

1 **Heightened perception of competition hastens courtship**

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12 Short title: Competition hastens courtship

13

14

15 **Abstract**

16 When animals use costly labile display or signal traits to display to the opposite  
17 sex, they face complex decisions regarding the degree and timing of their  
18 investment in separate instances of trait expression. Such decisions may be  
19 informed by not only the focal individual's condition (or pool of available  
20 resources), but also aspects of the social environment, such as perceptions of  
21 same-sex competition or the quality of available mates. However, the relative  
22 importance of these factors to investment decisions remains unclear. Here we  
23 use manipulations of condition (through dietary nutrition), recent social  
24 environment (exposure to a silenced male, non-silenced male, female, or  
25 isolation), and female mating history (single- or multiple-male) to test how  
26 quickly male decorated crickets (*Gryllodes sigillatus*) decide to begin courting an  
27 available female. We find that males that were previously housed with non-  
28 silenced males started courting the female earlier than other males. Females only  
29 mounted males after courtship began. Our results suggest a strong effect of the  
30 perception of competition on the decision to invest resources in sexual signalling  
31 behaviour, and that females might exert directional selection on its timing.

32

33 **Keywords:** *Sexual selection, courtship, condition-dependence, social experience,*  
34 *sexual signalling, phenotypic flexibility, Gryllodes sigillatus.*

35 **Introduction**

36

37 Sexual signalling displays are some of the most extravagant and diverse traits  
38 observed in nature, and both sexual selection and life history theory inform us as  
39 to their evolution and consequences. Investment in mate attraction displays  
40 often confers a much higher mating success, but at some cost to their bearer  
41 (Darwin 1871; Andersson 1994). A ubiquitous cost of sexual trait investment is  
42 simply that any individual has only a finite pool of resources that can be  
43 allocated to its various traits. Thus, investment in one trait also represents a loss  
44 of potential investment in all other competing traits. Individuals that can acquire  
45 more resources have a greater pool from which to allocate, meaning the  
46 marginal costs of additional investment in condition-dependent displays should  
47 be lower (Van Noordwijk and De Jong 1986). This may be why males in high  
48 condition tend to spend more on mate attraction (Hunt et al. 2004), and  
49 experimental manipulations of diet have resulted in increased signalling or  
50 display effort in a variety of taxa (e.g., fiddler crabs *Uca beebei*, Backwell et al.  
51 1995; wolf spiders *Hygrolycosa rubrofasciata*, Kotiaho 2000; field crickets *Gryllus*  
52 *campestris*, Holzer et al. 2003).

53

54 In species where individuals express their sexual trait repeatedly across their  
55 lifetime, resource-based trade-offs occur between not only the focal trait and  
56 other components of life history, but also current and future expression of the  
57 focal trait. Given that resources invested at one stage are unavailable for  
58 investment at another, individual condition is therefore critical in determining

59 both the intensity of male signalling and the most suitable allocation pattern of  
60 current versus future reproductive effort (Bretman et al. 2011).

61

62 If the signal trait in question is a behavioural display, the resolution of trade-offs  
63 can be highly dynamic and responsive to short-term changes in the local  
64 environment (Bretman et al. 2011). The ability of individuals to respond  
65 plastically to immediate changes in the local environment should be selected for  
66 in species living in very unpredictable habitats, or where mating success may be  
67 highly dependent on the number of mating rivals and mating opportunities.

68 Social cues influencing male behaviour can range from the population sex ratio  
69 and density, to encounters and matings with females (Bateman and Fleming  
70 2006; Bailey et al. 2010; Bretman et al. 2011). However, the importance of the  
71 social environment relative to other factors affecting plastic reproductive effort  
72 (e.g., diet or mate quality) remains unclear. Furthermore, tracking one's own  
73 status and predicting fitness is likely quite complicated in natural environments  
74 (Kasumovic et al. 2012). Simple behavioural rules (e.g., "spend resources if you  
75 have them, and if not, focus on acquiring resources instead"; Houslay *et al.*, 2017)  
76 may be more likely than complex adaptive plasticity to explain variation in  
77 social, age, and status-dependent reproductive effort.

78

79 In this study we quantify how resource acquisition and cues of the local social  
80 environment influence the timing and intensity of sexual trait investment in the  
81 decorated cricket, *Gryllodes sigillatus*. Male crickets signal to females using  
82 stridulation of their hardened forewings, through which they can produce two  
83 types of calls: a long-range call to attract females from far away, and a close-

84 range courtship-call just before mating (Ketola et al. 2007). Time spent signalling  
85 (typically referred to as 'calling effort') is a strong predictor of mating success in  
86 nature (Hunt et al. 2004; Bentsen et al. 2006; Rodriguez-Munoz et al. 2010), but  
87 is energetically expensive (e.g., Kavanagh 1987; Hunt et al. 2004; Ophir et al.  
88 2010; Mowles 2014) and may increase mortality risk from both intrinsic (Hunt  
89 et al. 2004) and extrinsic sources (Cade 1975; Walker 1979). Previous work on  
90 *G. sigillatus* has shown that increased dietary nutrition leads to an increase in  
91 both the likelihood and amount of signalling in early adulthood, as well as to  
92 greater investment in energy stores (Houslay et al. 2017). The same study  
93 demonstrated that signalling investment is highly responsive to the availability  
94 of a potential mate: males are more likely to signal, and signal for longer, if a  
95 female is present relative to when absent. In related species of crickets, the  
96 recent or current presence of rival males at adulthood can increase calling effort  
97 (Callander et al. 2013; Noguera 2018), suggesting plasticity of signalling  
98 behaviour based on perceived competition. Manipulations of the juvenile social  
99 environment have also indicated that crickets can perceive future competition  
100 rates and adjust investment in reproductive tissues (Bailey et al. 2010) and age-  
101 specific calling effort (Kasumovic et al. 2013) accordingly.

102

103 Here we manipulate resource acquisition ability in males from the day of  
104 eclosion to adulthood using diets that vary in nutritional content. We then use a  
105 2-day 'social environment' manipulation, providing cues of future competition or  
106 mating opportunities by exposing males to either another male, a female, or  
107 keeping them isolated. We monitor their calling effort during this period to  
108 estimate their immediate response to the social situation. We then provide each

109 male access to a female cricket and observe their latency to begin courtship. To  
110 assess the importance of female reproductive value in determining courtship  
111 speed, female crickets were all mated previously, having been given the  
112 opportunity to mate with either only a single male or multiple males.

113

114 We hypothesise that male *G. sigillatus* housed with other males may perceive a  
115 greater level of competition relative to isolated males, and should thus begin  
116 courtship more quickly when next encountering a female. Males who are  
117 exposed to a female during the social environment manipulation instead might  
118 perceive greater mating opportunities and low competition, and thus begin  
119 courting later. We predict that increased dietary nutrition level should decrease  
120 the latency to call, as males with a greater pool of resources have less incentive  
121 to conserve resources. We also hypothesise that males may start courting  
122 females with only one previous partner more quickly than those with prior  
123 access to multiple potential partners, as females with multiple previous mates  
124 may present heightened levels of sperm competition in this highly polyandrous  
125 species (Sakaluk 1987). *G. sigillatus* appear able to detect previous mating  
126 partners using cuticular hydrocarbons (CHCs) that are transferred during  
127 mating, and that vary according to both sex and genotype (Ivy et al. 2005;  
128 Weddle, Mitchell et al. 2012; Weddle, Steiger et al. 2012). We expect a positive  
129 relationship between male courtship latency and female mounting latency, such  
130 that female *G. sigillatus* are likely to more quickly mount males that begin  
131 courting earlier. Females may also prefer males on higher-quality diets as they  
132 should be in better condition, and multiple-male females might be less keen to  
133 mate than their single-male counterparts (based on the observation that female

134 receptivity tends to decrease somewhat after the first mating, even for highly  
135 polyandrous species; Jennions and Petrie 2000; Wedell 2005; Judge et al. 2010).

136

## 137 **Methods**

138 Study species and mating behaviour

139 The decorated cricket (*G. sigillatus*) is probably native to South Asia, but is  
140 common to tropical and subtropical regions worldwide (Otte 2006). Females  
141 respond phonotactically to calling songs of conspecifics (Champagnon and Cueva  
142 del Castillo 2008). Mating involves a female mounting the male in order to attach  
143 his spermatophore (comprising a sperm ampulla and gelatinous  
144 spermatophylax) to her (Alexander and Otte 1967), and forced copulations are  
145 not possible in this species. The spermatophore comprises a sperm ampulla  
146 surrounded by a spermatophylax (Sakaluk 1987; Ivy and Sakaluk 2005), which is  
147 a gelatinous mass that the female separates from the ampulla and feeds on while  
148 the sperm is transferred into her sperm receptacle (Sakaluk 1987). After  
149 finishing eating or discarding the spermatophylax, the female removes the  
150 ampulla too, therefore terminating the transfer of sperm. Females have a high  
151 remating rate (Sakaluk 1987), and polyandry improves survival prospects of  
152 offspring (Ivy and Sakaluk 2005). Males have a lower maximal mating frequency  
153 than females (Sakaluk 1987), apparently due to the time required to build a new  
154 spermatophore (Sakaluk 1985). The potential nutritional benefits to females of  
155 the spermatophylax are controversial (Will and Sakaluk 1994; Warwick et al.  
156 2009), although spermatophylax consumption provides a water-stressed female  
157 with great hydration (Ivy et al. 1999), possibly representing one of the  
158 advantages of mating multiply.

159

160 Cricket rearing and maintenance

161 Experimental *G. sigillatus* were the 55<sup>th</sup> generation of a laboratory stock

162 composed by 5000 individuals originally from Las Cruces, New Mexico, USA. The

163 previous generations were allowed to breed freely, with periodic new

164 introductions from cultures of other research institutions. The crickets used in

165 this study were born and maintained at 31±1 °C on a 14:10 hr light:dark

166 photoperiod in a controlled-temperature room set for lights off at 2:30 PM. We

167 reared the newly hatched nymphs in clear plastic containers (30 × 30 × 15 cm),

168 each containing several dozen individuals of the same age born from mixed

169 parents. We provided cricket nymphs with *ad libitum* water in 47 mL vials

170 stoppered with dampened cotton wool, ground dry cat-food (Friskies Go-Cat

171 Senior, Purina®, London, UK), and egg cartons for shelter. We cleaned the

172 containers and replaced food and water weekly throughout the experimental

173 period. Males and females were reared together until the day of their eclosion, at

174 a density of approximately 200-300 crickets per container.

175

176 Experimental design and manipulations

177 We checked all nymphs every morning, collecting any individuals that had

178 eclosed to adulthood overnight. On the day of its eclosion we weighed every

179 individual with an electronic balance (PI-225DA, Denver Instrument, Bohemia,

180 NY). We isolated each new adult male individually in a small plastic container (7

181 × 7 × 7 cm). These containers were supplied with 7 mL water vials plugged with

182 cotton wool, and plastic mesh attached to the sides of the container as substrate.

183 To manipulate the crickets' nutritional condition, we haphazardly assigned each



184 adult male to one of five dry and granular artificial diets differing in energy  
185 content. All the diets had a 1:8 ratio of protein:carbohydrate, and the total  
186 protein and carbohydrate content of the food mass ranged from 36% to 84% of  
187 (the rest being a mix of vitamins and indigestible crystalline cellulose). Previous  
188 studies have shown that these diets affect individual condition and allocation to  
189 competing traits in male *G. sigillatus* (Rapkin et al. 2016; Houslay et al. 2017).  
190 The experimental crickets had access to their assigned diet *ad libitum* for 10  
191 days, until the behavioural trials. We measured body mass again at the end of the  
192 first adult week (day 7) in order to test for effect of diet treatment on any change  
193 in body mass.

194

#### 195 Social experience manipulation

196 From their day of eclosion onwards, we isolated individuals acoustically by  
197 placing their containers into cubes of acoustic foam (Houslay et al. 2015; Houslay  
198 et al. 2017). Each foam lid had a small opening to allow light from the chamber  
199 inside the box, and we tested that this opening did not allow cross-talk from  
200 other cricket containers to contaminate our estimate of calling effort for a focal  
201 male (see below). At seven days post-eclosion (by which time males have greatly  
202 increased both the likelihood and intensity of calling effort; Houslay et al., 2017),  
203 we exposed males to one of four treatments designed to manipulate their social  
204 experience for a period of 2 days (i.e., days 8 and 9 post-eclosion): 1) control  
205 (maintained in isolation); 2) housed with a female; 3) housed with a silenced  
206 male (wings clipped); or 4) housed with an injured male as a sham, which was  
207 subject to autotomy of a single limb (a common escape mechanism in  
208 Orthopterans; Bateman and Fleming, 2008) that did not affect calling ability. The

209 sham treatment was used instead of an unmanipulated treatment to account for  
210 the potential effect of injury on male calling. We were unsure if wing clipping (for  
211 silenced males) might reduce the activity of crickets, and whether any such  
212 reduced activity might be perceptible to focal males. Since the sham treatment  
213 was meant to reflect differences in calling attributable to calling by the non-focal  
214 male, we wanted that male to have been similarly handled (and injured) in a way  
215 that mirrored the silenced male, but without directly affecting calling. The social  
216 partners of focal males were introduced to the experimental crickets' individual  
217 containers inside pierced 60 mL transparent plastic vials containing some  
218 soaked cotton for water and a pellet of commercial cat food. Tactile contact  
219 between crickets was possible only through the holes pierced on the sides of the  
220 vials, which were not large enough to allow mating. The crickets for this social  
221 manipulation were stock individuals of the same generation, but not taking part  
222 in the experiment.

223

224 We recorded calling effort over both nights of the social manipulation  
225 experiment by inserting a microphone (C1163, Dick Smith Electronics) into the  
226 lid of each individual male container, which we connected to an Electronically  
227 Activated Recorder (EAR; Bertram and Johnson 1998). The EAR samples each  
228 microphone 10 times per second to determine whether the assigned cricket was  
229 calling or not. We started recording every day as the lights went out (2:30 PM)  
230 and stopped the following day (9:30 AM). On the morning of day 9 post-eclosion,  
231 we ended the social experience manipulation, weighing the experimental males  
232 and returning them to their original container for a day of isolation before the  
233 courtship behaviour trials.

234

235 Females mating history manipulation

236 After collecting their morphological measures upon eclosion, we housed females  
237 of similar age together in a 30 × 30 × 15 cm plastic container. We provided them  
238 with ground dry cat food and water *ad libitum* until day 7 post-eclosion. At day 7,  
239 we randomly assigned each female to one of two treatment groups: single-male  
240 (SM) and multiple-males (MM). We placed a female assigned to the SM treatment  
241 in a 7 × 7 × 7 cm plastic container with food and water, one stock male, and  
242 moistened cotton on a petri dish as oviposition substrate. We placed a female  
243 assigned to the MM treatment in a bigger plastic container (12 x 12 x 12 cm),  
244 containing up to three females and many stock males (3-4 males for every  
245 female), as well as food, water and oviposition substrate. We left each female in  
246 either of these groups for 2 days, after which we weighed her and placed her in  
247 isolation for one day in a 7 x 7 x 7 cm container supplied with food and water.  
248 The following day, we performed the courtship behavioural trials. SM and MM  
249 treatments therefore differ in potential mating frequency and probable number  
250 of mates; however, we did not verify mating frequency for individual females.  
251 Nevertheless, it is very likely that in two days there would be more than one  
252 mating due to *G. sigillatus* females' typical mating frequency (Sakaluk 1987).

253

254 Mating behaviour trials

255 At 10 days post-eclosion we randomly paired one experimental male with either  
256 an SM or MM female, using a no-choice experimental paradigm (e.g., Shackleton  
257 et al. 2005; Judge et al. 2010). All the mating trials took place soon after the main  
258 lights went out, under illumination from a 25 W red incandescent bulb held

259 about 40 cm from the cricket containers to minimise any possible visual  
260 disturbance. The female was introduced to the male in his individual container,  
261 which was supplied with water but no food. We noted the time elapsed until the  
262 male's first call (latency to call), as well as how long the female took to mount the  
263 male after his courtship started (latency to mount). Each trial lasted a maximum  
264 of 30 minutes, after which we ended the observation regardless of the state of  
265 courtship.

266

#### 267 Statistical analysis

268 We performed all analyses using R version 3.4.2 (R Core Team 2017), with the  
269 'tidyverse' set of packages for data cleaning and visualisation (Wickham 2017).  
270 For normally distributed response variables, we used generalised linear mixed  
271 effects models (GLMMs) with restricted maximum likelihood (REML)  
272 approaches in lme4 (Bates et al. 2015). In lme4, we checked model fit visually  
273 through diagnostic plots, and used parametric bootstrapping (with 1000  
274 simulations) to assess the difference between nested models refitted with ML for  
275 hypothesis testing (R package pbkrtest; Halekoh and Højsgaard 2014). For  
276 overdispersed count data (see below) we used Bayesian estimation in  
277 MCMCglmm (Hadfield 2010). Here we checked model fit visually through plots of  
278 MCMC chains for both variance components and fixed effects, in addition to  
279 testing that multiple runs converged to similar results via the Gelman-Rubin  
280 diagnostic (Gelman & Rubin 1992) and that models were robust to different  
281 priors. We used 95% credible intervals of posterior distributions for hypothesis  
282 testing in these models. For pairwise comparison of groups within categorical  
283 predictors, we subtracted the posterior distribution of one group from another,

284 and inspected the 95% credible interval of the resulting ‘difference’ distribution  
285 (such that if 0 is excluded then the difference between those groups is nominally  
286 significant). In all models, the effect of diet was centred and scaled to single unit  
287 increments (i.e., the 5 diets were treated as a continuous sequence from -2 to 2).  
288 The ‘social manipulation’ and ‘female mating status’ manipulations were treated  
289 as 4- and 2-level categorical variables respectively.

290

291 We assessed the effect of the nutritional manipulation using a mixed effects  
292 model fit in lme4, where our response variable was ‘body mass’ and predictors  
293 were diet, time period (0 and 1 to reflect start and end of first week post  
294 eclosion), and their interaction. We also included a random effect of male cricket  
295 ID. A significant positive interaction between time period and diet would  
296 indicate that males on diets containing greater nutritional content increased  
297 body mass at a higher rate over the course of the week.

298

299 Calling effort data during the social experience manipulation roughly  
300 approximated a Poisson distribution and was highly overdispersed, so we  
301 elected to use Bayesian methods, as MCMCglmm includes a vector of residuals  
302 that handles overdispersion. Unlike previous studies of calling effort in this  
303 species (e.g., Houslay et al. 2015; Houslay et al. 2017), the level of zero-inflation  
304 was fairly low (less than 15%) and so we used the overdispersed Poisson  
305 distribution rather than a more specialist hurdle or zero-altered model. Our  
306 model included fixed effects of the social manipulation, diet and their interaction,  
307 day of observation (mean-centred) as a further covariate, and a random effect of  
308 male ID.

309

310 We also used overdispersed Poisson models fit in MCMCglmm to analyse the  
311 effects of our treatments on (i) latency to begin courtship, and (ii) latency to  
312 mount. For courtship latency, predictors included diet, social manipulation  
313 treatments, and female mating status. As data were right-censored, the latency to  
314 call was set to the maximum value (1800s) for males that did not call (49/217).  
315 For mounting latency, we excluded those 49 males that did not call, and used the  
316 same predictors as above (male diet, male social manipulation treatments, and  
317 female mating status) with the addition of log-transformed call latency (mean-  
318 centred and scaled to standard deviation units after the log-transformation) as a  
319 further predictor. Data were right-censored, so we set the latency to mount to  
320 the maximum value (1800s) for females that did not mount the courting male  
321 (43/168).

322

### 323 **Results**

324 A total of 217 male crickets completed the experiment and were included in our  
325 final data set. Our experimental design resulted in 40 diet × social environment ×  
326 female status combinations, each cell of which contained a minimum of 3 and  
327 maximum of 11 individuals after excluding those that died during the  
328 experiment (see Table S1 for full breakdown of sample size by experimental  
329 manipulations).

330

331 Males tended to gain body mass over the course of the first week post-eclosion  
332 on average, and mass increase was greater in those with access to diets of higher  
333 nutritional content (parametric bootstrap  $P < 0.001$ ; Figure 1, Table S2). Calling

334 effort was higher in all treatments relative to control, although only significantly  
 335 so in the two male treatments (Figure 2, Table 1). We found no interaction  
 336 between diet and the manipulations of social environment (95% CIs were large  
 337 and centred close to zero for each term), and so refit the model excluding this  
 338 interaction. Excluding the interaction had negligible effect on the coefficients for  
 339 the main effects. Increased nutritional content tended to increase the level of  
 340 calling effort, but this was not significant. We found that males decreased calling  
 341 effort from the first day to the second of the social manipulation experiment.

342

343 Table 1: Coefficients and 95% credible intervals for the analysis of calling  
 344 effort. Social manipulation treatment levels show deviations from the  
 345 reference group ('isolated male').

Parameter	Estimate	95% CI	pMCMC
(Intercept)	3.42	2.66, 4.13	<0.001
Diet	0.21	-0.07, 0.47	0.14
Social: Female	0.33	-0.63, 1.31	0.49
Social: Silenced male	1.15	0.08, 2.22	0.04
Social: Sham male	1.39	0.31, 2.48	0.01
Day	-0.72	-1.08, -0.29	0.002

346

347

348 The only significant predictor of the latency to call in the courtship behaviour  
 349 trials was the 'sham male' treatment group, during which the focal male had  
 350 been housed with a male that was able to call (Table 2). These males show a

351 marked decrease in the time taken to begin courting the available female,  
352 relative to isolated males (Figure 3). Previous housing with a silenced male also  
353 tended to decrease latency to call, although the effect size was smaller and the  
354 95% credible intervals did extend beyond zero (95% CI: -1.22, 0.04; Table 2).  
355 Dietary nutrition tended to reduce call latency, but again the credible intervals  
356 (just) included zero (95% CI: -0.33, 0.01; Table 2).

357

358 Pairwise comparisons of treatment groups indicated that the sham male  
359 treatment group had a significantly shorter latency to begin calling relative to the  
360 female treatment group (95% CI: -1.62, -0.26). There was no significant  
361 difference between sham male and silenced male treatment groups (95% CI: -  
362 1.14, 0.28), nor between silenced male and female treatment groups (95% CI: -  
363 1.15, 0.17).



364

365

Table 2: Coefficients and 95% credible intervals for the analysis of

366

courtship latency. Higher values indicate increased time for the focal male

367

to begin courting the available female in the behavioural trial. Categorical

368

variables show deviations from the reference group (isolated for social

369

manipulation; single male for female mating status).

Parameter	Estimate	95% CI	pMCMC
(Intercept)	4.94	4.40, 5.46	<0.001
Diet	-0.16	-0.33, 0.01	0.07
Social: Female	-0.11	-0.74, 0.51	0.73
Social: Silenced male	-0.60	-1.22, 0.04	0.06
Social: Sham male	-1.08	-1.77, -0.42	0.003
Mating: Multiple-males	0.08	-0.37, 0.59	0.73

370

371

372

We found no significant effects of our experimental manipulations on female

373

decision time (Table 3). Females mounted males only after courtship had been

374

initiated (Figure 4).

375 Table 3: Coefficients and 95% credible intervals for the analysis of  
 376 mounting latency. Higher values indicate increased time for the female to  
 377 mount the male in the behavioural trial. Categorical variables show  
 378 deviations from the reference group (isolated for social manipulation;  
 379 singly mated for female mating status).

Parameter	Estimate	95% CI	pMCMC
(Intercept)	5.02	4.47, 5.64	<0.001
Diet	-0.08	-0.26, 0.10	0.40
Social: Female	0.37	-0.36, 1.02	0.30
Social: Silenced male	0.16	-0.50, 0.89	0.66
Social: Sham male	0.28	-0.42, 0.95	0.41
Mating: Multiple-males	0.46	-0.04, 0.96	0.09

380

381

## 382 Discussion

383 A male's decision to invest in sexual signalling may be informed by both his  
 384 energy budget and his experience of the social environment. Few studies have  
 385 manipulated an animal's resource acquisition and social experience  
 386 simultaneously, to assess their relative importance in mating interactions. Here,  
 387 we find that the recent social environment plays a large role (seemingly larger  
 388 than that of diet) in determining how quickly male *G. sigillatus* begin courting an  
 389 available female. Males that were housed recently with another male began  
 390 courting the female earlier, although this effect was strongest when the other  
 391 male was not silenced. The idea that male calling behaviour is strongly

392 influenced by the perception of competition is reinforced by our observations of  
393 calling effort during the social environment manipulation, where males that  
394 were housed with other males (whether silenced or not) appear to call more  
395 than males housed with females or by themselves. Our results suggest that male  
396 crickets show both immediate and lasting (at least in the short-term)  
397 behavioural plasticity, based on their experience of the social environment. The  
398 importance of this effect is highlighted by the consequence of courtship latency  
399 on mating, as females mounted males only after they commenced calling.

400

#### 401 *Exposure to rival males increases calling effort*

402 Previous studies have shown that male calling effort is strongly affected by the  
403 social environment, whether that be developmental plasticity caused by the  
404 perception of future competition at the juvenile stage (Kasumovic et al. 2012;  
405 Kasumovic et al. 2013), flexibility caused by recent or current exposure to rival  
406 males (Callander et al. 2013; Noguera 2018), or current access to potential mates  
407 (Houslay et al. 2017). Here we show that males also exhibit an increase in calling  
408 effort when exposed directly to another male. This does not appear to be due  
409 solely to some 'chorus effect' (i.e., being provoked into calling via the calling of a  
410 competitor), as we saw an increase in calling effort among males exposed to  
411 silenced (i.e., wings removed) males as well as to the 'sham' injured males. We  
412 note that while a sham male may be contributing to the observed calling effort  
413 assigned to a focal male, in the silenced male treatment all calling must be from  
414 the focal male. While this result indicates males do respond to perceived  
415 competitors, males placed with females did not call more than those kept in  
416 isolation – a result seemingly at odds with those of Houslay *et al.* (2017), in

417 which exposure to female crickets greatly increased both the likelihood and  
418 amount of male calling effort. However, males in our current study did not have  
419 full physical access to the other individual (instead being separated by a plastic  
420 barrier, albeit with holes to allow some degree of contact and airflow). As  
421 posited by Houslay *et al.* (in whose study males had full physical access to  
422 females and were able to mate), the positive feedback from females may drive  
423 the increased calling by males. We note also that the calling effort recorded in  
424 that study was far beyond that which was seen in ours, despite a similar nightly  
425 recording period and similar dietary regimen. An open question concerns how  
426 males were distinguishing differences in social environment in our study, which  
427 could be based on visual or auditory, touch, or chemosensory (via CHC) cues.

428

#### 429 *Exposure to rival males decreases courtship latency*

430 We originally hypothesised that males might be able to use a mechanism such as  
431 information from CHCs to infer a female's number of previous matings, thus  
432 enabling discrimination against females in the multiple-male treatment group  
433 (who would likely present a higher intensity of sperm competition (Sakaluk  
434 1986) and/or higher likelihood of carrying sexually transmitted nematodes  
435 (Luong *et al.* 2006)). However, even if this is possible, we found no difference in  
436 how quickly males started to court a female. We also found no effect of the  
437 'female exposure' social manipulation on courtship latency. While males  
438 exposed to females previously took longer to begin courting an available female  
439 in the courtship trials relative to those exposed previously to males, there was no  
440 difference between those exposed to females and those held in isolation.

441 Therefore, these results do not support our *a priori* hypothesis that the exposure

442 to a female might alter behaviour due to a male's perception of mate availability,  
443 causing a decrease in his signalling effort and urge to start courting the next  
444 female encountered.

445

446 We do, however, see a strong response in courtship latency as a result of a male's  
447 own prior exposure to other males, particularly those that were not silenced (i.e.,  
448 the 'sham' male treatment). Combined with the strong increase in calling effort  
449 during the social environment manipulation for males exposed to sham and  
450 silenced rivals, our results suggest a strong and lasting effect of a short-term  
451 change in the competitive social environment in this species. This effect held  
452 despite a day spent in isolation between the social treatment and the mating  
453 trial. Previous studies have shown that manipulations of the juvenile social  
454 environment (using recordings of males played to mimic different densities)  
455 induce developmental plasticity that affects how males invest in calling at  
456 adulthood in a related species of cricket (*Teleogryllus commodus*; Kasumovic et  
457 al. 2012; Kasumovic et al. 2013). Our results add support to the notion that male  
458 crickets are highly tuned to their social environment, and likely use multiple  
459 sources (including acoustic and chemical) to gather information regarding  
460 potential competition for mating opportunities.

461

462 *Mounting latency is related to calling latency*

463 Females that had had access to multiple potential mates prior to the mating trials  
464 showed only a small and non-significant increase in mounting latency,  
465 suggesting that availability of multiple males previously did not greatly diminish  
466 a female's receptivity. This result is in line with previous work in this species

467 indicating that female *G. sigillatus* have a high re-mating rate, averaging 22 times  
468 every 20 days (Sakaluk 1987). Despite the lack of evidence for substantial  
469 nutritional benefits of the spermatophylax (Will and Sakaluk 1994), previous  
470 work has indicated that such a high re-mating rate may be offsetting any costs of  
471 reproduction via some benefits of nuptial gifts provided by males (Burpee and  
472 Sakaluk 1993). In our experiment, around 75% of males that started courting in  
473 trials were mounted by the female during their behavioural trial, with similar  
474 proportions of callers mounted across single- and multiple-male females: 18/78  
475 (77%) and 25/90 (72%) respectively. This high female re-mating rate could be  
476 selecting for responsiveness to mating opportunities in males, who would  
477 benefit from advertising their availability as quickly as possible. These results  
478 are also in line with patterns found by Houslay *et al.* (2017): over the course of a  
479 week of continued access to females there was a decrease in calling effort (which  
480 appeared due largely to declining energy reserves), but not of the likelihood of  
481 calling. This pattern suggests that males have a strong inclination to court  
482 females, even if they are in lower condition. We note that the dietary nutrition  
483 manipulation used in the study of Houslay *et al.* (2017) did not show a  
484 statistically significant effect of diet on the likelihood of calling, and here we find  
485 a small and non-significant decrease in calling latency due to dietary nutrition.

486

#### 487 *Concluding remarks*

488 Overall, our results show that male energy reserves tended to increase sexual  
489 signalling duration and hasten the decision to start courting an available female,  
490 but these effects were fairly weak and not statistically significant. Variation in  
491 male signalling effort can be driven strongly by variation in the current or recent

492 competitive environment, suggesting that males are gathering information from  
493 various sources to determine their behaviour. Our results provide further  
494 evidence for the flexibility of sexual signalling behaviour, which in turn suggests  
495 that a male's ability to respond to current opportunities has been shaped by  
496 substantial past selection. Additional investigation of how individuals gather  
497 information and make decisions to outcompete their rivals and take advantage of  
498 potential mating opportunities – and how this affects patterns of age-dependent  
499 variation, as well as allocation to competing life history traits – might be a  
500 fruitful avenue of research. More broadly, the field would benefit from more  
501 quantitative assessments of the relative importance of multiple contributing  
502 factors to behavioural variation.

503

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506

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512 reviewers for constructive comments on our manuscript.

513

#### 514 **Data Accessibility**

515 Analyses reported in this article can be reproduced using the data provided by  
516 Santori *et al.* (2019).

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643

644

645 **Figure legends**

646 Figure 1: The effect of dietary nutritional content on the change in body mass  
647 over the first week post-eclosion. Dark points and vertical bars show raw means  
648 and standard errors; light points show individual data (jittered on x-axis with a  
649 small amount of random noise). The horizontal dotted line at 0 indicates no  
650 change in body mass.

651

652 Figure 2: The effect of social environment manipulation on calling effort during  
653 the treatment period. The 'isolation' treatment represents our control treatment  
654 for analysis. Grey points are raw data (lightly jittered on x axis with small  
655 amount of random noise); black points and line ranges show estimates and 95%  
656 credible intervals from MCMCglmm analysis (averaging over effects of day and  
657 diet), plotted on log scale.

658

659 Figure 3: Effects of the social environment manipulation on latency to call in the  
660 courtship behavioural trials. Males in the 'sham male' treatment group show a  
661 significant reduction in call latency relative to isolated males. Grey points are  
662 raw data; black points and line ranges are estimates and associated 95% credible  
663 intervals from MCMCglmm analysis, plotted on log scale. Note that males  
664 assigned a censored score of 1800 s did not call before the end of the trial period.

665

666 Figure 4: Males were only mounted after they started actively courting females,  
667 shown by the positive relationship between (log-transformed) latency to male  
668 calling (x-axis) and (log-transformed) total time to mating (i.e., calling latency +  
669 mounting latency; y-axis). Open circles indicate observations where males called

670 but were not mounted by the female (and were assigned a censored score of  
671 1800 s for latency to mount). Dotted line shows the 1:1 relationship between  
672 latency to call and total time to mating. Males that did not call were not mounted,  
673 and are not shown.

674

675 **Supplementary material**

676

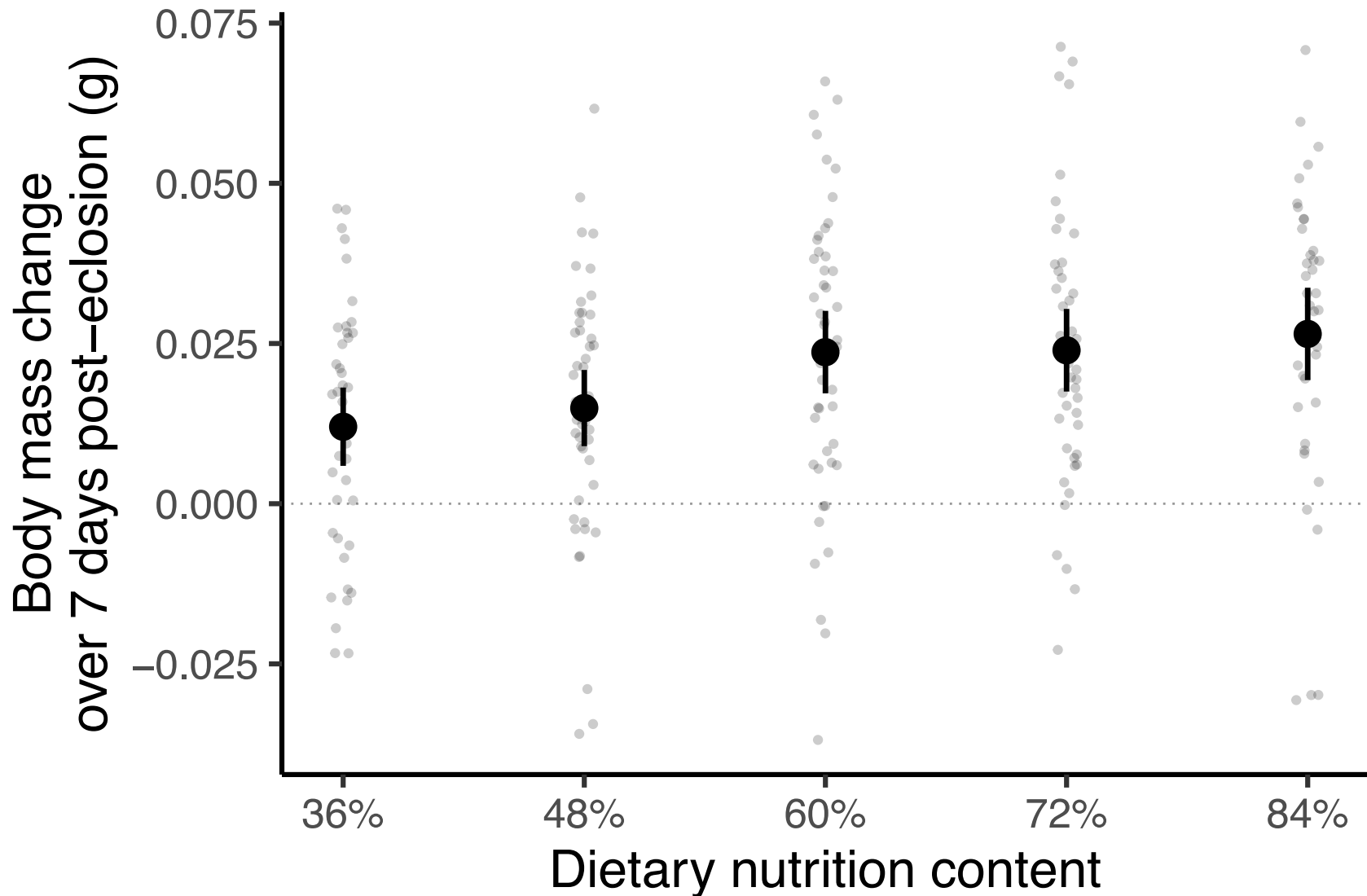
677 Table S1: Sample sizes of males completing the experiment across each  
678 combination of social manipulation, female mating status and dietary nutrition.

679

680 Table S2: Summary of mixed model (fitted in lme4 with Gaussian error family)  
681 for analysis of the change in body mass over the first week post-eclosion.

682

683





Calling effort (s)

10000  
1000  
100  
10  
1

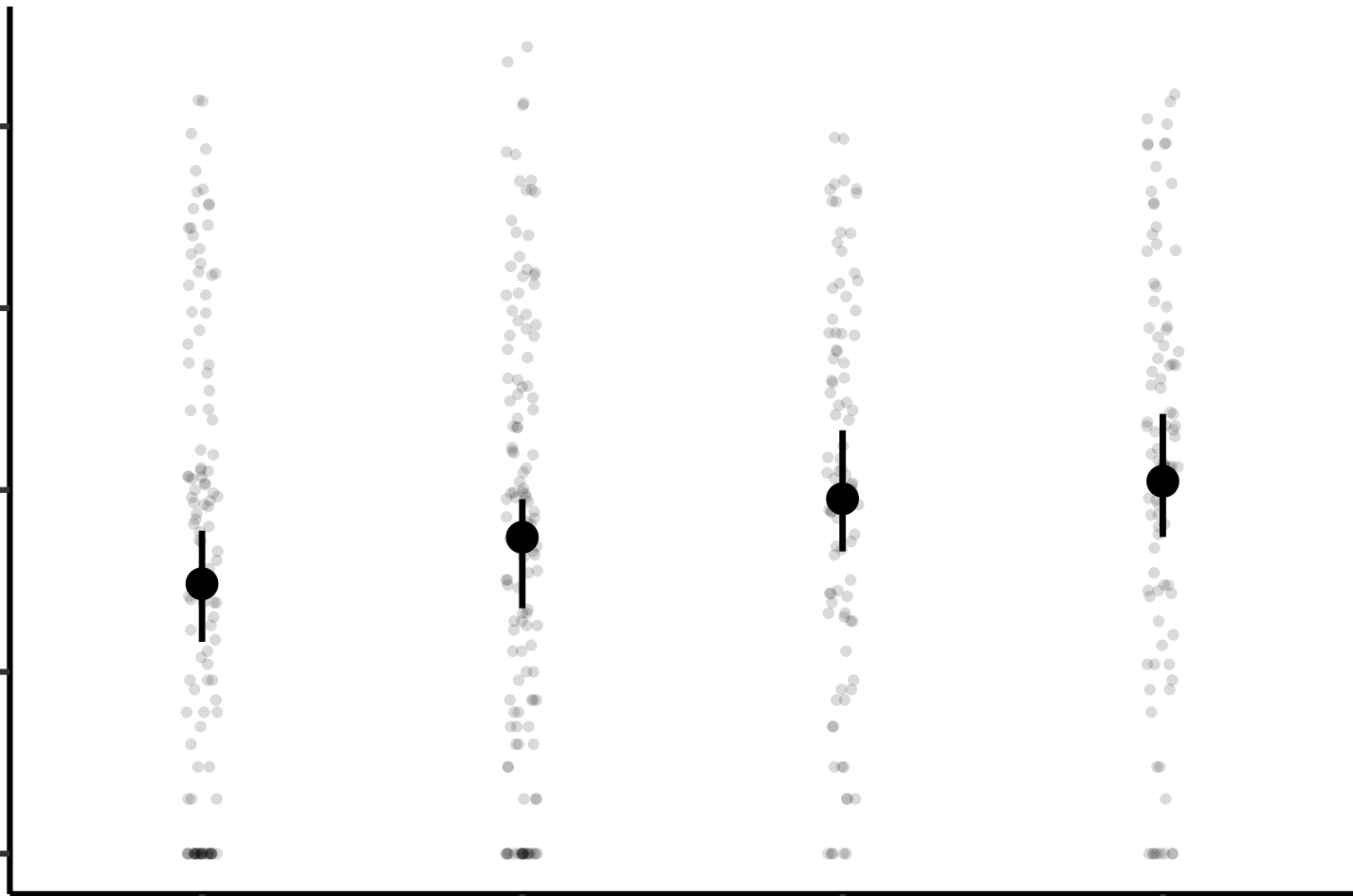
Isolation

Female

Silenced male

Sham male

Social environment manipulation



Latency to call (s)

1800  
1000  
100  
10  
1

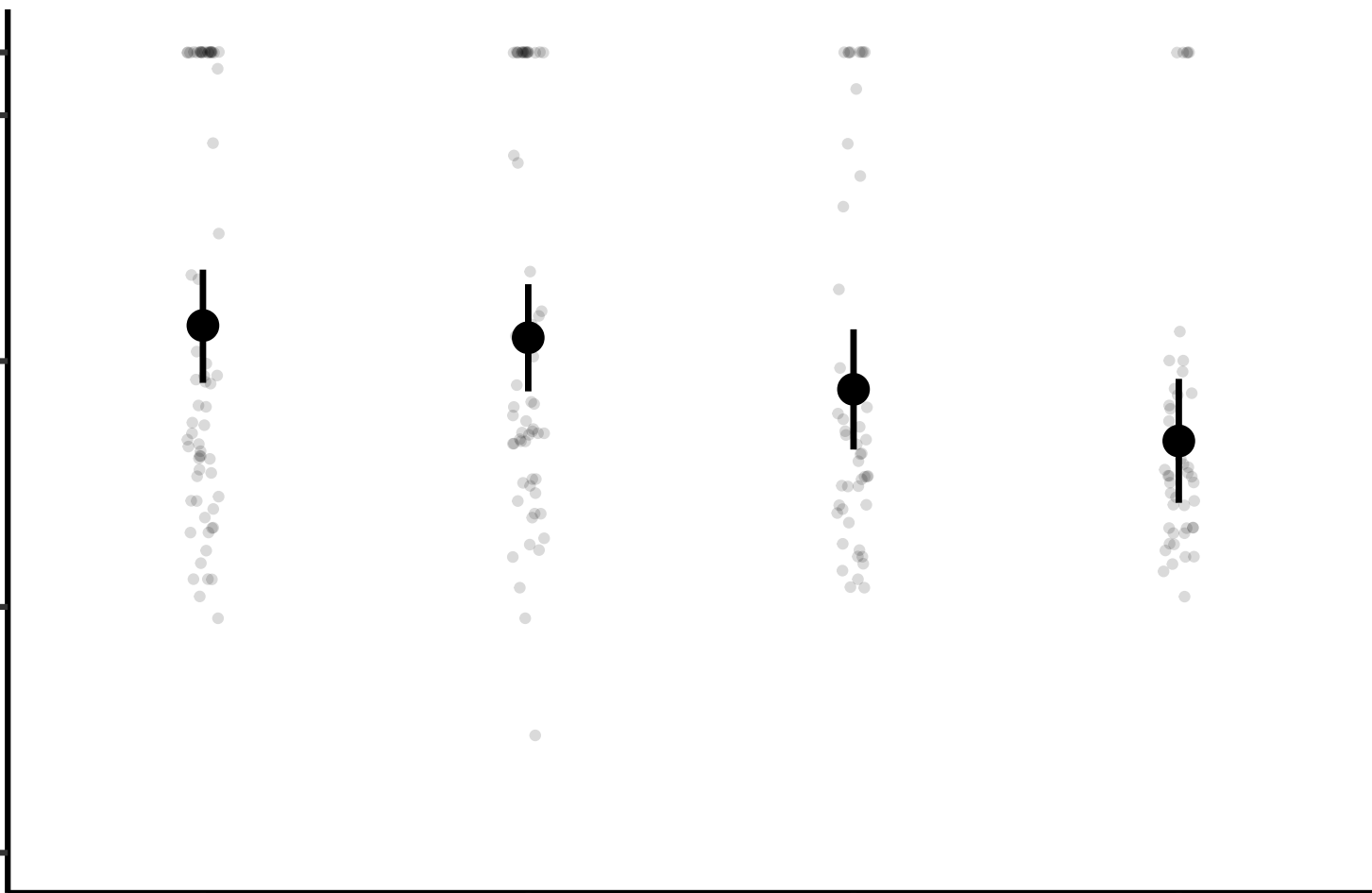
Isolation

Female

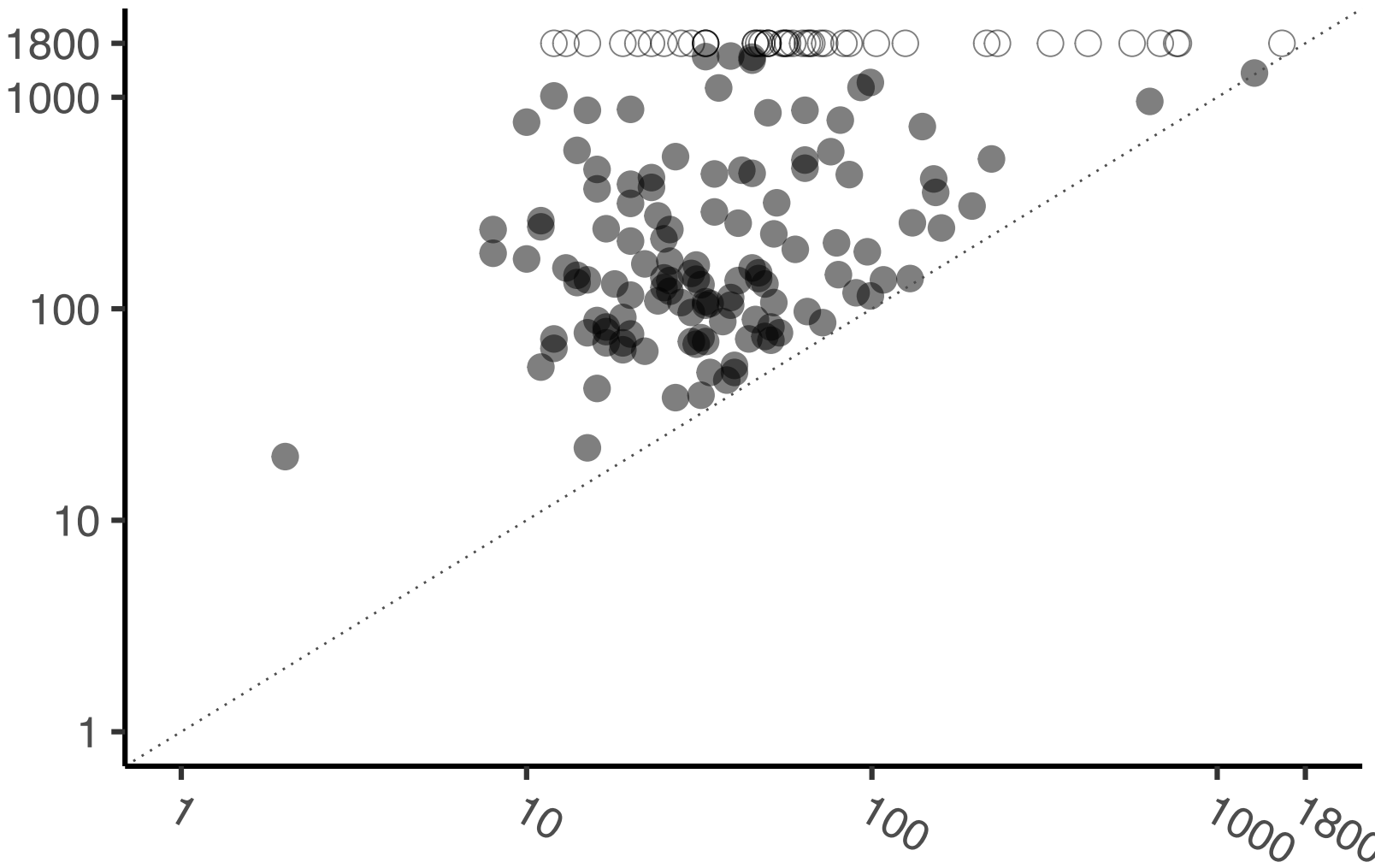
Silenced male

Sham male

Social environment manipulation



Total time to mating (s)



Latency to male call (s)