1 TITLE: No support for intra-nor inter-locus sexual conflict over mating latency and copulation duration in a
2 polyandrous fruit fly
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21 carried out, and collected the data for Population 1. JMC analyzed the data in MCMCglmm that is

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25

Abstract

The total strength of sexual selection on males depends on the relationship between various components of pre- and post-copulatory fitness. Misalignment between male and female interests creates inter-locus sexual conflict, where the fitness of one sex is increased at the expense of the other. Although rarely considered, mating behaviours can also be genetically correlated between males and females, creating intra-locus sexual conflict, where beneficial alleles in one sex are costly when expressed in the other sex. How inter- and intra-locus sexual conflicts operate on the expression of mating behaviours remains little understood. Here, we study male attractiveness, mating latency and copulation duration in two populations of the polyandrous *Drosophila serrata*. Univariate analyses show little genetic variance in mating latency, and that males, but not females, contribute to copulation duration genetic variance. Further, multivariate analyses revealed little covariance between the studied traits. However, analyses considering male and female contribution in a single framework supported genetic contributions from both sexes for mating behaviours and complex patterns of between sexes correlations. Finally, our study did not find any association between those mating behaviours and fitness component, specifically (i) no phenotypic covariance between male attractiveness and mating latency and, (ii) longer copulations did not result in the production of more offspring. With no detectable fitness benefits in any sexes for shorter mating latency or longer copulation duration, our results do not support the presence of inter- nor intra-locus sexual conflict for these mating traits.

Keywords: mating latency, copulation duration, quantitative genetics, indirect genetic effect, sexual conflict, drosophila serrata, multivariate analyses, Cuticular HydroCarbons

48 ## Introduction

49   The total strength of sexual selection on males depends on the relationship between various

50 components of pre- and post-copulatory fitness (Collet et al., 2014). Theoretical expectations of how

51 pre- and post-copulatory fitness covary, however, are unsettled. On the one hand, the phenotype-linked

52 fertility hypothesis predicts that male ornaments reflect their fertility, and thus male pre- and post-

53 copulatory success are predicted to be positively correlated (Sheldon, 1994). On the other, trade-offs

54 may exist between pre- and post-copulatory traits, for example, mating success and sperm

55 competitiveness, resulting in a negative correlation between pre- and postcopulatory fitness (Parker &

56 Pizzari, 2010). The empirical data are also mixed: substantial experimental and observational studies

57 support both negative (e.g. Danielsson, 2001) and positive (e.g. Collet et al., 2012) phenotypic

58 correlations (See metanalysis Mautz et al., 2013). Despite the interest in, and the large number of

59 phenotypic studies, there have been fewer to focus on the genetic covariances between pre- and post-

60 copulatory traits (but see Taylor et al., 2013, Hall et al., 2013). Genetic covariances, however, are the

61 currency for evolution, and in order to understand the evolution of copulatory traits in males, we must

62 understand how they genetically covary.

63   In addition to within-sex genetic covariances, correlations of homologous traits between the

64 sexes have an important role in determining the evolutionary trajectories of fitness-related and other

65 traits (e.g. Gosden et al., 2012, McGlothlin et al., 2019, Sztepanacz & Houle, 2019, Holman & Jacomb,

66 2017). When the largely shared genome of males and females is subject to divergent selection to sex-

67 specific phenotypes, intralocus sexual conflict can arise (Bonduriansky & Chenoweth, 2009). For

68 example, when the alleles that underlie traits which increase male reproductive fitness, such as

69 attractiveness, come at a cost when they are expressed in females, and vice versa. Sexual antagonism

70 can also arise when selection is sexually concordant, suggesting that it is an inevitable by-product of

71 species with separate sexes (Connallon & Clark, 2014). Intralocus sexual conflict results in a reduction of

72 population mean fitness because neither sex can reach their fitness optima. Although sexual conflict

73 may be resolved by the evolution of sexual dimorphism (Collet et al., 2016, Bonduriansky & Chenoweth,

74 2009), the pervasiveness of negative intersexual genetic correlations for fitness indicate that these

75 conflicts are often unresolved (Chippindale et al., 2001, Brommer et al., 2007, Foerster et al., 2007,

76 Kohorn, 1994, Cox & Calsbeek, 2009) .

77   Another form of sexual conflict, interlocus sexual conflict, can arise from the interactions between the

78   sexes, which increase the fitness of one sex at the expense of the other (Arnqvist & Rowe, 2005). These

79   interactions result in a coevolutionary arms race where adaptation of a trait in one sex results in

80   counteradaptation of the interacting trait in the other sex (Parker, 1979, Moore & Pizzari, 2005, Rowe et

81   al., 2005, Arnqvist & Rowe, 2005, Chapman et al., 2003, Clutton-Brock & Parker, 1995). Such

82   evolutionary arms races can lead to the rapid evolution of interacting traits. Historically, intra- and inter-

83   locus sexual conflict have been treated as separate evolutionary processes. More recently, however, the

84   potential for an interaction between intra- and inter-locus sexual conflict has been highlighted (Pennell

85   & Morrow, 2013). In particular, theory has shown that when reproductive traits are involved in both

86   intra- and inter- locus sexual conflict their interactions can lead males and females into a repeating cycle

87   of conflict escalation followed by resolution, preventing them from reaching a stable equilibrium

88   (Pennell et al., 2016).

89   The extent of between-sex pleiotropy (intra-locus) for traits that are also involved in inter-locus

90   sexual conflict is scarce, and in general, we lack a comprehensive study of traits that may experience

91   both intra- and inter-locus sexual conflict in a single framework. Copulatory traits are one class of traits

92   that may be genetically correlated between the sexes and also interact during mating, leading to the

93   potential for both intra- and inter- locus sexual conflict. Here, we used a quantitative genetic breeding

94   design to investigate the within and between sex heritabilities and correlations of copulatory traits in

95   *Drosophila serrata*. Specifically, we estimated the heritability of male attractiveness, male and female

96   mating latency and copulation duration, and the within and across- sex genetic correlations of these

97   traits. Furthermore, we investigated whether any of these traits predicted male and female fitness,

98   indicating the potential for sexual conflict.

99   The relationship between attractiveness and mating latency has been particularly well described

100   in *Drosophila melanogaster*, where males that carry the cuticular hydrocarbon (CHC) pheromone, 7-

101   tricosene, are more attractive (as measured by their mating rate), and mate faster, than males that do

102   not (Grillet et al., 2006). In one study, variation in mating latency was explained to a similar extent by

103   the genetic identity of both males and females, suggesting a role of both sexes in determining this

104   phenotype (Tennant et al., 2014). The relationship between male attractiveness and mating latency is

105   not universal, however. In *D. bunnanda*, males that were artificially selected to carry a more attractive

106   combination of cuticular hydorcarbons (as measured by their mating rate) did not mate faster than

107   males selected for a less attractive combination (McGuigan et al., 2008), and in *D. melanogaster,*

108   MacKay *et al* (2005) found that the genetic background of females was the best predictor of mating

109   latency. In *D. serrata,* male attractiveness has also been well described, where females have been shown

110   to prefer a particular combination of CHC contact pheromones in males (Chung et al., 2014, Hine et al.,

111   2002, Hine et al., 2011, Chenoweth & Blows, 2003). In a number of studies, these CHCs have consistently

112   predicted male mating success, explaining up to 46% of its variance (Rundle et al., 2005). CHCs also

113   genetically covary between the sexes, and may experience intralocus sexual conflict (Gosden et al.,

114   2012). The relationship between male CHCs and mating latency, however, has not been established in

115   this species.

116   In many insects, once mating starts, a cocktail of proteins (Seminal fluid proteins, Sfps) are

117   transferred along with sperm during copulation (Poiani, 2006). These proteins trigger an array of

118   behavioural and physiological responses in females which increase male fitness, for example, decreasing

119   female receptivity to subsequent mating, stimulating egg laying, facilitating sperm storage, forming a

120   mating plug, and displacing sperm from other males (Chen et al., 1988, Gioti et al., 2012, Chapman &

121   Davies, 2004). These responses come at a cost to females, as Sfps reduce female lifespan and their

122   lifelong reproductive success (Wigby & Chapman, 2005, Fowler & Partridge, 1989). Mating in *D.*

123   *melanogaster* takes ~20 minutes, twice the time needed to transfer sperm alone (Gilchrist & Partridge,

124   2000), and mating duration has a genetic basis (Moehring & Mackay, 2004), and is phenotypically plastic

125   depending on the social environment (Rouse et al., 2018). In a rare study investigating sex-specific

126   genetic contributions to copulation duration, Edward at al. (2014) found significant genetic variance for

127   copulation duration in both males and females *D. melanogaster*, although male's contribution was

128   higher than female's.

129   Copulations in *D. serrata* are markedly different. In a modest study of 31 individuals, median

130   mating duration was 4 minutes, with 157s recorded as the shortest mating duration that could lead to

131   progeny (Hoikkala et al., 2000). Males in this species appear to transfer sperm in a single clump (J

132   Sztepanacz pers observation), suggesting that longer copulation durations would not equate to more

133   sperm transferred. Whether males also transfer seminal fluid proteins that affect female physiology or

134   behaviours is unknown. Hoikkala et al (2000) observed a weak and non-significant negative relationship

135   between copulation duration and the number of progeny produced by females, suggesting that Sfps

136   may not have a large role in this species.

137   In this study we estimated the phenotypic and genetic correlations between male

138   attractiveness, mating latency, duration, and productivity, in two independent data sets from the same

139   species. Each data set was a large quantitative genetic breeding design with 80 sires and phenotypes on

140   over 3,000 flies measured in each experiment. These independently replicated data provide

141   unprecedented power to identify the genetic basis of copulatory behaviours and their effects on fitness.

142

Materials and methods

143

Experimental and Breeding Design

144

145   The data that we analyse here come from two independent populations and experiments in *D. serrata*

146   performed under similar conditions. The data from Population 1 allows us to estimate the heritabilities

147   for and genetic correlations between male attractiveness, mating latency, and mating duration. The data

148   from Population 2 allows us to estimate the phenotypic correlations between male attractiveness and

149   mating latency, and heritabilities for and genetic correlations between male and female mating latency

150   and duration, and their cross-sex genetic correlations. Further, this experiment measured productivity of

151   the resulting matings, allowing us to directly estimate the relationship between mating behaviour and

152   fitness. Therefore, the combination of these two experiments enable us to track the outcomes of mating

153   interactions from pre- to post-copulatory fitness.

154   *Population 1*

155   Experiments in population 1 were conducted on an outbred laboratory population of *D. serrata* (Rundle

156   et al., 2006) maintained at a large population size (N>2000) under standard laboratory conditions. A half

157   sibling breeding design was carried out to estimate additive genetic variances and covariances in traits

158   of interest. The details of the breeding design are described in detail in Sztepanacz and Rundle (2012).

159   Briefly, eighty sires were each mated to three virgin dams, which were allowed to oviposit for 72h after

160   mating. Upon emergence of their offspring, male offspring were collected from these families using CO_2

161   anaesthesia and were held as virgins at a density of 6 flies per vial for 5-7 days prior to their use in

162   experimental assays. The breeding design was conducted in two blocks of 40 sires, spanning two

163   generations of the laboratory population, and resulted in 2941 individuals from 240 full- and half-sibling

164   families from 80 sires. Brothers from each family were used in either a (1) a competitive mating trial

165   where their cuticular hydrocarbons (CHCs) were subsequently extracted or (2) in a behavioural assay

166   which measured mating latency and duration.

167   The details of the binomial mate choice and CHC assay are described in detail in Sztepanacz and Rundle

168   (2012), where these data were first published. They found that male CHCs were under significant

169   directional sexual selection which explained 9.1% of the variance in male mating success. In order to

170   obtain an 'attractiveness' score for these males, here we applied the standardized sexual selection

171   gradient β, presented in their paper, to the individual pheromone profiles of each male (n=1979 males).

172   $$Attractiveness\ score = \ \beta^T \mathrm{CHC}_i \beta$$

173   Where β is the standardized sexual selection gradient, ^{T} denotes the transpose of the vector, and CHC_i

174   indicates the vector of 8 CHC traits for an individual.

175   To measure mating latency and duration a single male fly was introduced in a vial with a single female

176   which had the same genetic background as the male, but was fixed for a recessive orange-eye mutation.

177   The time it took before mating began was recorded as mating latency. Once mating started, copulation

178   duration was recorded as the time during which genital contact could be observed (n=962 males). If the

179   pair did not mate during the first two hours, the pair was discarded from the measure of mating latency

180   and copulation duration.

181   *Population 2*

182   Experiments in population 2 were conducted on an outbred laboratory population of *D. serrata*

183   described elsewhere (Hine et al., 2014), maintained with a large population (N>2000) under standard

184   laboratory conditions. First, we carried out a paternal half-sibling breeding design to estimate the

185   genetic variances and covariances between mating latency, duration, and productivity in males and

186   females. Eighty sires were each mated with three females and up to 10 female and 10 male virgin

187   offspring of each pair were collected using ice anaesthesia. Males and females were kept separately (up

188   to four males per vial, up to six females per vial) in vials containing 7mL of standard food, for four to six

189   days before mating trials. The day before the experiment, males and females from the breeding design

190   were transferred in an individual vial with fresh food. In total 1558 males and 1726 females, were

191   produced across three blocks from 80 sires and 220 dams. Males and females from each family were

192   used in (1) behavioural assays to measure mating latency and duration, and (2) had their productivity

193   subsequent measured.

194   On the day of the experiment, each focal female was put together with a virgin male from the same

195   population and time until mating started was recorded as mating latency. If the pair did not mate during

196   the first two hours, the pair was discarded from the measure of mating latency and copulation duration.

197   Once mating started, copulation duration was recorded as the time during which genitalia contact could

198   be observed. At the end of the mating, males were discarded, and females placed in individual 10mL

199   vials with standard food and without live yeast. We left females lay eggs for 24 hours, after which

200   females were discarded and offspring were left to develop. We chose 24h to capture the potential male

201   effect of mating on the number of eggs laid, that was shown to occur when sex peptide was injected

202   into female *D. melanogaster* (Aigaki et al., 1991). After offspring emergence, vials with adult offspring

203   were frozen at -20C and the number of adults that had emerged was counted as the measure of

204   productivity.

205   Males from the paternal half-sibs were tested in similar conditions. The day before mating trial, males

206   from the breeding design were isolated in a fresh vial with 10mL standard food. On the mating trial day,

207   each male was put together with a virgin female that was fed with *ad libitum* yeast. Mating latency and

208   copulation duration were recorded the same way as for females. Male productivity was also recorded as

209   the number of offspring that emerged from the vial of their female mate.

210   When recording productivity from the frozen vials, there was a few instances where the number of

211   offspring was impossible to individually count, as they emerged in the stopper. In a few vials, we

212   proceeded to careful visual inspection to estimate that around five flies must have been caught in the

213   stopper. Thus, five additional offspring were systematically added to the productivity obtained in

214   subsequent vials in which those emergence were encountered.

215   Finally, we measured whether attractive males mated faster. We randomly collected virgin males and

216   females at emergence from the population. Four to six days after collection, to guarantee sexual

217   maturity, two virgin males were put together with a virgin female. The time between the introduction of

218   the female in the vial with both males and the beginning of the mating was recorded as mating latency.

219   After copulation started, the mating pair was separated and the male who mated (chosen) and the other

220   male (rejected) had their CHCs extracted and assayed using gas chromatography following standard

221   procedure as in population 1 (Blows et al., 2004, Sztepanacz & Rundle, 2012). We then followed the

222   same method as in population 1 to obtain attractiveness scores; we determined the selection gradients

223   for male CHC profile by using the partial regression coefficients of the linear regression of standardized

224   mating success on the standardized log contrasts of CHCs peaks, and applied it to the individual

225   pheromone profile of each male.

226

227   Statistical analyses

228   *Quantitative genetic analyses in Populations 1 and 2: heritabilities and genetic covariances*

229   All models estimating quantitative genetic parameters in both populations were performed on

230   standardized (z-score) data using animal models (Lynch & Walsh, 1998, De Villemereuil, 2012) with the R

231   package MCMCglmm (Hadfield, 2010). In all models, blocks were entered as fixed effects and additive

232   genetic effects (using the pedigree information) were entered as random effects. In models that

233   included attractiveness in population 1, a supplementary *column* effect was added to account for the

234   use of two different gas chromatography columns for this experiment. Error distributions were

235   Gaussian. Number of iterations, burn in, and thinning intervals varied for each model, and they were set

236   to achieve convergence by using the Heidelberger and Welch test in the coda R package (Heidelberger &

237   Welch, 1981, Plummer et al., 2006) and a minimum effective sample of 1000 for all studied parameters.

238   All credible intervals are 89% (Makowski et al., 2019). Analyses of Population 1 and Population 2 were

239   performed independently.

240   First, univariate animal mixed models were performed on mating latency, copulation duration, and

241   attractiveness, in females and in males. Prior distributions were sets to inverse-Gamma, with

242   parameters V=1 and nu=0.002. Heritabilities were calculated from the posterior distribution as the

243   median proportion of phenotypic variance explained by the animal factor. To confirm whether there

244   was additive genetic variance, we compared the Deviance Information Criterion (DIC) of these univariate

245   models to the DIC of models that did not include the 'animal' random effect. DIC are useful to select the

246   model which best describes the data (as for example the AIC), when the posterior distribution is well

247   summarized by its mean (Spiegelhalter et al., 2002, Gelman et al., 2014).

248   To investigate covariance between pre- and post-copulatory traits, and between the sexes, we ran

249   multivariate animal mixed models including the traits for which we tested genetic covariance with

250   MCMCglmm. Additive genetic variance-covariance G matrices were inferred from the median posterior

251   distribution of genetic variances and covariances of the animal effect. To test the extent of genetic

252   covariance, we compared the DIC of those models to the DIC of models in which the genetic covariance

253   was set to 0 (*idh*, off-diagonal of the G matrix =0).

254   *Phenotypic analyses in population 2: relationships between copulatory and fitness traits*

255 We used population 2 to investigate whether mating latency or copulation duration could predict the

256 measured components of pre- and post-copulatory fitness. First, we used the mate choice experiment to

257 see whether more attractive males mated faster. We tested whether the log transformed mating

258 latency could predict the attractiveness score with a linear model (lm in R-Core-Team, 2014) in males

259 that were successful at mating.

260 Further, we tested whether mating latency or copulation duration predicted the pair's productivity.

261 Because the dataset was obtained in the paternal half-sibs breeding design, we accounted for

262 relatedness between tested individuals with the following mixed model (Pinheiro et al., 2020):

263 $$Y_{ijk} = \beta_0 + \beta_1\ block + \beta_2\ trait + b_{k(j(i))}$$

264 where Y_{ijk} is the standardized productivity of the k th male, son of the j th dam and i th sire, $block$ is to

265 account for the three blocks performed, $trait$ either mating latency or copulation duration. b_{ijk} is an

266 observation (k)-level random effects nested in dams (j), themselves nested in sires (i). Statistical

267 significance for the $trait$ effect was tested with Log-Likelihood ratio tests comparing two models where

268 the $trait$ fixed effect was included or not.

269

270 ## Results

271 ### Heritability of attractiveness, mating latency, and mating duration

272 There was substantial phenotypic variation in male attractiveness scores in both populations, which had

273 a heritability of 0.31 in population 1 (estimated using REML, genetic effect vs. no genetic effect

274 ΔAIC=75.2, Suppl Materials, MCMCglmm ΔDIC=1018, Table 1). Mating occurred shortly after males and

275 females were put together in the vial. Over a quarter of the pairs mated in the first 5 minutes in

276 Population 1 and in the first 7 minutes in Population 2, however the distribution was skewed with a long

277 tail extending to 120min, which is when the experiment was stopped (Fig. 1). The median mating latency

278 in Population 1 was 10.2 minutes, and in Population 2 was 22.4 minutes (Fig. 1). We estimated the

279 heritability of mating latency using the observed variance standardised data, and using log-transformed

280 values. Overall, the estimates of heritability were similar, so we report the non-transformed values here

281 and heritability of log-latency in the supplementary material (Supplementary Table 2). Univariate

282 analyses in population 2 found no evidence for heritable variation in mating latency in males (h^2=0.007;

283 CI [0.000 ; 0.046]) nor in females (h^2= 0.009; CI [0.001 ; 0.050]). In population 1, however, there was

284 some evidence that latency was heritable in males (h^2= 0.068; CI [0.003 ; 0.175]) and evolvable (e =

285 1.3%). In *D. melanogaster*, single phenotypic observations of latency have been shown to be a highly

286 noisy measure (Hoffmann, 1999), which may explain why we were able to detect heritable variation in

287 one population but not the other.

288 Once mating started, the median copulation duration in Population 1 was 5.8 minutes and 4.6 minutes

289 in Population 2 (Fig. 2). This is consistent with the durations observed in *D. serrata* by Hoikkala et al

290 (2000), which ranged from 2.62 to 7.87 minutes with a median of 4 minutes. Copulation duration was

291 heritable in males from Population 1 with an estimated heritability of 0.15 and an evolvability of 1.5%,

292 suggesting that this trait has a genetic basis and can respond to selection (Table 1). In Population 2,

293 however, heritabilities were low in both sexes, although higher in males (male h^2 = 0.043 [0.002 ; 0.043];

294 female h^2 = 0.005 [0.000 ; 0.031]), with little statistical support (male ΔDIC = 6; female ΔDIC = -1, Table

295 1).

Relationships between copulatory traits

297 There was no genetic correlation between male attractiveness score and mating latency, with a credible

298 interval that overlapped zero (Bivariate model in Population 1: r_G 0.11; [-0.17 ; 0.37]; ΔDIC = 21, Table

299 2). The genetic covariance matrix for male attractiveness, mating latency, and mating duration in

300 Population 1 is shown in Table 2. Overall, the genetic correlations were low between all traits with

301 confidence intervals of the estimates overlapping 0 and ΔDIC between models with and without genetic

302 covariance equal to -2.

Cross-sex relationships for interactive traits

304 In population 2 we were able to estimate both within-sex and across-sex genetic covariances for mating

305 latency and duration. The full genetic correlation matrix is shown in Table 3. Similar to population 1,

306 genetic correlations between latency and duration within each sex were modest with credible intervals

307 of the estimates overlapping 0. However, the overall G matrix contained some level of genetic

308 covariance, as ΔDIC between models with and without genetic covariance equal to 95.

309 Consistent with other studies that estimate cross-sex genetic covariance matrices (Gosden &

310 Chenoweth, 2014, Sztepanacz & Houle, 2019), we found that estimates of cross-sex cross-trait

311   correlations were asymmetric. The point estimate of the correlation between latency in males and

312   duration in females was negative (r_G = -0.056; [-0.379 ; 0.283]), while the correlation between latency in

313   females and duration in males was positive (r_G = 0.094; [-0.260 ; 0.416]). The confidence intervals of

314   these estimates were large and overlapping however, so we cannot say with confidence that they differ

315   from each other

Relationships between copulatory traits and fitness components

317   In population 2 we were able to determine the relationship between mating latency, copulation

318   duration, and two fitness components: male attractiveness (determined from mating success assays)

319   and productivity. There was no phenotypic relationship between male attractiveness and mating latency

320   (Population 2: $F_{1,199}$ = 0.83, p=0.36, Fig. 3A) nor with the pair productivity ($F_{1,1781}$ = 0.14, p=0.71, Fig. 3B).

321   Finally, copulation duration showed a tendency to be negatively associated with the pair productivity

322   ($F_{1,1754}$=3.6, p=0.06, Fig. 4).

323

Discussion

325   This study provides a unique overview of the fitness effects and genetic contributions of two widely

326   studied interactive traits, mating latency and copulation duration, in a species with higher polyandry and

327   shorter copulations than traditional *Drosophila* model systems. We found that, in *Drosophila serrata*, (i)

328   copulation duration was heritable in males in both population, (ii) multivariate analyses provided more

329   power to detect male and female contributions to interactive traits, and (iii) mating latency and

330   copulation duration did not predict the two measured fitness components, namely male attractiveness

331   and short-term productivity.

332   Quantitative genetic analyses showed that variation in copulation duration was heritable in males from

333   both populations. When only one of both sexes genetically contribute to an interactive trait, it limits the

334   opportunity for arm race, as adaptation would only respond to selection on one of the sexes (although

335   plastic behaviours may still enable conflictual interactions, Moore & Pizzari, 2005). However, female

336   genetic contribution to copulation duration is significantly higher than 0, as revealed by multivariate

337   analyses (Table 3), so the opportunity for conflict is not absent.

338   Male and female genetic contributions to copulation duration were very similar in this study than in *D.*

339   *melanogaster* (h^2_{female} = 0.08 ± 0.04 in *D. melanogaster vs* h^2_{female} = 0.08 ± 0.05 in *D. serrata*, and h^2_{male} =

340   0.13 ± 0.05 in *D. melanogaster vs* h^2_{male} = 0.15 ± 0.10 and h^2_{male} = 0.12 ± 0.06 in population 2 of *D.*

341   *serrata*). To obtain these results in *D. melanogaster*, Edward *et al.* (2014) also used a paternal half sibs,

342   but they mated pairs that both came from the breeding design in a full-factorial design. Thus, their

343   multivariate model incorporated both male and female genetic contributions, as did our second,

344   multivariate model. Sex-specific genetic contributions to copulation duration may thus be conserved

345   between species.

346   The differences in heritability estimates between univariate and multivariate models that incorporated

347   both sexes in population 2 were important, underscoring the value of incorporating both sexes' genetic

348   contributions in a single model when studying traits that can cause sexual conflict. However, we did not

349   find any significant pairwise correlations between pre- and post-copulatory traits, or across sexes. Other

350   studies of multivariate cross-sex genetic covariances between copulation behaviours are rare. Edward et

351   al (2014), found support for within female genetic covariance for number of eggs laid before and after

352   mating in *D. melanogaster*, whilst Han et al. (2020) found support for inter sexual genetic correlation for

353   male mate guarding and female latency to mate in field crickets. Our comprehensive approach

354   considering several traits in both sexes in a single framework showed that the cross-sex G matrix carried

355   complex and statistically supported covariance between interactive traits and sexes.

356   Male attractiveness was not correlated with mating latency at the phenotypic or genetic level in our

357   study. Consistent with this result, several experiments selecting for more attractive and more

358   competitive males have failed to detect a change in their mating latency. In *D. pseudoobscura*

359   (Bacigalupe et al., 2008), *Callosobruchus maculatus* (Maklakov et al., 2010), and even *D. melanogaster*

360   (Nandy et al., 2013), males under male biased sex ratio, an experimental condition expected to favour

361   attractive males, did not have a different mating latency than males evolved under equal or female

362   biased sex ratios. In *D. bunnanda*, artificially selected attractive males also failed to mate faster

363   (McGuigan et al., 2008), which the authors suggest was due to a lack of variation in attractiveness rather

364   than latency being a non-heritable trait in males. Mating latency may also vary with female

365   attractiveness, but we could not test this using our data. Mating latency could also be due to female

366   responsiveness and choosiness, however, the heritability of mating latency in females was as low as in

367   males. Finally, we may have failed to detect genetic contributions to our traits because of our

368   experimental protocol. Mating traits are notoriously difficult to capture in single assays. Hoffman (1999)

369   found that mating latency did not appear heritable after one mating trial, however when individuals
370   were tested several times for the same mating trait it was possible to detect non-zero heritability.
371   Although our experimental protocol created numerous repetitive measures within families (on average
372   28 sons tested per sire, 160 sires across populations), there was no repetition of individual measures
373   and environmental noise of single measures may have overwhelmed our ability to detect genetic
374   variation.

375   We did not find that longer matings resulted in more offspring. Indeed, our results showed a non-
376   significant trend that longer copulations resulted in fewer offspring produced, consistent with that
377   observed by Hoikkala et al (2000). In *D. melanogaster*, sperm transfer only takes half of the time of
378   copulation and the second half is used to transfer seminal fluid proteins (Gilchrist & Partridge, 2000).
379   Those proteins affect numerous female functions, in particular increase the number of eggs they lay and
380   reduce their receptivity to subsequent mating (Chen et al., 1988, Gioti et al., 2012). In *D. serrata*, sperm
381   are transferred in a single ball (Sztepanacz, pers. comm.), and little is known about Sfps transfer. Wing
382   song, however, has been shown to be an important component of copulatory courtship display, with
383   females discriminating against males that were not able to produce courtship song during copulation
384   (Hoikkala et al 2000.). Wing shape in male *D. serrata* is genetically variable (Sztepanacz & Blows, 2015),
385   and in other species there is weak evidence that shape variation may be associated with variation in
386   wing-song (Menezes et al., 2013, Snook et al., 2005). Whether *D. serrata* males with different wing
387   shapes produce different courtship songs, and whether song variation explains variation in copulation
388   duration is unknown. *D. serrata* males have also been shown to make active courtship and mounting
389   attempts after copulation (Hoikkala et al 2000.), which may keep the female unreceptive, preventing
390   further matings and sperm displacement (Alcock & Buchmann, 1985). Although relatively little is known
391   about male manipulation after mating, our results suggest that physiological effects of seminal fluid
392   proteins may be limited in this species.

393   Altogether, our comprehensive analyses of mating latency and copulation duration provide little support
394   for the hypothesis that these traits could be subject to intra- or inter-locus sexual conflict, as they were
395   not phenotypically or genetically associated with any pre- and copulatory components of fitness studied
396   here. We cannot rule out a lack of experimental power as a cause of low heritability estimates, however,
397   we analysed data from over 6000 flies and 160 sires, underscoring the difficulty in estimating
398   quantitative genetics parameters on behavioural traits. Multivariate cross-sex genetic analyses revealed

399 that complex patterns of between sexes correlations could set the scene for complex evolution for these

400 interactive traits.

Acknowledgements

407

Figures

409 **Figure 1:** Distribution of mating latencies (in seconds) in population 1 and population 2. In population 2,

410 results are shown in two different colours according to whether they were measured in females or

411 males of the breeding design.

412 **Figure 2:** Productivity of mating pairs according to the duration of their copulation. Darker colours

413 appear when several datapoints overlap. The line corresponds to a regression line obtained with the

414 mixed models accounting for the individuals relatedness.

415 **Figure 3**: Mating latency (in seconds) as a predictor of male fitness: either **A:** male attractiveness or **B:**

416 productivity of a pair. **A**: Each datapoint represents a successful male's latency from introduction into a

417 vial with a virgin female to copulation, and their attractiveness index based on their CHCs profiles. The

418 line corresponds to a linear model ("lm" in R). **B**: Each datapoint represents a mating pair. Darker colours

419 appear when several datapoints overlap. The line corresponds to the regression line obtained using a

420 mixed model accounting for the replication due to relatedness between individuals.

421 **Figure 4:** Distribution of copulation durations (in units of seconds) in population 1 and population 2. In

422 population 2, results are shown in two different colours according to whether they were measured in

423 females or males of the breeding design.

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TABLE 1: Summaries of univariate models. h^2 is the median narrow sense heritability, Eff. Samp. is the Effective Sample Size, DIC dif is thedifference in the deviance Information Criterion between the null model and the tested models. Credible intervals are 89 %. *models for those estimates did not converged according to the Heidelberger and Welch test.
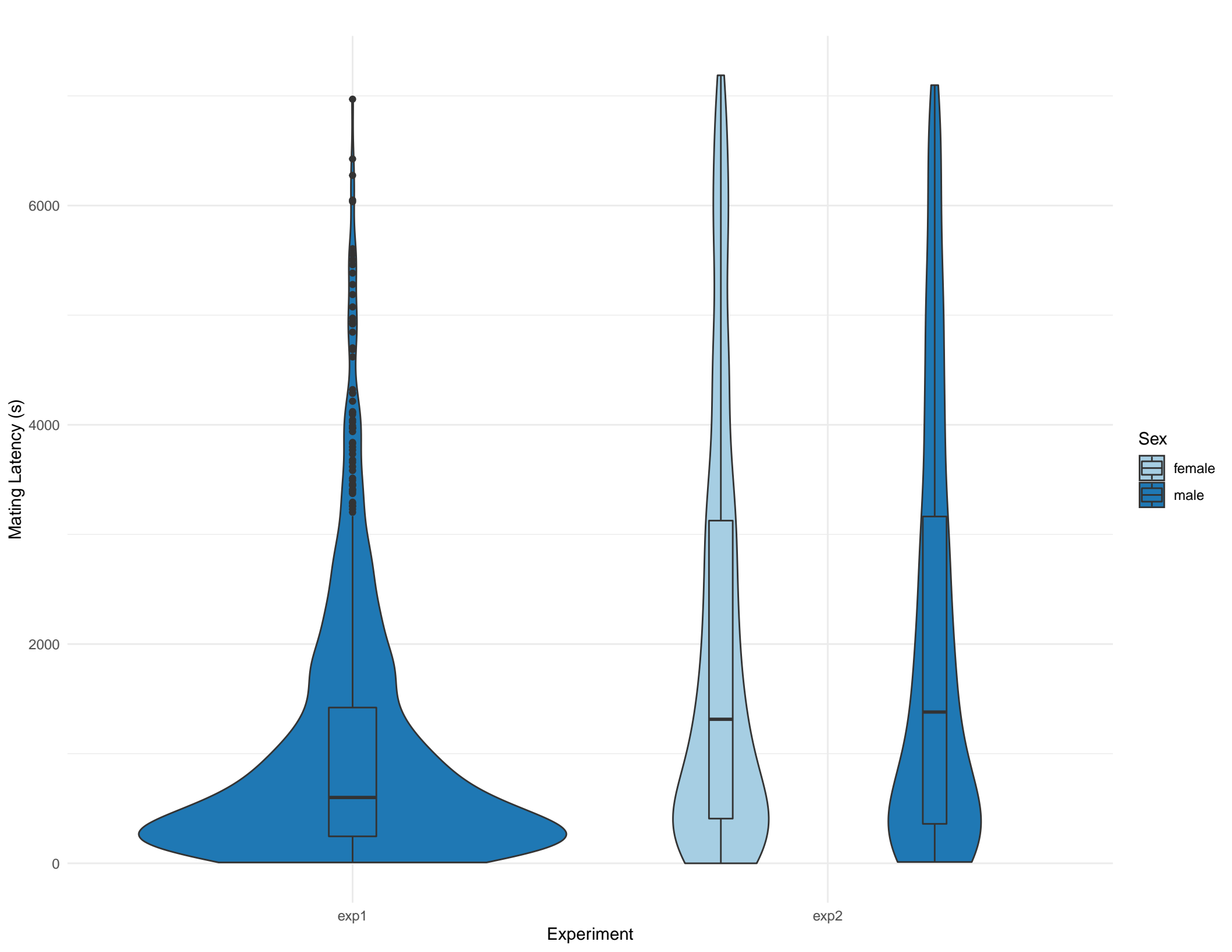
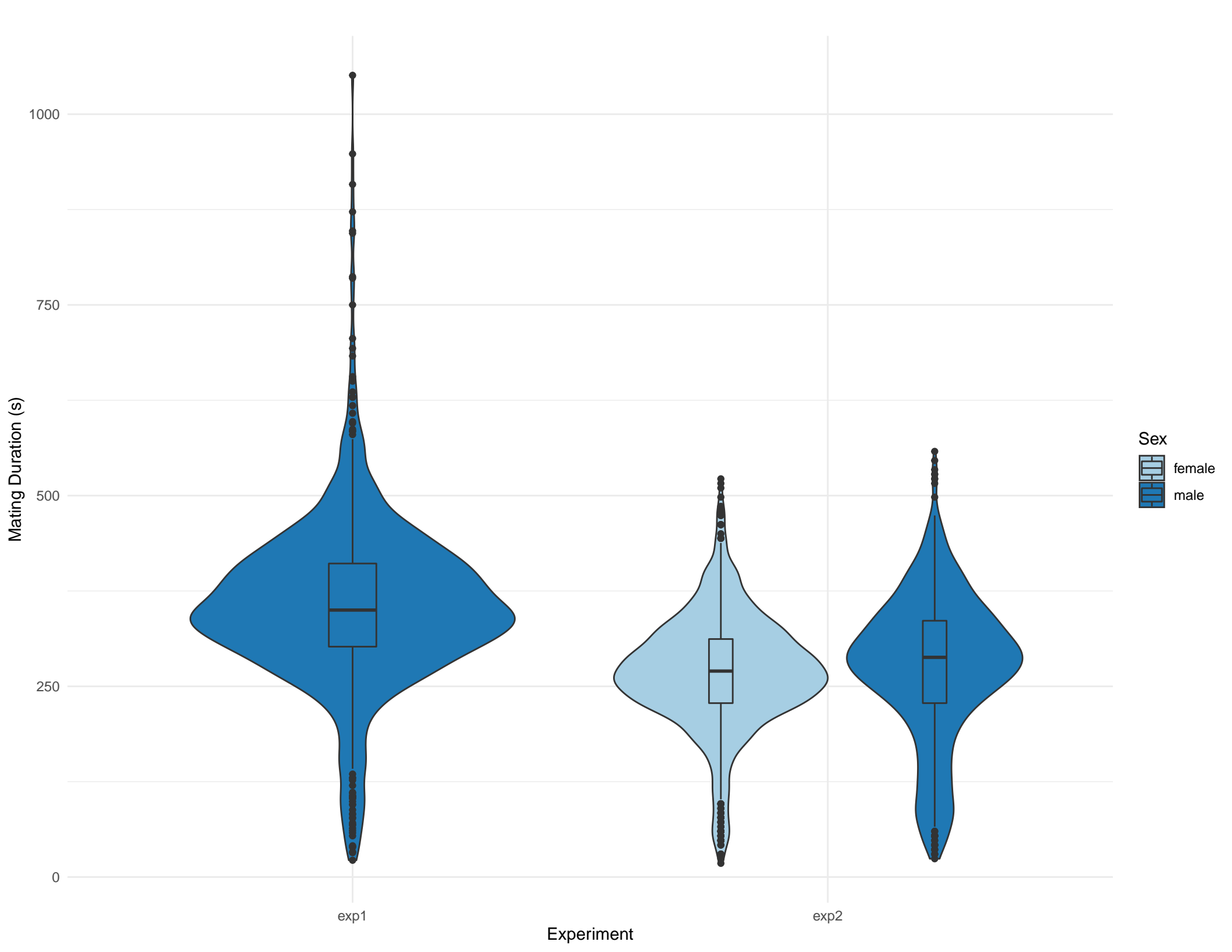| Population	Parameter	h^2	Credible interval	Eff. Samp.	DIC dif	evolvability (%)	Credible interval
1	Male Latency	0.068	[0.003 ; 0.175]	1871	10	1.3	[0.0 ; 26.0]
	Male Duration	0.153	[0.064 ; 0.257]	2737	35	1.5	[0.7 ; 2.6]
	Male attractiveness	0.678	[0.576 ; 0.787]	26397	1018		
2	Male Latency	0.007	[0.000 ; 0.046]	1138	-1	0.0*	[0.0 ; 2.1]*
	Male Duration	0.043	[0.002 ; 0.043]	1135	6	0.2	[0.0 ; 0.6]
	Female Latency	0.009	[0.001 ; 0.050]	1724	0	0.0*	[0.0 ; 2.5]*
	Female Duration	0.005	[0.000 ; 0.031]	1084	-1	0	[0.0 ; 0.2]

TABLE 2: The genetic variance-covariance matrix, G for male attractiveness, mating latency, and copulation duration in population 1. Heritabilities are in bold along the diagonal, covariances are below the diagonal, and correlations are in italic above the diagonal. 89% confidence interval for each value are in brackets.

	Attractiveness	Latency	Duration
Attractiveness	**0.636 [0.553 ; 0.716]**	*0.102 [-0.152 ; 0.347]*	*-0.059 [-0.281 ; 0.176]*
Latency	0.009 [-0.015 ; 0.035]	**0.133 [0.076 ; 0.217]**	*-0.062 [-0.421 ; 0.316]*
Duration	-0.006 [-0.032 ; 0.020]	-0.009 [-0.071 ; 0.050]	**0.179 [0.104 ; 0.276]**

TABLE 3: The genetic variance-covariance matrix, $G_f m$ for latency and mating duration in population 2. Heritabilities are in bold along the diagonal, covariances are below the diagonal, and correlations are in italic above the diagonal. 89% confidence interval for each value are in brackets.

		Female		Male	
		Latency	Duration	Latency	Duration
Female	Latency	**0.084 [0.051 ; 0.133]**	*-0.081 [-0.392 ; 0.251]*	*0.111 [-0.239 ; 0.431]*	*0.094 [-0.260 ; 0.416]*
	Duration	-0.006 [-0.033 ; 0.020]	**0.070 [0.044 ; 0.108]**	*-0.056 [-0.379 ; 0.283]*	*-0.170 [-0.473 ; 0.171]*
Male	Latency	0.010 [-0.021 ; 0.043]	-0.004 [-0.033 ; 0.023]	**0.087 [0.052 ; 0.142]**	*-0.126 [-0.456 ; 0.239]*
	Duration	0.009 [-0.026 ; 0.045]	-0.014 [-0.049 ; 0.015]	-0.012 [-0.053 ; 0.024]	**0.116 [0.069 ; 0.183]**

1