Reproductive plasticity in both sexes interacts to determine mating behaviour and fecundity

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Running head: Effects of behavioural plasticity in fruitflies

Keywords: phenotypic plasticity, cues, oviposition, mating duration, *Drosophila*

15   **Abstract**

16   Organisms alter their phenotype in response to variation in their environment by expressing phenotypic
17   plasticity. Both sexes exhibit such plasticity in response to contrasting environmental and social cues, and
18   this can reflect the influence of sexual conflict. However, theory predicts that plasticity expressed by both
19   sexes may *either* maximise the sex-specific fitness of both, or of one sex at the expense of the other. Hence
20   empirical tests of the predictions are sorely needed. Here we conducted novel tests of the fitness effects of
21   interacting reproductive plasticity in *Drosophila melanogaster*. First, prior to mating, males were kept
22   alone, or with same sex rivals, and females were kept alone, in same sex, or mixed sex groups. Second, we
23   conducted matings between individuals from all these social treatments under 'choice' and 'no choice'
24   scenarios. The results showed that males and females can both plastically respond to these socio-sexual
25   environments to influence the expression of mating duration, mating latency, and fecundity. These plastic
26   responses interacted significantly to determine mating latency and fecundity. Effects on mating latency
27   were also observed under both choice and no-choice conditions, but in opposing directions. Variation in the
28   outcome of interacting plasticity pivoted around the outcomes observed with focal females that had been
29   maintained in same-sex environments prior to mating. However, not all fitness-related traits examined
30   responded in the same way. Mating duration was determined largely by the social environment of the
31   male. Our results show that the expression of some, but not all fitness-related reproductive traits can be
32   determined by the outcome of interacting behavioural plasticity expressed by both sexes. This highlights
33   the need for new predictive theory informed by these empirically-derived parameters. Overall, we conclude
34   that variation in the expression of shared traits due to interacting plasticity represents an important and
35   novel facet of sexual interactions.

36   **Impact Summary**

37   Animals and plants are able to respond to variation in their environment by expressing phenotypic
38   plasticity. In sexual organisms, both males and females can exhibit such plasticity but the cues they respond
39   to and the fitness consequences of these actions may be different between the sexes, and even conflicting.
40   For example, males may respond to the presence of competitors by altering their mating behaviour or
41   ejaculate transfer to increase their own, but not necessarily their mate's reproductive output. However,
42   females may also express phenotypic plasticity in response to their social and sexual environment to
43   maximise their own fitness. Theory suggests that plasticity expressed by both sexes may either maximise
44   the sex-specific fitness of both, or of one sex at the expense of the other. So far, little experimental work
45   has been conducted to explore such interacting plasticity. Here we conducted novel tests of the fitness
46   effects of interacting plasticity in the fruit fly *Drosophila melanogaster*. In doing so, we provide novel
47   experimental evidence for interacting behavioural plasticity. We show that males and females can
48   plastically respond to their socio-sexual environment to influence the expression of mating duration,
49   mating latency, and fecundity. These plastic responses, while induced to increase the fitness interests of
50   each sex, interact in the case of mating latency and fecundity and may reflect the outcome of sexual
51   conflict. Our findings suggest that studies of reproductive behaviour should carefully consider the socio-
52   sexual environment of both males and females and highlight the need for new predictive theory informed
53   by empirically-derived parameters. Overall, we show that interacting plasticity between sexes represents
54   an important and novel facet of sexual interactions.

Introduction

Phenotypic plasticity is the ability of organisms to directly respond to biotic or abiotic changes in the environment by altering their phenotype (Komers et al., 1997; West-Eberhard, 2003; Fordyce, 2006; Dingemanse and Wolf, 2013). Potential benefits of plasticity lie in maximising fitness or survival in variable environments in which a fixed strategy may be costly (Bretman et al., 2013a). Therefore, strongly plastic individuals may be better able to match the conditions experienced in a heterogenous environment. The socio-sexual environment is an important stimulus for the expression of behavioural plasticity across different taxa (e.g. Han and Brooks, 2014; Dorset et al., 2017; Oku and van den Beuken, 2017). For example, information such as the number of rivals or mating opportunities can indicate to an individual the likely level of competition for resources and mates they will face (Davis et al., 2011; Bretman et al., 2011).

Behavioural plasticity has been well studied in fruitflies. Male *D. melanogaster* are observed to increase aggressive behaviour towards rival males when the number of rivals is low but decrease aggression when high (Nandy et al., 2016). This results in an increased chance of mating when competition is less likely, and avoids potentially damaging conflict or diminishing fitness returns when competition is strong (Nandy et al., 2016). *D. melanogaster* males may also alter their reproductive investment in response to perceived levels of sperm competition. For example, males exposed to rivals prior to mating extend mating duration and transfer into females more of two key seminal fluid proteins (SFPs), Ovulin and sex peptide (SP) (Wigby et al., 2009; Bretman et al., 2013a; Filice et al., 2020). Ovulin and SP induce important post-mating behavioural and physiological changes to females, including increased fecundity and decreased sexual receptivity (Chapman et al., 1993; Herndon and Wolfner, 1995; Heifetz et al., 2000; Liu and Kubli, 2003; Wigby et al., 2009). This is expected to benefit males as they invest in energetically costly SFPs that increase fecundity and decrease female re-mating receptivity only when required (Wigby et al., 2009). As the receipt of SFPs can be costly for females (Chapman et al., 1995; Wigby and Chapman, 2005) this may also exacerbate sexual conflict (Sirot et al., 2015).

Though understudied outside the context of mate choice, there are increasing reports of female plasticity. For example, females can observe and learn oviposition strategies from other females, choosing to lay eggs on a potentially 'good' food substrate (Sarin and Dukas, 2009). Mated females may also alter their egg laying behaviour in response to a male-derived pheromone, aggregating in order to lay eggs (Wertheim et al., 2006), and can also exhibit variation in fecundity according to the genetic diversity of the males in their social environment (Billeter et al., 2012). The intrasexual pre-mating environment can influence a female's behavioural plasticity. For example, female *D. melanogaster* housed with other females lay significantly more unfertilised eggs as virgins but are less fecund following mating, compared to socially isolated females (Fowler et al., 2020; Churchill et al., 2021).

Though increasing evidence shows that both sexes can express reproductive plasticity in response to the presence of conspecifics, we lack information on whether plastic responses can interact in determining the overall levels of reproductive investment made by each sex. For example, we do not yet know how the expression of plastic responses by one sex affects those of the other. This important omission is the main focus of the current study. Interactions between plasticity expressed by males and females are expected to be an important determinant of overall fitness. For example, we hypothesise that the plastic response of a male could trigger females to alter their own reproductive investment. However, we lack theory on which to base predictions, and that which does exist predicts variable outcomes. For example, interacting plasticity is predicted by theory to either maximise the sex-specific fitness of *both* sexes (McGhee et al., 2013), *or* of one at the expense of the other (Yamaguchi and Iwaga, 2015; McLeod and Day, 2017; Day and McLeod, 2018). Variation in outcomes might be predicted if the strength of sex-specific selection on the

99   relevant fitness traits involved differs, or if one sex has gained the upper hand in the expression of traits
100   subject to sexual conflict. However, to our knowledge, there are as yet no empirical tests of this theory or
101   of the effects of interacting plasticity on mating behaviour and fitness. Here we addressed this omission by
102   using the fruitfly *Drosophila melanogaster* to conduct novel tests of the effects on key fitness traits of
103   plasticity expressed by both sexes in response to their social and sexual environments.

104   We first varied the pre-mating social environments, by keeping focal flies alone, with same-sex or opposite
105   sex individuals. We predicted that focal females exposed to other females, vs to males would vary in their
106   perception of resource vs sexual competition (Table 1). Similarly, that males exposed to rivals would
107   perceive a high threat of sexual competition. We then mated males and females from each of the social
108   environments varying in competitiveness together in all combinations. This allowed us to test for
109   interacting effects of plastic response of both sexes to those environments on mating success, mating
110   latency, mating duration and fecundity.

111   We conducted mating tests in both traditional 'choice' and 'no-choice' mating scenarios. The choice assays
112   placed females from each of three social treatments (alone, same-sex or opposite-sex) with two males from
113   two different social treatments (alone with no rival, or with same-sex rivals). This introduced the effect of
114   direct competition between males of different environments and of direct comparison of those males by
115   females (Table 1). In the no-choice assays, females from each of the three social treatments were allocated
116   a single male from either social treatment. This removed the effect of direct competition and tested the
117   effect of responses to previous social environments (Table 1).

118

Methods

Stock Maintenance and Fly Collection

121   Wild type *D. melanogaster* flies were from a large laboratory population originally collected in the 1970s in
122   Dahomey (Benin). Flies were maintained in stock cages with overlapping generations on SYA medium
123   (Sugar Yeast Agar: 100 g brewer's yeast, 50 g sugar, 15 g agar, 30 ml Nipagin (10% w/v solution), and 3 ml
124   propionic acid, per litre of medium). SYA was used throughout the experiments and all flies were cultured
125   and reared, and all experiments performed, at 25°C, 50%RH, on a 12h:12h light:dark cycle. Eggs for all
126   experimental manipulations were collected from population cages using purple agar egg collection plates
127   (275ml H_2O, 12.5g agar, 250ml red grape juice, 10.5ml 10% w/v Nipagin solution) supplemented with live
128   yeast paste. First instar larvae were picked into 7ml SYA vials (75 x 25 mm) at a density of 100 larvae per
129   vial. Adults were collected within 8h of eclosion, separated into same sex groups using ice anaesthesia and
130   stored 10 per vial. Adults were stored under these conditions for four days and allowed to reach sexual
131   maturity until use in the experiments.

Manipulation of Focal Female and Male Pre-Mating Social and Sexual Environments

133   *Females*: The three female social environment treatments were: 'alone', 'group same sex' (GSS), and 'group
134   mixed sex' (GMS). The female social environment treatments were all set up in vials that were divided into
135   two chambers using perforated acetate (through which sound, smell and visual cues could be transmitted,
136   but flies could not physically pass). For the alone treatment, a single focal female was placed in one of the
137   vial chambers. For the GSS treatment, one focal female was placed in one chamber and three non-focal
138   females in the other. For the GMS treatment one focal female was placed in one chamber and three non-
139   focal males in the other. The plastic dividers limit physical contact between individuals, but we have found

140   previously that pre-conditioning vials by allowing non-focal flies access to the entire vial for 24h prior to
141   adding the focal female and dividers provides sufficient cues for focal females to accurately detect their
142   social environment (Fowler et al., 2020). We therefore preconditioned the vials in the GSS and GMS
143   treatments (figure 1A – 'Vial Pre-conditioning') by placing all non-focal individuals into the appropriate vial
144   24h before the introduction of the focal female and the acetate dividers.

145   *Males*: The two male social environment treatments were: 'rival' and 'no-rival'. The rival treatment
146   consisted of four males per vial, and the no-rival treatment a single male in a vial. Male social treatments
147   did not require pre-conditioning and so focal males were randomly allocated into one of the two male
148   treatments at the same time as the focal females.

149   All females and males were kept in the above pre-mating social environments for 48h before the mating
150   assays (figure 1A - 'Social Exposure'). We conducted matings between the males and females kept in all
151   these social environments in two separate experiments that varied only in whether they allowed for direct
152   competition / choice. In the first, the mating test was conducted using a traditional 'choice' design,
153   whereby each focal female was exposed to two males in the mating arena (one male from each of the rival
154   or no rival social treatments). In the second experiment, mating tests consisted of one female and one male
155   in a traditional 'no-choice' design (Table 1).

156   **1: Effect of male and female social environment on mating behaviour and fecundity under choice**
157   **conditions**

158   In the choice experiment, the mating and fecundity responses of alone, GSS and GMS females paired in the
159   mating arena with two males, one from the rival and one from the no-rival treatments, were recorded.
160   Males were wing marked with either red or black ink using Staedtler Lumocolor red/black marker pens.
161   Wing marking was balanced across rival and no-rival groups to ensure that any effect on female choice of
162   ink colour would not introduce any directional bias. On the day of the mating experiment, the focal female
163   from each treatment was aspirated into a vial with marked males from the rival and no-rival treatments
164   and given 90 minutes to mate (figure 1B – 'Choice Experiment'). Mating latency and mating duration were
165   recorded as was the ink colour of the male that was chosen / secured the mating. After mating had
166   finished, males were discarded and females were left in the vials for 24h to lay eggs. The sample sizes for
167   each mate choice scenario were ~50 per female treatment. Since only one male out of the pair secured a
168   mating, samples sizes for mating latency, duration and fecundity were approximately 25 per male and
169   female treatment, depending on how many matings occurred (see table S1 for final sample sizes).

170   **2: Effect of male and female social environment on mating behaviour and fecundity under no-choice**
171   **conditions**

172   In the no-choice experiment, the mating and fecundity responses of alone, GSS and GMS females placed
173   with either rival or no-rival condition males were recorded. Focal females and males were in set up in their
174   alone, GSS and GMS social environments, as described above, for 48h. In this set up females could mate or
175   not mate with a rival or no-rival male assigned at random to them, thus marking of focal males was not
176   required. All non-focal males were wing clipped under CO_2 anaesthesia 24h prior to setting up the social
177   exposure treatments. On the day of the mating experiment, focal females from each alone, GSS and GMS
178   treatment were each aspirated into an SYA vial with one focal male either from the rival or no-rival
179   treatment. Pairs were given 90 minutes to mate during which mating latency and duration were recorded
180   (figure 1B – 'No-Choice Experiment'). Post-mating egg data were collected as before. The starting sample
181   sizes for mating latency, duration and fecundity were approximately 50 per female and male treatment
182   (final sample sizes for each treatment are given in table S1.

183 **Statistical Analyses**

184 All analyses were carried out using R version 3.6.3 (R Core Team, 2020) (packages used: survminer, survival,
185 ggplot2, dplyr, stats). A Generalized Linear Model (GLM) with a quasi-Poisson error block was used to
186 analyse post-mating egg production and mating duration for the rival and no-rival groups for each alone,
187 GSS and GMS female social treatments. In these models, each female group was subset and tested
188 separately for their response to the rival/no rival treatment. A Cox Proportional Hazards Model was used to
189 test for differences in mating latency. Individuals that did not mate within 90 min were treated as censors.
190 A Chi Square analysis was used to test for the effect of wing colour on male mating success. A GLM with
191 binomial errors was used to test effect of male and female social treatment on proportion of matings
192 secured. A maximal model was fitted, and then simplified to remove the male:female treatment interaction
193 term. Models were compared using analysis of deviance (anova() in "stats" package) with a chi-squared
194 test. The final model retained the male and female social treatment terms (see table S1 for final sample
195 sizes).

196 ## Results

197 **1: Effect of male and female social environment on mating behaviour and fecundity under choice**
198 **conditions**

199 Each female was presented with males from rival or no-rival social treatments simultaneously, allowing
200 females to directly compare different males and allowing males to compete. Thus intersexual choice and
201 intrasexual competition was possible. Wing marking colour had no effect on male mating success within
202 any of the female treatments (table 2). There was a marginally non-significant trend for no-rival males to
203 secure more matings than rival males in each of the alone, GSS and GMS female social treatments (X^2 = 3.4,
204 df = (1,4), p = 0.06; table 2, figure 2). Matings occurred rapidly, with a median latency to mating of three
205 minutes across all treatments. The social environment of the males had no significant effect in the GMS (HR
206 = 0.861, 95% CI [0.462, 1.604], p = 0.637) and alone (HR = 0.841, 95% CI [0.466, 1.518], p = 0.566)
207 treatments. However, in the GSS treatment, rival males started mating significantly faster than no-rival
208 males (HR = 2.384, 95% CI [1.232, 4.612], p = 0.0099) (figure 3). Females in the alone and GMS treatments
209 mated for significantly longer with rival than with no-rival males (alone: t_{44} = 2.486, p = 0.0168; GMS: t_{41} =
210 2.799, p = 0.0078). The trend was in the same direction, but was not significant in the GSS treatment (t_{39} =
211 0.791, p = 0.434) (figure 4A). There was no significant difference in the number of eggs produced 24h after
212 mating between females that mated with rival male and no-rival males in any of the female social
213 environments (alone: t_{44} = 1.517, p = 0.136; GSS: t_{39} = -0.77, p = 0.446; GMS: t_{41} = -0.429, p = 0.67) (figure
214 5A).

215 Overall, in these choice tests, the results showed evidence for interacting plasticity effects, but only for
216 mating latency. Specifically, males previously exposed to rivals mated significantly faster only when paired
217 with females previously exposed to other females (GSS). In contrast, mating duration was determined
218 solely by the male's social environment, being longer following exposure to rivals prior to mating. There
219 was no effect of plasticity in either sex on fecundity.

220 **2: Effect of male and female social environment on mating behaviour and fecundity under no-choice**
221 **conditions**

222 Here each female was presented with a single male in the mating arena. This tested the effect of the pre-
223 mating social environment on mating behaviour and fecundity, in the absence of intrasexual competition
224 between males in the mating arena. Mating latency was slower than in the above choice experiment, with a

225 median mating latency of 8mins. Consistent with the choice trials, there were no significant differences in
226 mating latency between rival and no-rival males paired with alone (HR = 0.965, *95% CI* [0.636, 1.466], p =
227 0.868) or GMS (HR = 1, *95% CI* [0.654, 1.53], p = 0.999) females (figure 3). As with the choice trials, mating
228 latency was significantly different between male treatments for the GSS females, but the direction was
229 reversed - rival males took significantly longer to start mating with GSS females than the no-rival males (HR
230 = 0.519, *95% CI* [0.327, 0.825], p = 0.006). Females from all three treatments mated with rival males for
231 significantly longer than no-rival males (alone: t_{86} = 2.831, p = 0.0058; GMS: t_{83} = 2.838, p = 0.0057; GSS: t_{78}
232 = 2.218, p = 0.0295) (figure 4B). As with the choice experiment, there was no difference in post-mating egg
233 production following a mating with a rival and no-rival male in the alone and GMS female treatments
234 (alone: t_{88} = 0.368, p = 0.714; GMS: t_{81} = -0.181, p = 0.856). However, females in the GSS treatment that
235 mated with rival males produced significantly more eggs than those that mated with no-rival males (t_{78} =
236 2.279, p = 0.0254) (figure 5B).

237 Overall, in these no-choice tests there was again evidence for effects of interacting plasticity - on mating
238 latency, as above, but also upon fecundity. Interestingly, the mating latency effect was in the opposite
239 direction, with males previously exposed to rivals being slower to mate with GSS, but not other treatment
240 females. For fecundity, GSS, but not other treatment females laid significantly more eggs following matings
241 with rival males. Again, mating duration was determined only by the male's social environment, with rival
242 males mating for longer across all contexts.

243 **Overall patterns in choice and no-choice scenarios**

244 The results from the two separate experiments above show evidence for interacting effects of plasticity, on
245 mating latency across both scenarios and for fecundity in the no choice tests. The interactions pivoted
246 around divergent responses seen with females from the GSS female social environment. However, we note
247 potential limitations to the comparisons of the patterns obtained from the choice and the no choice tests.
248 First, the experiments were done separately and second, there were differences in sample sizes (each male
249 treatment in the choice experiment was approximately half that in the no-choice assays, as only half of the
250 choice males secured a mating). To explore the latter, we assessed the potential for the differences in
251 sample size to confound the patterns observed. Specifically, we considered whether the choice were
252 underpowered in comparison to those of the choice assays. We did this in a resampling exercise
253 (supplementary information) to explore the effect of sample size. The hypothesis tested was whether there
254 was any evidence for not observing an effect in the choice assays that was present in the no-choice data,
255 i.e. effects that 'disappeared' in the subsampled no-choice dataset.

256 For mating latency, the subsampled datasets showed broadly similar patterns and in no case was the
257 direction of the effect reversed from the full no-choice dataset (figure S1, S2, S3). We conclude that the
258 differences observed in both the choice and no-choice assays for mating latency were robust. For mating
259 duration, in the full no-choice dataset we observed consistent significant differences between rival and no-
260 rival males, and in the subsampled datasets showed significant differences in approximately half of the
261 cases (figure S4). This was in line with the pattern of results seen in the choice data, where differences were
262 either statistically significant or in the same direction. Hence, we conclude that the observation of male-
263 only plasticity affecting mating duration across both test environments was reasonably robust. Finally, for
264 fecundity, the observed difference between the male treatments in the no-choice assay interacting with
265 the GSS females was not evident in any subsampled datasets (figure S5). This does not indicate that the no
266 choice interacting plasticity effect was not robust, but could suggest that, had there been a comparable
267 effect in the choice assays, it might not have been detected.

268

Discussion

270   The major finding was that the interacting plastic responses of both sexes to their social and sexual
271   environment can influence the expression of fitness-related traits. We found that mating latency and
272   fecundity were sensitive to the interacting effects of responses to the socio-sexual environment made by
273   both sexes. However, not all traits were affected in this way and we found that mating duration was
274   determined largely by the social environment of the male. Effects of responses to the social environment
275   by both sexes on mating latency were observed under both choice and no-choice conditions, but were
276   manifested in opposing directions. Variation in the outcome of interacting plasticity pivoted around the
277   outcomes with focal females previous exposed to other females (the GSS treatment). Our results show that
278   the expression of some, but not all fitness-related reproductive traits can be determined by the outcome of
279   interacting behavioural plasticity between the two sexes, and highlight the need for new predictive theory.

280   **Interacting phenotypic plasticity: mating behaviour and fecundity were determined by the plastic**
281   **responses of both sexes**

282   Importantly, the study provides evidence for the interacting effects of phenotypic plasticity expressed by
283   both sexes on mating latency and fecundity. The direction of the interacting effect on mating latency as
284   also reversed under choice versus no choice conditions. All of the interacting effects were centred around
285   differences in the tests with the GSS, as opposed to other social treatment, females. This suggests that
286   there was something qualitatively or quantitatively distinct about the plastic responses of these females, or
287   the way they were perceived by males, in comparison to females kept on their own versus with males prior
288   to mating. These findings are explored further, below.

289   In the no-choice experiment, no-rival males secured matings faster than did rival males with the GSS, but
290   not other treatment, females. Mating latency of the rival vs no-rival males mating with GSS females in the
291   choice experiment also differed significantly, although in this case no-rival males were slower to mate. The
292   opposing direction of the response in the no choice vs choice experiments suggests the proximate mating
293   environment as well as previous social experience both influence mating latency. It also suggests mating
294   latency may be uncoupled from overall mate choice preference, since fewer rival males secured matings
295   than no-rivals overall in the choice experiment, even though the rival males that did mate were faster to
296   start copulating with GSS females. The design of mate choice experiments can affect preference outcomes,
297   with females of many species generally exhibiting stronger preference when mates are presented
298   simultaneously compared to sequentially (Dougherty and Shuker, 2014). Differences in preference between
299   the designs could be driven by increased costs of rejection in a no-choice scenario (Dougherty and Shuker,
300   2014). It is possible that the outcomes we observed here could be a result of differential costs of rejection
301   between the two mating regimes, combined with expectations of mate or resource competition from
302   previous and proximate environments.

303   Overall, the differences in mating latency were focussed around the interactions with the female GSS
304   treatment in both experiments. This suggests that the female socio-sexual environment can affect mating
305   latency, but that variation in this trait is also influenced by interacting plasticity between both sexes. Given
306   that the GSS females may perceive that mating opportunities are low, they may be less resistant to mating
307   attempts than females from other treatments. The potentially lower choosiness of GSS females could
308   exacerbate differences between rival and no-rival males in this treatment. Similarly, it was only females in
309   the GSS treatment in the no-choice experiment that showed elevated fecundity when mating to rival, in

310 comparison to no-rival, males. This implies that fecundity is influenced by both male and female pre-mating
311 social environments. Female fecundity is affected by the receipt of SFPs. Processing of SP and ovulin is
312 dependent on a network of female-expressed proteins and so there is opportunity for the female to exert
313 control over the effects of SFPs (Sirot et al., 2015). Indeed, it may benefit the female to precisely control
314 the level of SFP processing in response to her own perception of the chances of re-mating and the
315 availability of resources such as nutrients and oviposition sites. For example, the costs of receiving SP are
316 likely caused by a lower re-mating rate and increased short-term investment in egg production which may
317 trade off against somatic investment. However, if opportunities for re-mating are low, as might be signalled
318 in the GSS treatment when females are exposed to other females, then increased short-term fecundity
319 mediated through SFPs may benefit females, or at least be costly to resist.

320 **Interacting plasticity and sexual conflict**

321 The interactions described above between the behavioural plasticity expressed by each sex, have
322 previously been investigated through theoretical modelling and are predicted to be an important facet of
323 intra- and interspecific interaction dynamics (Yamaguchi and Iwaga, 2015; McLeod and Day, 2017; Day and
324 McLeod, 2018). Specifically, sexual conflict is predicted under some circumstances to drive interacting
325 plasticity to reach a fitness optimum between the interests of the male and the female, though the extent
326 to which this occurs will depend upon the strength of sex-specific selection and thus whether either sex has
327 the upper hand in any conflict.

328 We can consider whether there is any evidence to support this scenario in the findings we observed here.
329 We saw that males that perceived themselves to be at high risk of sperm competition mated for
330 significantly longer with females from all social environments. It has previously been observed that longer
331 matings under these circumstances can transfer more cost-inducing SFPs (Wigby et al., 2011). Thus, the
332 heightened SFP allocation by such males should be evident in plastic responses in females to resist SFP
333 effects. This could be evident as reduced willingness to mate with such males (slow mating latency or lower
334 mating success) potentially modified by the female's own information on the likelihood of meeting any
335 additional males (as signalled by their pre-mating and / or mating social environment). The results are
336 generally in line with this expectation. Specifically, in the choice scenarios, rival males were generally less
337 successful at mating than were no rival males and there were significant interactions of mating latency with
338 female social status. Rival treatment males were significantly slower to mate with GSS females under no
339 choice, but significantly faster under choice conditions. The data suggest that females may be able to assess
340 their own social environment and respond in a manner that potentially mitigates SFP effects. We suggest
341 that these findings indicated that selection may have favoured males that can increase their mating
342 propensity while also favouring females that can effectively assess their environment. This would be
343 interesting to investigate in future studies.

344 **Non-interacting phenotypic plasticity: mating duration was determined primarily by plasticity expressed**
345 **by males**

346 In contrast to the interacting effects described above, plasticity in mating duration was primarily
347 determined by the responses of males to their social environments. In both the choice and no-choice
348 assays, males of the rival treatment always mated for longer than males of the no-rival treatment (though
349 not always significantly so). Therefore, we conclude the mating duration effect was independent of female
350 social environment in both mating scenarios, indicative of a 'one-sex' plasticity exhibited by the male. This
351 is consistent with previous findings (Lizé et al., 2011; Price et al., 2012; Bretman et al., 2013b). Under
352 elevated sperm competition, mating duration may be correlated with an increased transfer of SFPs to the
353 female (Bretman et al., 2009). As a result, females may experience reduced mating propensity (Mazzi et al.,

354 2009). This is advantageous for the male, by preventing females from re-mating and thus increasing
355 paternity share. However, these extended post-mating effects may also be costly to females if re-mating
356 opportunities with higher quality males are lost. Plasticity in mating duration in response to rival exposure
357 has previously been shown to be under male control (Bretman et al., 2013b). However, *Drosophila* females
358 do have the ability to exert some influence on mating duration (Mazzi et al., 2009), and so the fact that the
359 social environment of the female did not affect mating duration in our study suggests that the costs to
360 males from sperm displacement are greater than costs to females from missed matings. Hence, we suggest
361 there may be greater selection acting on males to guard females through increased mating duration than
362 on females to resist longer matings.

363 **Non-interacting phenotypic plasticity: mate choice**

364 In each of the three female social environment treatments within the choice experiment, no-rival males
365 secured more matings than did rival males, although this was marginally non-significant. The potentially
366 higher mating success of no-rival males over rival males could be due to female preference or to male
367 competition. To respond to sperm competition, rival males increase the transfer of SFPs during mating
368 (Wigby et al., 2009; Hopkins et al., 2019). No-rival males may be more attractive to females if they have
369 remained in better condition. For example, rival treatment males may have experienced aggressive
370 interactions resulting in physical damage (Davis et al., 2018). This could decrease their perception as high
371 quality males or compromise their ability to court females (e.g. via wing damage). Such males could suffer
372 reduced mating success (Chen, 2002; Davis et al., 2018). Alternatively, no-rival males may also secure more
373 matings due to their ability to outcompete rival males. There are two possible explanations. First, territorial
374 aggressive behaviour can occur between rival males in close proximity (Chen, 2002). This would be
375 detrimental to their ability to compete for mates as they would have far less energy than the no-rival mate
376 to successfully court the female. Second, rival males may be less willing to court due to the perception of
377 high competition (Weir et al., 2011). Courtship behaviour is energetically costly (Bretman et al., 2013a;
378 Cordts and Partridge, 1996) and rival males may benefit from withholding courtship until competition is
379 reduced.

380 **Conclusions**

381 We have provided the first experimental evidence for interacting behavioural plasticity in the model
382 organism *Drosophila melanogaster*. We showed that males and females can plastically respond to their
383 socio-sexual environment to influence the expression of mating duration, mating latency, and fecundity.
384 These plastic responses, while induced to increase the fitness interests of each sex, interact in the case of
385 mating latency and fecundity and may reflect the outcome of sexual conflict. Our findings suggest that
386 studies of reproductive behaviour should carefully consider the socio-sexual environment of both males
387 and females.

388 **Acknowledgements**

391 **Author contributions**

392 EF, AB and TC devised the experiments, EF and SL conducted the research, collected and analysed the data;
393 EF, SL and TC wrote the paper; all authors contributed to the final draft.

394 **Data accessibility**

395 The raw data are deposited in the DRYAD data depository, doi to be added in proof and raw data were
396 made available for reviewers.

397 **Conflict of interest statement**

398 The authors declare no conflict of interest.

399 **References**

400 Bateman, A.J., (1948). Intra-sexual selection in *Drosophila*. *Heredity*, 2(3), pp.349-368.

401 Billeter, J.C., Jagadeesh, S., Stepek, N., Azanchi, R. and Levine, J.D., 2012. Drosophila melanogaster females
402   change mating behaviour and offspring production based on social context. *Proceedings of the Royal*
403   *Society B: Biological Sciences*, 279(1737), pp.2417-2425.

404 Bretman, A., Fricke, C., & Chapman, T. (2009). Plastic responses of male *Drosophila melanogaster* to the
405   level of sperm competition increase male reproductive fitness. *Proceedings of the Royal Society B:*
406   *Biological Sciences*. 276(1662), pp.1705-1711.

407 Bretman, A., Fricke, C., Hetherington, P., Stone, R. and Chapman, T., (2010). Exposure to rivals and plastic
408   responses to sperm competition in *Drosophila melanogaster*, *Behavioral Ecology*, 21(2), pp.317-321.

409 Bretman, A., Westmancoat, J. D., & Chapman, T. (2013b). Male control of mating duration following
410   exposure to rivals in fruitflies. *Journal of Insect Physiology*. 59(8), pp.824-827.

411 Bretman, A., Westmancoat, J. D., Gage, M. J. G., & Chapman, T. (2011). Males use multiple, redundant cues
412   to detect mating rivals. *Current Biology*. 21(7), pp.617-622.

413 Bretman, A., Westmancoat, J. D., Gage, M. J. G., & Chapman, T. (2013a). Costs and benefits of lifetime
414   exposure to mating rivals in male *Drosophila melanogaster*. *Evolution*. 67(8), pp.2413-2422.

415 Chapman, T., Hutchings, J., & Partridge, L. (1993). No reduction in the cost of mating for *Drosophila*
416   *melanogaster* females mating with spermless males. *Proceedings of the Royal Society B: Biological*
417   *Sciences*. 253(1338), pp.211-217.

418 Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F. & Partridge, L. (1995). Cost of mating in *Drosophila*
419   *melanogaster* females is mediated by male accessory gland products. *Nature*, 373(6511), pp.241-244.

420 Chen, P.S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M. and Böhlen, P., (1988). A male accessory
421   gland peptide that regulates reproductive behavior of female *D. melanogaster*. *Cell*, 54(3), pp.291-
422   298.

423 Chen, S., Lee, A.Y., Bowens, N.M., Huber, R. and Kravitz, E.A., (2002). Fighting fruit flies: a model system for
424   the study of aggression. *Proceedings of the National Academy of Sciences*, 99(8), pp.5664-5668.

425 Churchill, E.R., Dytham, C., Bridle, J.R. and Thom, M.D.F. (2021). Social and physical environment
426   independently   affect   oviposition   decisions   in   *Drosophila   melanogaster*.   BioRxiv,
427   https://www.biorxiv.org/content/10.1101/2021.01.27.428449v1.

428 Cordts, R. and Partridge, L., (1996). Courtship reduces longevity of male *Drosophila melanogaster*. *Animal*
429   *Behaviour*, 52(2), pp.269-278.

430 Davis, J.M., Nufio, C.R. and Papaj, D.R., (2011). Resource quality or competition: why increase resource
431   acceptance in the presence of conspecifics? *Behavioral Ecology*, 22(4), pp.730-737.

432   Davis, S.M., Thomas, A.L., Liu, L., Campbell, I.M. and Dierick, H.A., (2018). Isolation of aggressive behavior
433   mutants in Drosophila using a screen for wing damage. *Genetics, 208*(1), pp.273-282.

434   Day, T., & McLeod, D. V. (2018). The role of phenotypic plasticity in moderating evolutionary conflict.
435   *American Naturalist. 192*(2), pp.230-240.

436   Dingemanse, N. J., & Wolf, M. (2013). Between-individual differences in behavioural plasticity within
437   populations: Causes and consequences. *Animal Behaviour. 85*(5), pp.1031-1039.

438   Dorset, E. E., Sakaluk, S. K., & Thompson, C. F. (2017). Behavioral Plasticity in Response to Perceived
439   Predation Risk in Breeding House Wrens. *Evolutionary Biology. 44*(2), pp.227-239.

440   Dougherty, L. R. & Shuker, D.M. (2014). The effect of experimental design on the measurement of mate
441   choice: a meta-analysis. *Behavioural Ecology. 26*(2), pp.311-319.

442   Filice, D.C. & Long, T.A., (2017). Phenotypic plasticity in female mate choice behavior is mediated by an
443   interaction of direct and indirect genetic effects in *Drosophila melanogaster. Ecology and
444   Evolution*, 7(10), pp.3542-3551.

445   Filice, D. C. S., Bhargava, R., & Dukas, R. (2020). Plasticity in male mating behavior modulates female life
446   history in fruit flies. *Evolution. 74*(2), pp.365-376

447   Fordyce, J. A. (2006). The evolutionary consequences of ecological interactions mediated through
448   phenotypic plasticity. In *Journal of Experimental Biology. 209*(12), pp.2377-2383.

449   Fowler, E. F., Leigh, S., Rostant, W. Thomas, A., Bretman, A., & Chapman, T., (2020). Female fruitflies use
450   gustatory cues to physically alter fecundity in response to the social environment. In review.

451   Garbaczewska, M., Billeter, J. C., & Levine, J. D. (2013). *Drosophila melanogaster* males increase the
452   number of sperm in their ejaculate when perceiving rival males. *Journal of Insect Physiology. 59*(3),
453   pp.306-310.

454   Han, C. S., & Brooks, R. C. (2014). Long-term effect of social interactions on behavioral plasticity and
455   lifetime mating success. *American Naturalist. 183*(3), pp.431-444.

456   Heifetz, Y., Lung, O., Frongillo, E. A., & Wolfner, M. F. (2000). The *Drosophila* seminal fluid protein Acp26Aa
457   stimulates release of oocytes by the ovary. *Current Biology. 10*(2), pp.99-102.

458   Herndon, L. A., & Wolfner, M. F. (1995). A *Drosophila* seminal fluid protein, Acp26Aa, stimulates egg laying
459   in females for 1 day after mating. *Proceedings of the National Academy of Sciences of the United
460   States of America. 92*(22), pp.10114-10118.

461   Hollis, B., Koppik, M., Wensing, K.U., Ruhmann, H., Genzoni, E., Erkosar, B., Kawecki, T.J., Fricke, C. and
462   Keller, L., 2019. Sexual conflict drives male manipulation of female postmating responses in
463   *Drosophila melanogaster*. Proceedings of the National Academy of Sciences, 116(17), pp.8437-8444.

464   Hopkins, B.R., Sepil, I., Thézénas, M.L., Craig, J.F., Miller, T., Charles, P.D., Fischer, R., Kessler, B.M.,
465   Bretman, A., Pizzari, T. and Wigby, S., 2019. Divergent allocation of sperm and the seminal proteome
466   along a competition gradient in *Drosophila melanogaster*. Proceedings of the National Academy of
467   Sciences, 116(36), pp.17925-17933.

468   Komers, P. E. (1997). Behavioural plasticity in variable environments. In *Canadian Journal of Zoology. 75*(2),
469   pp.161-169.

470 Lefevre Jr, G. and Jonsson, U.B., (1962). Sperm transfer, storage, displacement, and utilization in *Drosophila*
471   *melanogaster*. Genetics, 47(12), p.1719.

472 Liu, H., & Kubli, E. (2003). Sex-peptide is the molecular basis of the sperm effect in *Drosophila*
473   *melanogaster. Proceedings of the National Academy of Sciences of the United States of America.*
474   *100*(17), pp.9929-9933.

475 Lizé, A., Doff, R. J., Smaller, E. A., Lewis, Z., & Hurst, G. D. D. (2011). Perception of male-male competition
476   influences *Drosophila* copulation behaviour even in species where females rarely remate. *Biology*
477   *Letters. 8*(1), pp.35-38.

478 Mazzi, D., Kesäniemi, J., Hoikkala, A., & Klappert, K. (2009). Sexual conflict over the duration of copulation
479   in *Drosophila montana*: Why is longer better? *BMC Evolutionary Biology. 9*(1), p.132.

480 McGhee, K. E., Pintor, L. M., & Bell, A. M. (2013). Reciprocal behavioral plasticity and behavioral types
481   during predator-prey interactions. *American Naturalist. 182*(6), pp.704-717.

482 McLeod, D. V., & Day, T. (2017). Female plasticity tends to reduce sexual conflict. *Nature Ecology and*
483   *Evolution. 1*(3), pp.1-4.

484 Nandy, B., Dasgupta, P., Halder, S. and Verma, T. (2016). Plasticity in aggression and the correlated changes
485   in the cost of reproduction in male *Drosophila melanogaster. Animal Behaviour, 114*, pp.3-9.

486 Oku, K., & van den Beuken, T. P. G. (2017). Male behavioural plasticity depends on maternal mating status
487   in the two-spotted spider mite. *Experimental and Applied Acarology. 71*(4), pp.319-327.

488 Price, T. A. R., Lizé, A., Marcello, M., & Bretman, A. (2012). Experience of mating rivals causes males to
489   modulate sperm transfer in the fly *Drosophila pseudoobscura. Journal of Insect Physiology. 58*(12),
490   pp.1669-1675.

491 R Development Core Team. (2020). R: a language and environment for statistical computing. In *R: A*
492   *Language and Environmental for Estatistical Computing*.

493 Sarin, S. and Dukas, R., 2009. Social learning about egg-laying substrates in fruitflies. *Proceedings of the*
494   *Royal Society B: Biological Sciences, 276*(1677), pp.4323-4328.

495 Sirot, L. K., Wong, A., Chapman, T., & Wolfner, M. F. (2015). Sexual conflict and seminal fluid proteins: A
496   dynamic landscape of sexual interactions. *Cold Spring Harbor Perspectives in Biology. 7*(2), p.a017533.

497 Wigby, S., & Chapman, T. (2005). Sex peptide causes mating costs in *female Drosophila melanogaster.*
498   *Current Biology. 15*(4), pp.316-321.

499 Wigby, S., Sirot, L. K., Linklater, J. R., Buehner, N., Calboli, F. C. F., Bretman, A., Wolfner, M. F., & Chapman,
500   T. (2009). Seminal Fluid Protein Allocation and Male Reproductive Success. *Current Biology. 19*(9),
501   pp.751-757.

502 Weir, L.K., Grant, J.W. and Hutchings, J.A., 2011. The influence of operational sex ratio on the intensity of
503   competition for mates. *The American Naturalist, 177*(2), pp.167-176.

504 Wertheim, B., Allemand, R., Vet, L.E. and Dicke, M., 2006. Effects of aggregation pheromone on individual
505   behaviour and food web interactions: a field study on Drosophila. *Ecological Entomology, 31*(3),
506   pp.216-226.

507   West-Eberhard, M.J., (2003). *Developmental plasticity and evolution*. Oxford University Press

508   Yamaguchi, S., & Iwasa, Y. (2015). Phenotype adjustment promotes adaptive evolution in a game without
509   conflict. *Theoretical Population Biology. 102*, pp.16-25.

510   **Table 1. Description of the pre- and post-mating treatments and mating environments used in this study.**

511   The environments and the expected effects of those environments are described.

Treatment		Environment description	Expected effect of treatment
Females, pre-mating	Alone	One focal female per vial.	Signals absence of competition.
	Group same sex (GSS)	One focal female and three non-focal females per vial.	Signals competition between females for food, oviposition sites, and potentially males, is likely.
	Group mixed sex (GMS)	One focal female and three males per vial.	Signals females likely to experience effects of competition between males for matings and fertilisations.
Males, pre-mating	No rival	One focal male per vial.	Signals low competition for matings and fertilisations.
	Rival	One focal male and three non-focal males per vial.	Signals competition for matings and fertilisations likely.
Mating arena	Choice	One focal female and two focal males in the mating arena. One male is from the no-rival treatment and one from the rival treatment.	Females can simultaneously assess different males, who can directly compete. Assessment of competition based on previous and current experience of both sexes.
	No choice	One focal female and one focal male in the mating arena.	No opportunity for direct comparisons. Assessment of competition, and choice of whether to mate at all, is indirect and based upon previous experience only.

512

513   **Table 2:** Number of matings by male social treatment and wing colour in the choice experiment. Shown are
514   the results of a Chi-squared analysis of male mating success by male social treatment (no-rival or rival) or
515   male wing colour (black or red).

	Female social treatment								
	alone			Group Same Sex (GSS)			Group Mixed Sex (GMS)		
Male social treatment	Number of maters	Number of non maters	Chi square	Number of maters	Number of non maters	Chi square	Number of maters	Number of non maters	Chi square
no-rival	25	21	$X^2 = 0.39$ $df = 1$ $p = 0.53$	24	18	$X^2 = 1.19$ $df = 1$ $p = 0.27$	24	19	$X^2 = 0.74$ $df = 1$ $p = 0.39$
rival	21	25		18	24		19	24	
	Female social treatment								
	alone			Group Same Sex (GSS)			Group Mixed Sex (GMS)		
Male colour mark	success	fail	Chi square	success	fail	Chi square	success	fail	Chi square
black	26	20	$X^2 = 1.09$ $df = 1$ $p = 0.30$	21	21	$X^2 = 0$ $df = 1$ $p = 1$	25	18	$X^2 = 1.67$ $df = 1$ $p = 0.20$
red	20	26		21	21		18	25	

516

517
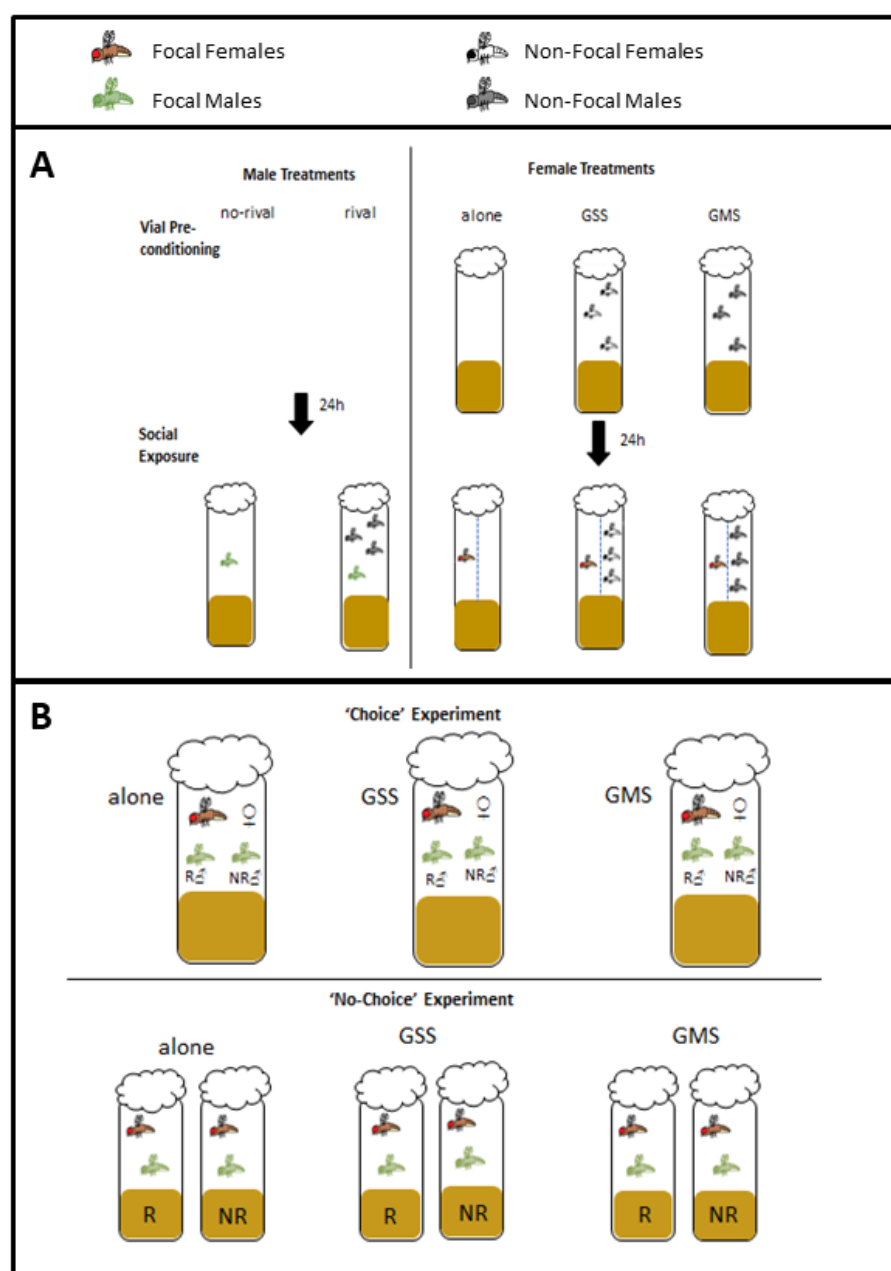
Figure 1. Pre- and post-mating environment manipulations. A: Set up for the pre-mating social environment manipulations. In the female treatments, non-focal flies were placed into vials 24h before the introduction of the focal female in order to transfer residual social cues in the group same sex (GSS) and group mixed sex (GMS) treatments (vial pre-conditioning). After 24h, pre-conditioned GSS and GMS and alone treatment vials were divided using perforated acetate sheets (blue dashed line) to separate focal females from the non-focal flies. **1B: Mating assay set up for the choice and no-choice experiments**. Vials in the choice experiment consisted of a single female from one of the three social treatments in a vial with both a rival (R) and no-rival (NR) male. The no-choice experiment consisted of a single female from one of the three social treatments in a vial with either a rival or no-rival male.
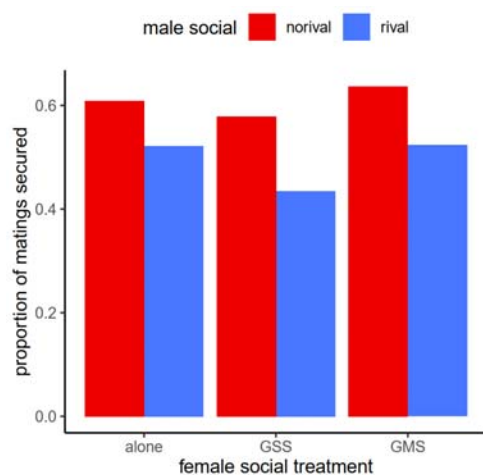
Figure 2: Proportion of matings secured by males from 'no-rival' and 'rival' male social treatments within each female treatment in the choice experiment. Results are shown for the 3 different female social treatments: alone, Group Same Sex (GSS), and Group Mixed Sex (GMS). Sample sizes in table S1.
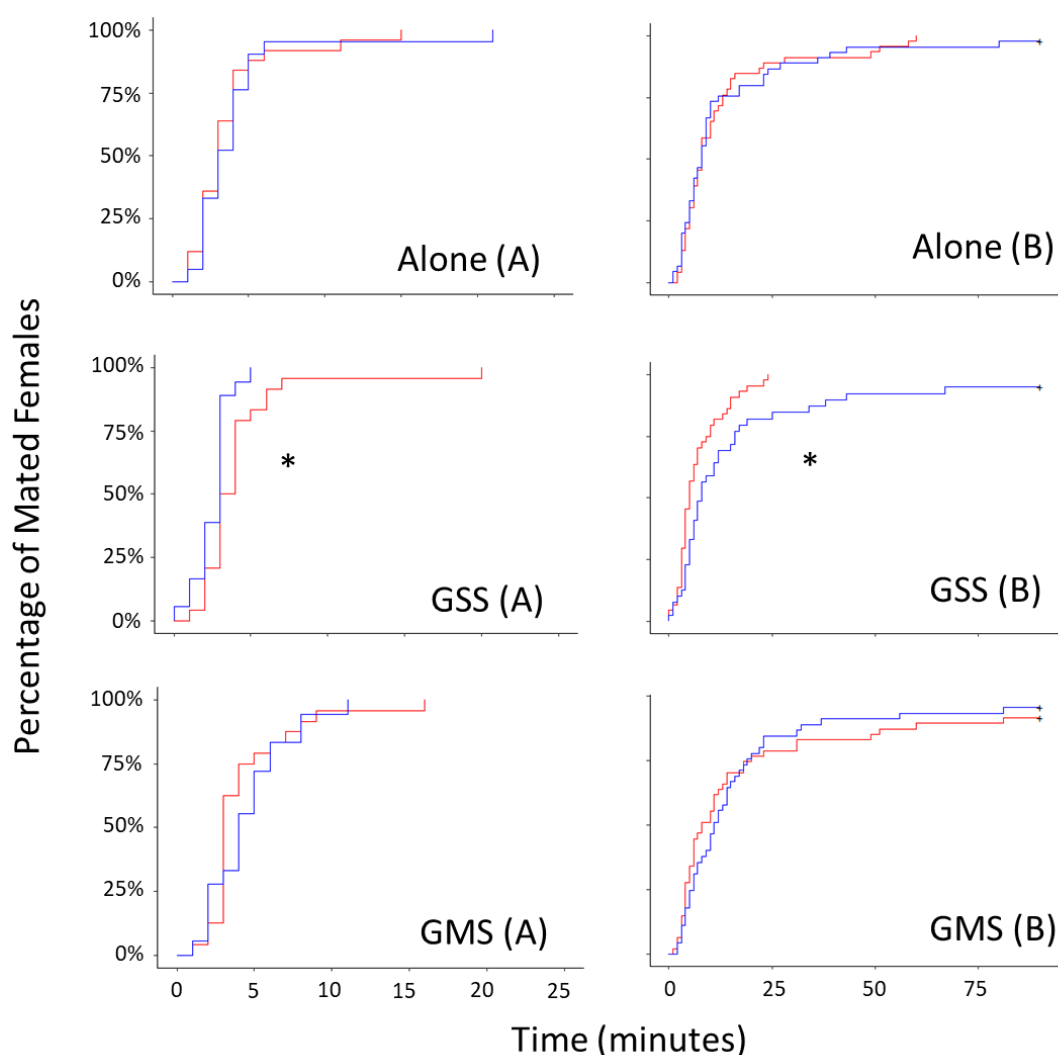
531

Figure 3: Mating latency of no-rival (red) and rival (blue) males with females from each of the female social treatments: Alone, Group Same Sex (GSS), Group Mixed Sex (GMS)) in the choice (A) and no-choice (B) experiments. Females and males used in these assays were raised in the different pre-mating social environments indicated (Figure 1A) for 48h prior to mating. Females were placed into a vial with both a rival and no-rival male in the choice experiment or either a rival or no-rival male in the no-choice experiment (Figure 1B) and observed for 90 minutes for mating to begin and end. Matings occurred more rapidly in the choice experiment, hence note the different X axis in A vs B. Asterisks indicate significant differences between treatments ($p < 0.05$). Sample sizes in table S1.
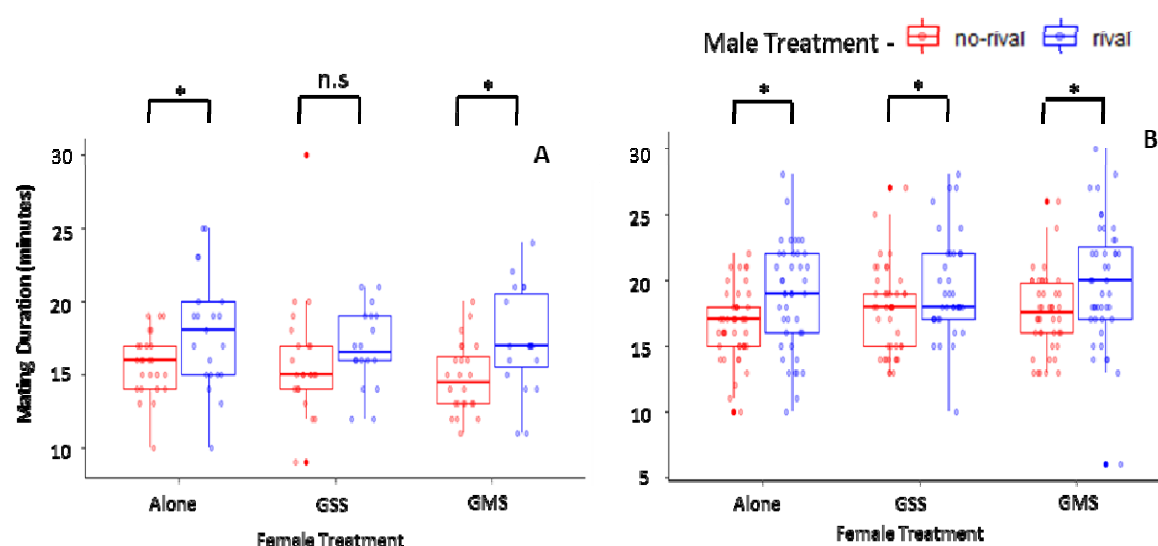
540

Figure 4: Mating duration (minutes) in the choice (A) and no-choice (B) experiments. Females and males
used in these assays were raised in different pre-mating social environments for 48h prior to mating (Figure
1A). Female social environments: alone, group same sex (GSS), group mixed sex (GMS). Male social
environments: no rival (red), rival (blue). Asterisks indicate significant differences between treatments
(p<0.05). Shown are box plots (median, 25-75% IQ range, whiskers (1.5 x IQR) and outliers). Sample sizes in
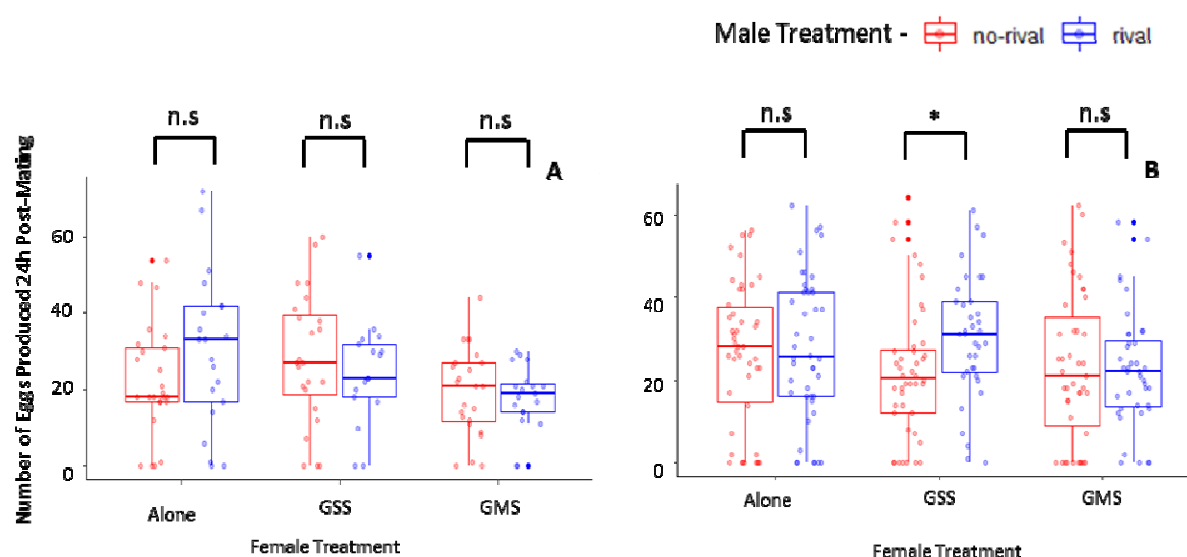table S1.

Figure 5: Number of eggs produced in the separate choice (A) and no-choice (B) experiments, in the 24h after mating. Females and males used in these assays were raised in different pre-mating social environments for 48h prior to mating (Figure 1A). Female social environments: 'alone', group same sex (GSS), group mixed sex (GMS). Male social environments: no rival (red), rival (blue). Asterisks indicate significant differences between treatments (p<0.05). Box plots as defined in figure 4. Sample sizes in table S1.