (Keogh et al. 2013;

462   Rose et al. 2013; Flanagan et al. 2014; McDonald et al. 2017). This may indicate that investment

463   in mate acquisition does not trade off with investment in fertilization success, possibly due to the

464   predicted low cost of producing ejaculates when these are distributed across several matings

465   (Hayward and Gillooly 2011; Parker 2016; Kahrl et al. 2021; *but see* Kahrl and Cox 2015).

466   One caveat is that our measure of competitive fertilization success required us to exclude

467   all instances in which a single male sired all of the offspring produced by a female, potentially

468   excluding extremely strong or weak sperm competitors from our analysis (Fig. S2D, S3B).

469   However, failure to account for the number of competing males in this way may result in

470   spurious correlations. This is because the estimated proportion of offspring sired by a male will

471   increase, regardless of the focal male's competitive ability, if females produce offspring with

472   fewer mates (Rose et al. 2013; Devigili et al. 2015; McCullough et al. 2018). Indeed, when we

473   used unadjusted fertilization success in brown anoles, we found significant, albeit very weak,

474   positive selection on male body size (Fig. S3). Thus, post-copulatory selection on body size may

475   be weaker in natural populations than previously reported by studies using unadjusted measures

476   of male fertilization success (Preston et al. 2001; Hosken et al. 2008; Turnell and Shaw 2015;

477   House et al. 2016). Our study suggests that, at least for body size, post-copulatory selection is

478   negligible compared to pre-copulatory selection. It is more likely that post-copulatory selection

479   acts primarily on male ejaculate traits, as has been demonstrated in brown anoles (Kahrl and Cox

480   2015), and that it may operate independent of male body size (Kahrl et al. 2021).

481

482   *Comparing behavioral and genetic measures of mating success*

483   We found a close association between measures of size-specific mating success derived

484 from genetic parentage and those inferred from copulations in the field, particularly in July (Fig.

485 5B). This highlights the utility of fluorescent powder transfer as a relatively inexpensive and

486 effective method for detecting copulations, particularly in natural populations, and for linking

487 mating success to broad categories of phenotypic variance. Our findings are in line with other

488 studies that have found behavioral proxies, such as the frequency of male-female associations in

489 space and time, to be closely predictive of the realized mating and reproductive success of males

490 (Kamath and Losos 2018; Olsson et al. 2019; Baird and York 2021). However, our technique is

491 much easier to execute compared to detailed observations of individual copulations or

492 movements, at least in our focal species. Thus, it can be used to uncover associations between

493 mating success and categorical simplifications of continuous traits (as in this study), naturally

494 categorical traits or groups (e.g., morphs), or experimental treatments (e.g., Wittman et al. 2022).

495 It can also be used to uncover mating patterns of secretive or spatially dispersed species that can

496 be difficult to observe in the wild for long hours (Gosden and Svensson 2007; Johnson et al.

497 2014). Nonetheless, behavioral estimates of size-specific mating success based on powder

498 transfer only corresponded closely with genetic mating success when extensive sampling of the

499 female population was possible and when mating rate was high (Fig. 5A-B). For example, in

500 May, we only sampled females for 1 day and the inferred mating rate was half of that seen in

501 July, when we sampled for 5 days (Fig. 5A-B). Perhaps as a result, the relatively low number of

502 observed copulations in May differed significantly from our expected distribution of size-

503 specific mating success, which was likely more accurate because it was based on a much larger

504   number of inferred copulations from genetic parentage (Fig. 5A). Thus, behavioral observations

505   or genetic parentage alone may not adequately capture fitness when populations are partially

506   sampled or if mating is infrequent within a short sampling period.

507

508   **Conclusions**

509   Overall, our study confirms that large body size is associated with higher reproductive

510   success in brown anoles, and that this is primarily due to the increased mating success of large

511   males. Although previous work has suggested that sexually antagonistic viability selection may

512   favor large male size and promote male-biased sexual size dimorphism in this species (Cox and

513   Calsbeek 2010a; *but see* Cox and Calsbeek 2015), our results support a parallel body of recent

514   work suggesting that sexual selection also strongly favors large male size (Tokarz 1985; Jenssen

515   et al. 2005; Duryea et al. 2016; Kamath and Losos 2018). Importantly, we extend this work by

516   specifically resolving the importance of pre-copulatory sexual selection and linking large male

517   size to both behavioral and genetic measures of mating success. Our results further illustrate that

518   strong pre-copulatory sexual selection and extremely male-biased sexual size dimorphism can

519   occur even in promiscuous mating systems in which access to females cannot be monopolized

520   and multiple paternity is common. Finally, our findings emphasize the importance of

521   incorporating both behavioral and genetic methods in the same study to achieve a more robust

522   understanding of the roles of pre- and post-copulatory processes in sexual selection.

523

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Data Availability

All code and data needed to reproduce the results presented in this paper will be available on

Dryad Digital Repository at https://doi.org/10.5061/dryad.c866t1gbb

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Figure legends

Fig. 1: Linear selection on adult body mass as a function of different components of male fitness, including relative measures of (A) reproductive success (total number of offspring), (B) mating success (total number of mates), (C) average mate fecundity (mean fecundity across all mates), and (D) competitive fertilization success (mean proportion of offspring sired across all mates, adjusted for the number of competing males). For each fitness component estimated from genetic parentage, we divided individual fitness by the population mean to obtain relative measures. Adult body mass was measured at the start of the breeding season in March and standardized to a mean of 0 and standard deviation of 1. Trendlines with 95% confidence intervals (CI) from linear regressions are used to visualize linear selection. Solid lines and asterisks in panels A-B indicate significant selection differentials while dotted lines in panels C-D indicate non-significant selection differentials (*** $P < 0.001$).

Fig. 2: Procedure for detecting copulations in the wild. (A) Males were dusted with one of four colors of fluorescent powder based on size quartiles for body mass in (B) May, and (C) July, with colors alternated among size classes between months. Boxplots in B and C depict medians (lines), interquartile ranges (boxes), and minimum and maximum values (whiskers), with the number of males in each quartile shown above each boxplot. After males were released and allowed to interact freely with females for two days, females were captured and (D) inspected under UV light for the presence and color of any powder transferred near their cloaca. The proportions of total copulations detected among females that we correctly attributed to males from each size category are shown separately for (E) May, and (F) July. The number of females with each color of powder is indicated within each bar. The dotted lines give the expected

857   proportion of copulations in each size quartile if mating is random with respect to male size.

858   Colors of bars and box plots indicate the color of powder used for that size quartile.

859

860   **Fig. 3:** Tests for size-assortative mating with respect to (A-B) body mass or (C-D) snout-vent

861   length (SVL)) of females that mated with males from each size quartile in May (left panels) and

862   in July (right panels), based on the color of fluorescent powder detected on the female. Small

863   filled circles (light grey) are individual values and larger overlaid symbols are mean ± SD values

864   for each quartile. Mating was not strongly size assortative in either May (left) or in July (right),

865   as shown by F statistics from a type II ANOVA.

866

867   **Fig. 4:** Distribution of (A-B) reproductive success (total number of offspring), (C-D) mating

868   success (total number of mates), (E-F) average mate fecundity (mean fecundity across all mates),

869   and (G-H) competitive fertilization success (mean proportion of offspring sired across all mates,

870   adjusted for the number of competing males), for males powdered in May (left panels) and July

871   (right panels) as a function of their corresponding size quartile. Fitness components were

872   determined using genetic parentage analysis. Small symbols are individual values and larger

873   overlaid symbols are mean ± SD values for each quartile. Large males had significantly higher

874   reproductive success and mating success than small males in May, but not in July. Average mate

875   fecundity and competitive fertilization success did not differ as a function of size quartile in May

876   or in July.

877

878   **Fig. 5:** Comparison between behavioral and genetic estimates of male mating success in (A)

879   May and (B) July. Bars represent the proportion of copulations observed for males in each size

880   quartile based on transfer of powder, and dotted black lines indicate the expected proportion of

881   copulations in each size quartile based on the number of mates inferred from genetic parentage

882   data for the same males. Numbers (n) indicate the total number of copulations observed in each

883   size quartile. The observed number of copulations based on powder transfer differed

884   significantly from the expected number based on genetic parentage in May, but not in July.

885

886

887 **Tables**

888 **Table 1:** Summary of coefficient estimates from generalized linear regressions, carried out

889 separately for each measure of fitness as a function of size quartile (ordinal), month (categorical),

890 and their interaction as predictors, when pooling data from May and July: reproductive success

891 (total number of offspring), mating success (total number of mates), average mate fecundity

892 (mean fecundity across all mates), and competitive fertilization success (mean proportion of

893 offspring sired across all mates, adjusted for the number of competing males).

Coefficient	Reproductive success	Mating success	Average mate fecundity	Competitive fertilization success
	Incidence Rate Ratios (95% CI)	Incidence Rate Ratios (95% CI)	Incidence Rate Ratios (95% CI)	β (95% CI)
Intercept	2.87 *** (2.38 – 3.47)	1.92 *** (1.63 – 2.25)	2.76 (2.51 – 3.03)	0.51 *** (0.48 – 0.54)
Size Quartile (Linear - L)	1.56 * (1.06 – 2.30)	1.44* (1.04 – 2.00)	0.81* (0.67 – 0.98)	0.04 (-0.02 – 0.09)
Size Quartile (Quadratic - Q)	0.75 (0.52 – 1.10)	0.90 (0.65 – 1.23)	1.12 (0.93 – 1.35)	-0.06 * (-0.11 – -0.005)
Size Quartile (Cubic - C)	1.02 (0.71 – 1.47)	0.96 (0.70 – 1.30)	1.00 (0.83 – 1.21)	-0.01 (-0.06 – 0.05)
Month [May]	0.54 *** (0.41 – 0.71)	0.63 *** (0.50 – 0.80)	0.95 (0.82 – 1.10)	-0.02 (-0.06 – 0.02)
Size (L) x Month	1.64 (0.95 – 2.85)	1.45 (0.91 – 2.34)	1.09 (0.83 – 1.44)	-0.00 (-0.08 – 0.08)
Size (Q) x Month	2.20 *** (1.27 – 3.82)	1.64* (1.01 – 2.65)	1.12 (0.83 – 1.50)	0.09 * (0.01 – 0.17)
Size (C) x Month	1.47 (0.85 – 2.56)	1.46 (0.90 – 2.37)	0.98 (0.72 – 1.34)	0.02 (-0.06 – 0.10)
n	262	262	176	135
R^2	0.279	0.225	0.090	0.081 / 0.030

Estimates indicate the relative increase (Incident Rate Ratio > 1 or β > 0) or decrease (Incident Rate Ratio < 1 or β < 0) with an increase in size quartile for each predictor variable.
Terms are significant if the 95% confidence intervals indicated in brackets do not overlap Incident Rate Ratios at 1 or β at 0 (*** P <0.001; *P <0.05)
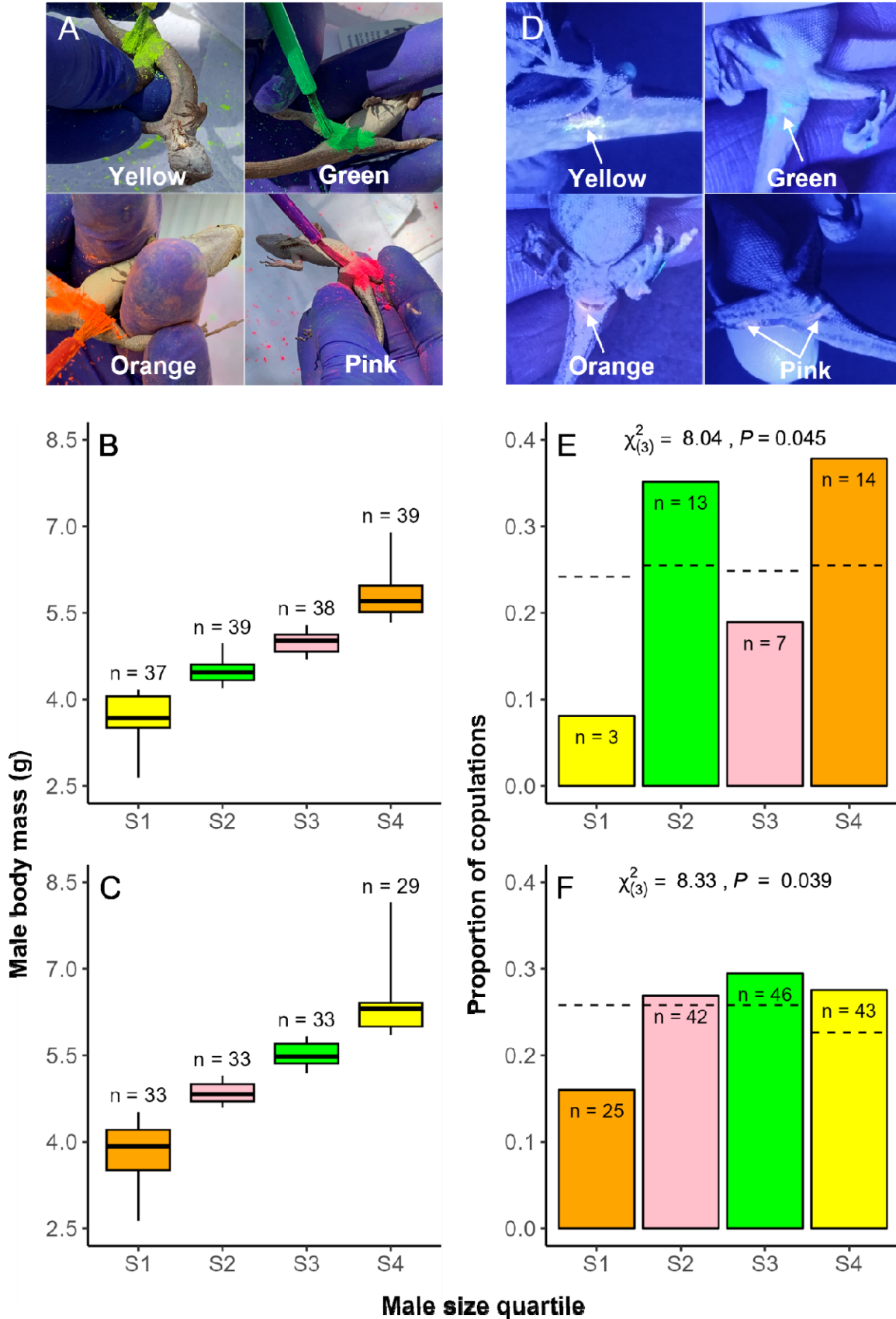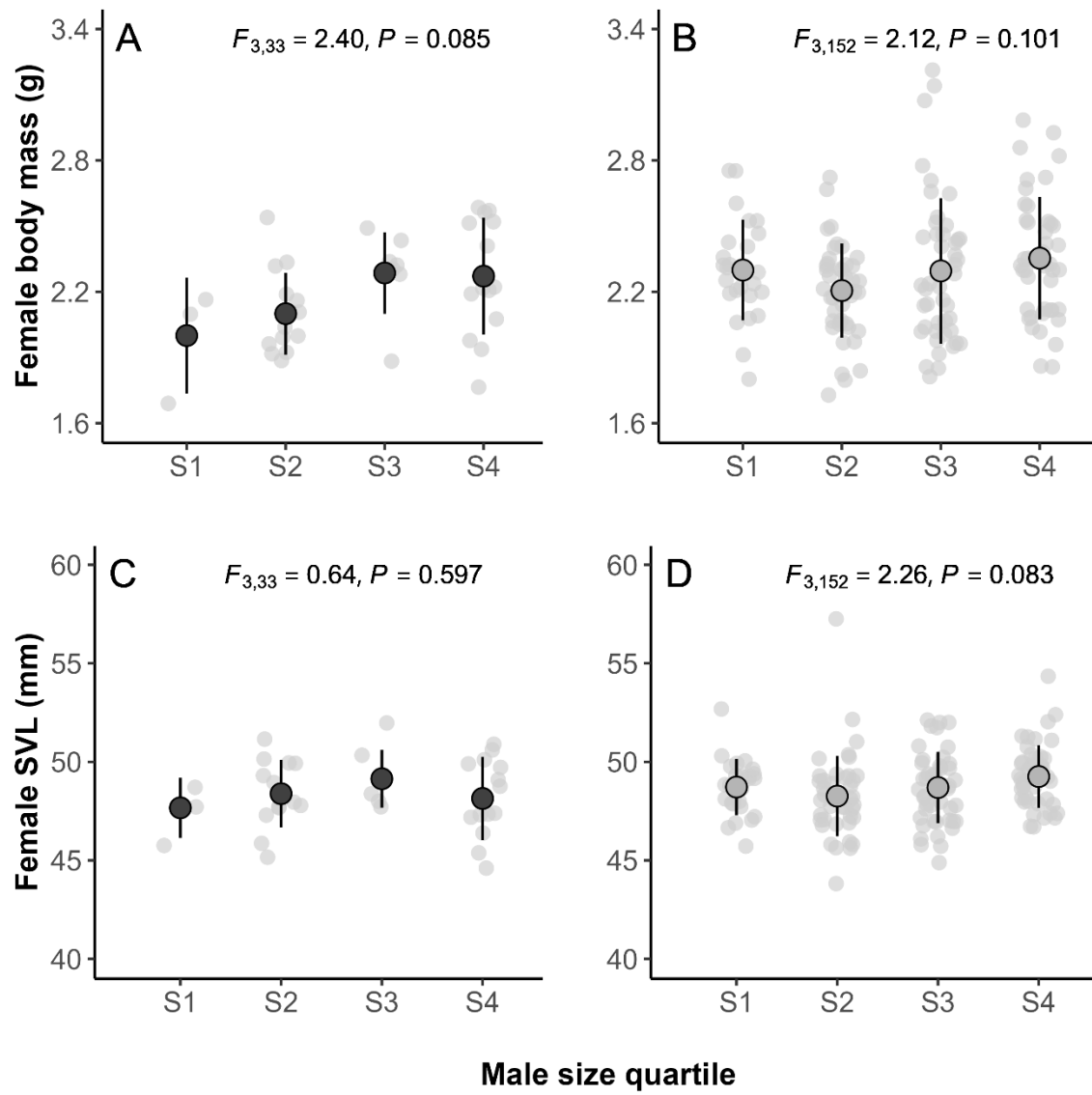
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895 **Figures**

896 **Fig 1.**
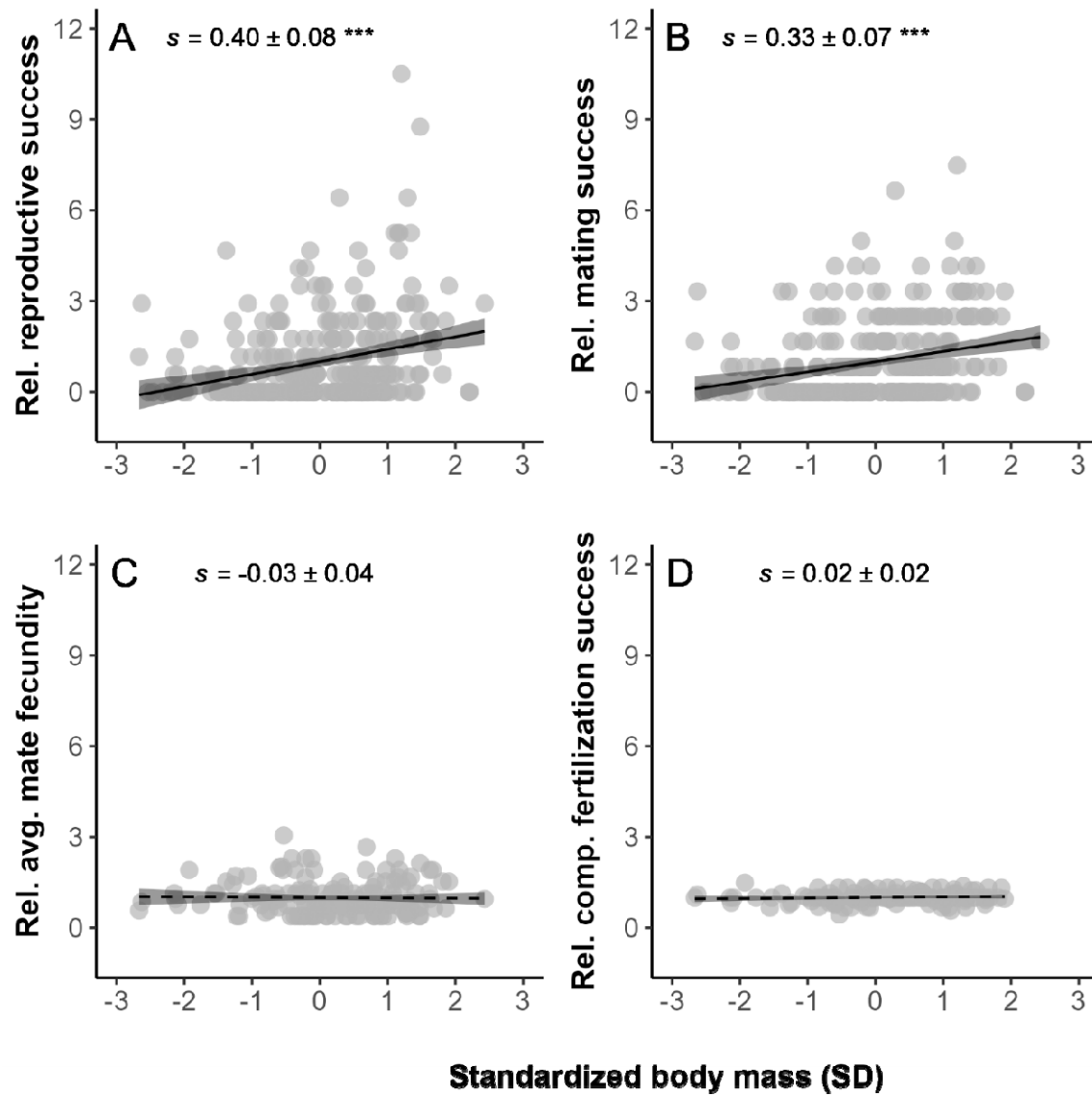
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Fig 2.

899 **Fig 3.**

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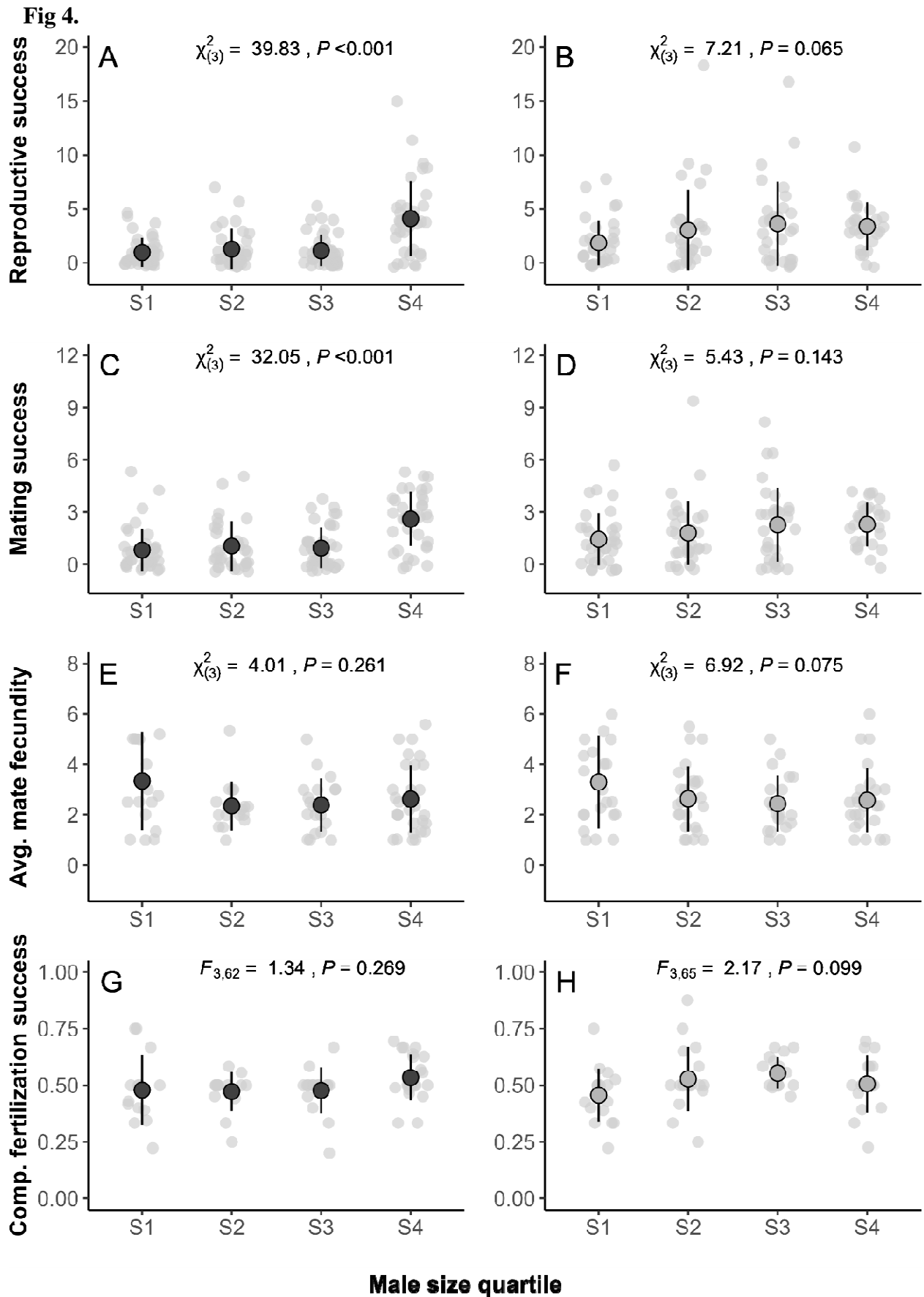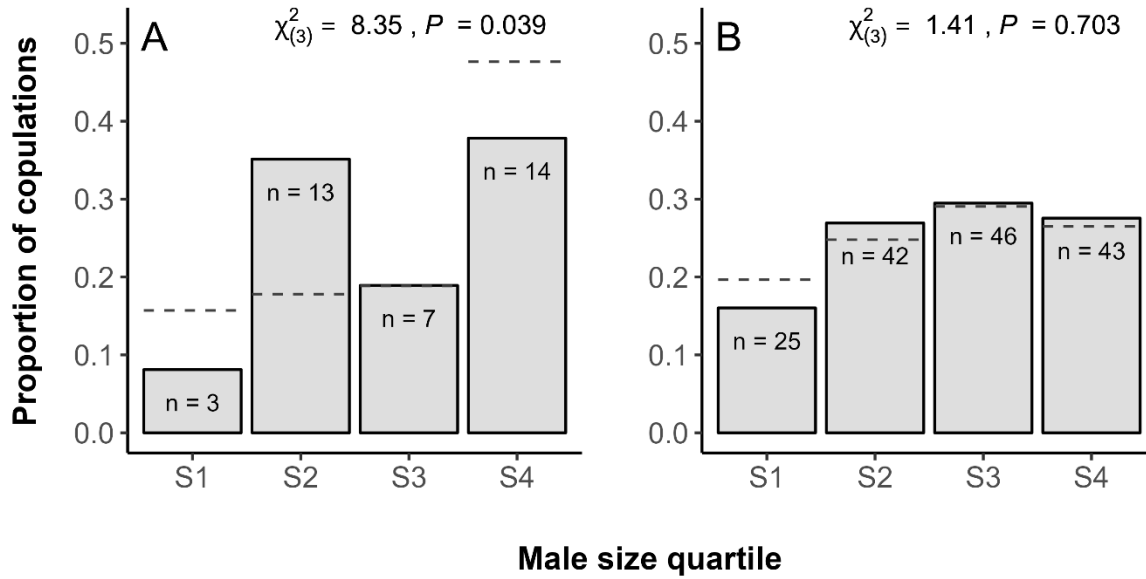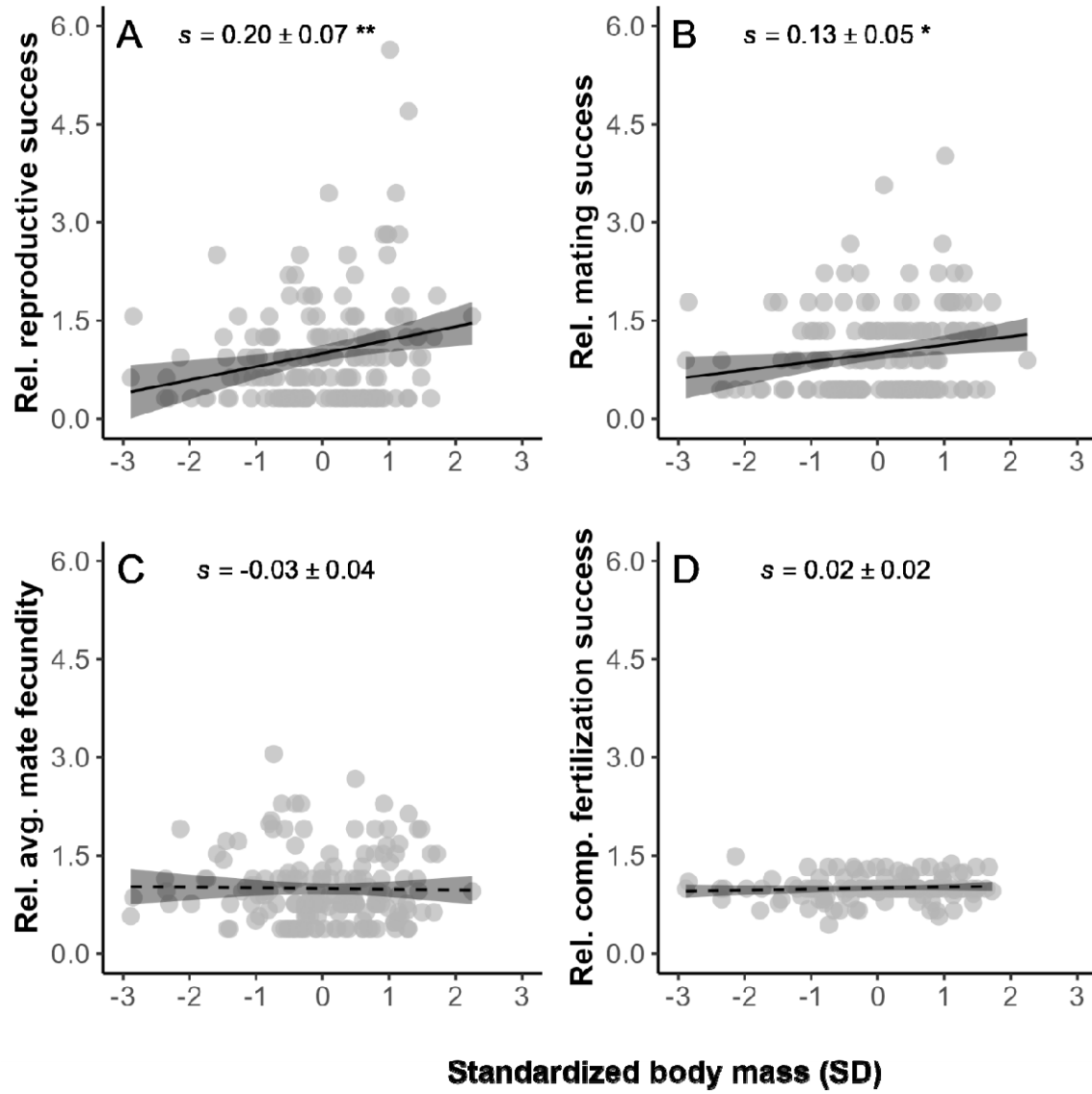
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Fig 4.

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903 **Fig 5.**

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Supplementary Figures

Fig. S1 Linear selection on standardized adult body mass as a function of different components of male fitness, including relative measures of (A) reproductive success (total number of offspring), (B) mating success (total number of mates), (C) average mate fecundity (mean fecundity across all mates), and (D) competitive fertilization success (mean proportion of offspring sired across all mates, adjusted for the number of competing males). Here, individuals that were not assigned offspring in parentage analysis have been excluded from all regressions to avoid inflating the relative contribution of mating success to total reproductive success by assuming that these males failed to mate. Trendlines (with 95% CI) from linear regressions are used to visualize selection. Solid lines and asterisks in panels A-B indicate significant selection differentials (s) while the dotted lines indicate non-significant selection differentials (** $P <$ 0.01; * $P < 0.05$). Excluding males without any assigned progeny reduces the overall strength of selection on size by 50% (from $s = 0.40$ to 0.20, compare with Figs. 1 or S3), but only slightly reduces the proportion of that selection attributable to variance in mating success (from $s = 0.33$ or 82.5% to $s = 0.13$ or 65%, compare with Figs. 1 or S2).
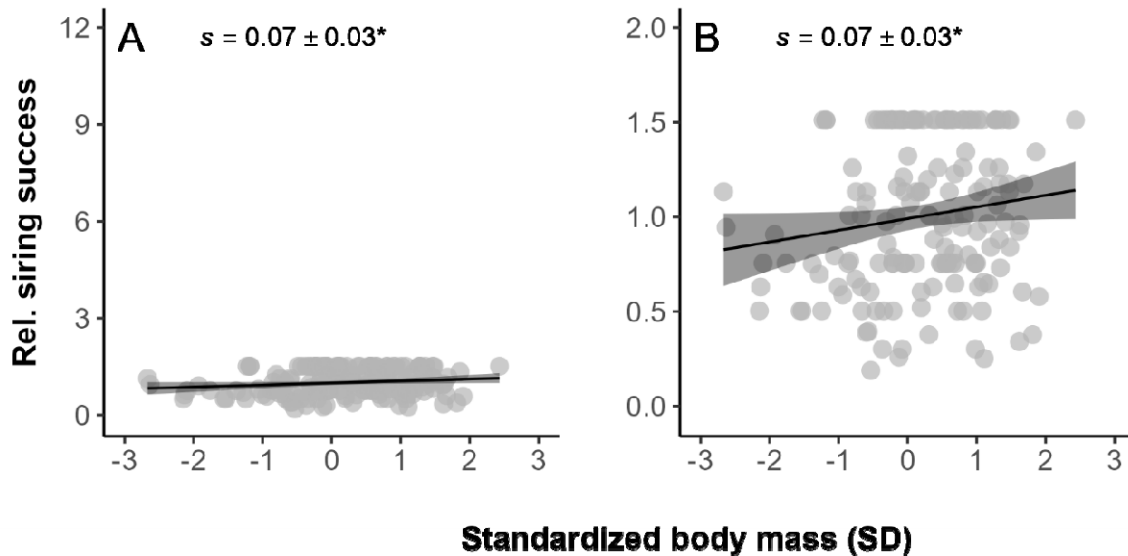
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927   **Fig. S2** Linear selection on standardized adult body mass as a function of different components

928   of male fitness, including relative measures of (A) reproductive success (total number of

929   offspring), (B) mating success (total number of mates), (C) average mate fecundity (mean

930   fecundity across all mates), and (D) competitive fertilization success (mean proportion of

931   offspring sired across all mates, adjusted for the number of competing males). Here, the y-axis

932   has been scaled to include only the range of data points for each fitness component to clearly

933   visualize the selection differentials estimated in Fig 1. Trendlines (with 95% CI) from linear

934   regressions are used to visualize selection. Solid lines and asterisks in plots A-B indicate

935   significant selection differentials (*** $P < 0.001$) while the dotted lines indicate non-significant

936   selection differentials ($P > 0.05$).
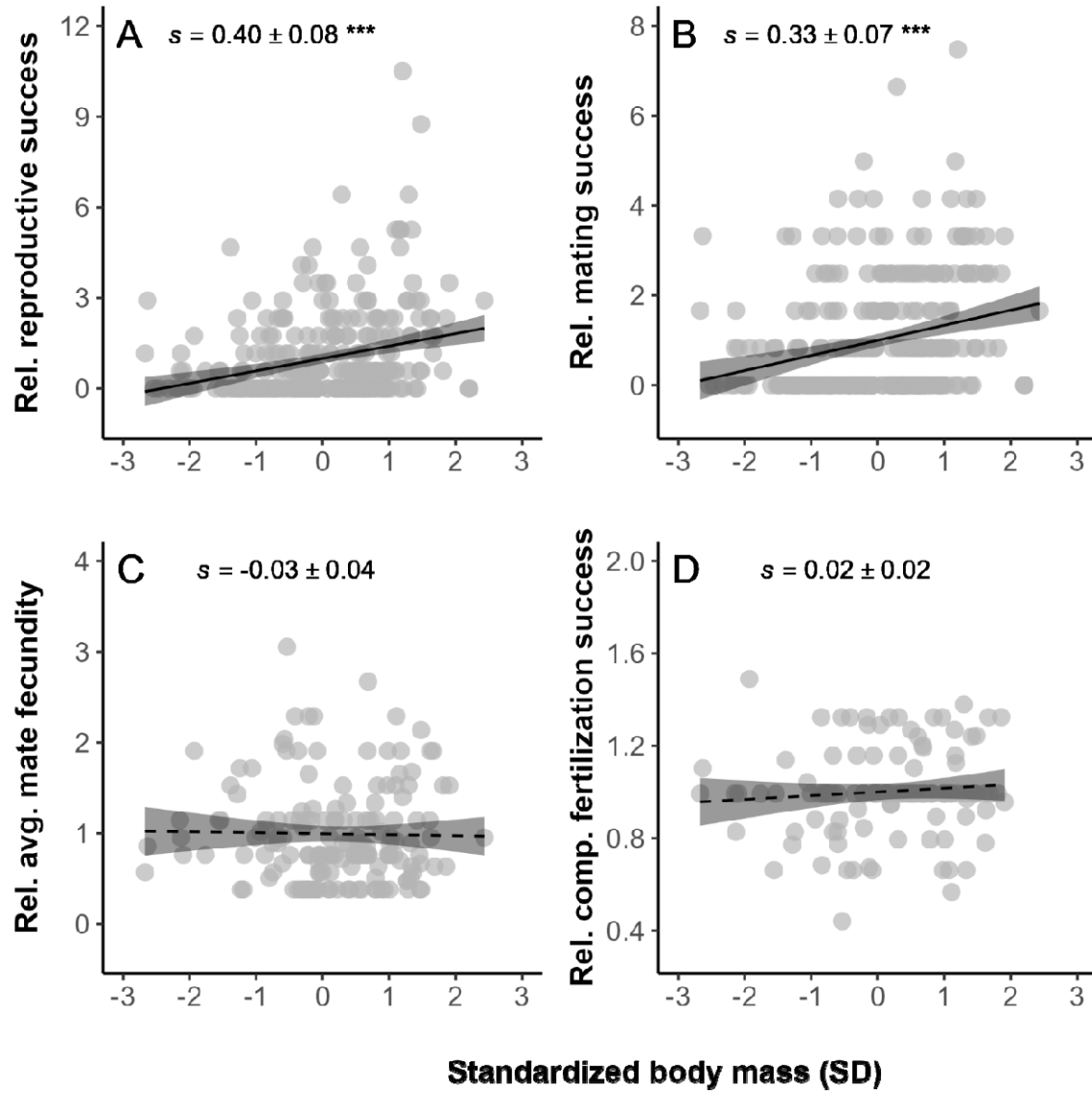
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939   **Fig. S3** Linear selection on standardized adult body mass as a function of relative siring success

940   of males (not adjusted for competitor males) (A) along the same scale as the y-axis in Fig 1 or

941   (B) with y-axis scaled to include only the range of data points for the fitness component to

942   clearly visualize the same selection differential. Trendlines (with 95% CI) from linear

943   regressions are used to visualize selection. Solid lines and asterisks indicate significant selection

944   differentials (* $P < 0.05$). Selection due to unadjusted measures of fertilization success on male

945   body mass was significant and three times stronger than that due to competitive fertilization

946   success (from s = 0.02 to 0.07, compare with Figs. 1D or S2D)

947

948