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Heightened perception of competition hastens courtship

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Abstract

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

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

Introduction

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

In species where individuals express their sexual trait repeatedly across their lifetime, resource-based trade-offs occur between not only the focal trait and other components of life history, but also current and future expression of the focal trait. Given that resources invested at one stage are unavailable for 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

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

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

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

Methods

Study species and mating behaviour

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

159

160 Cricket rearing and maintenance

161 Experimental *G. sigillatus* were the 55th 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

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

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

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

Statistical analysis

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

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

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

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

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

Results

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

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

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

Table 1: Coefficients and 95% credible intervals for the analysis of calling effort. Social manipulation treatment levels show deviations from the 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

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

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

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

Table 2: Coefficients and 95% credible intervals for the analysis of courtship latency. Higher values indicate increased time for the focal male to begin courting the available female in the behavioural trial. Categorical variables show deviations from the reference group (isolated for social 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

We found no significant effects of our experimental manipulations on female decision time (Table 3). Females mounted males only after courtship had been initiated (Figure 4).

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

Discussion

A male's decision to invest in sexual signalling may be informed by both his energy budget and his experience of the social environment. Few studies have manipulated an animal's resource acquisition and social experience simultaneously, to assess their relative importance in mating interactions. Here, we find that the recent social environment plays a large role (seemingly larger than that of diet) in determining how quickly male *G. sigillatus* begin courting an available female. Males that were housed recently with another male began courting the female earlier, although this effect was strongest when the other 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

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

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Data Accessibility

Analyses reported in this article can be reproduced using the data provided by 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

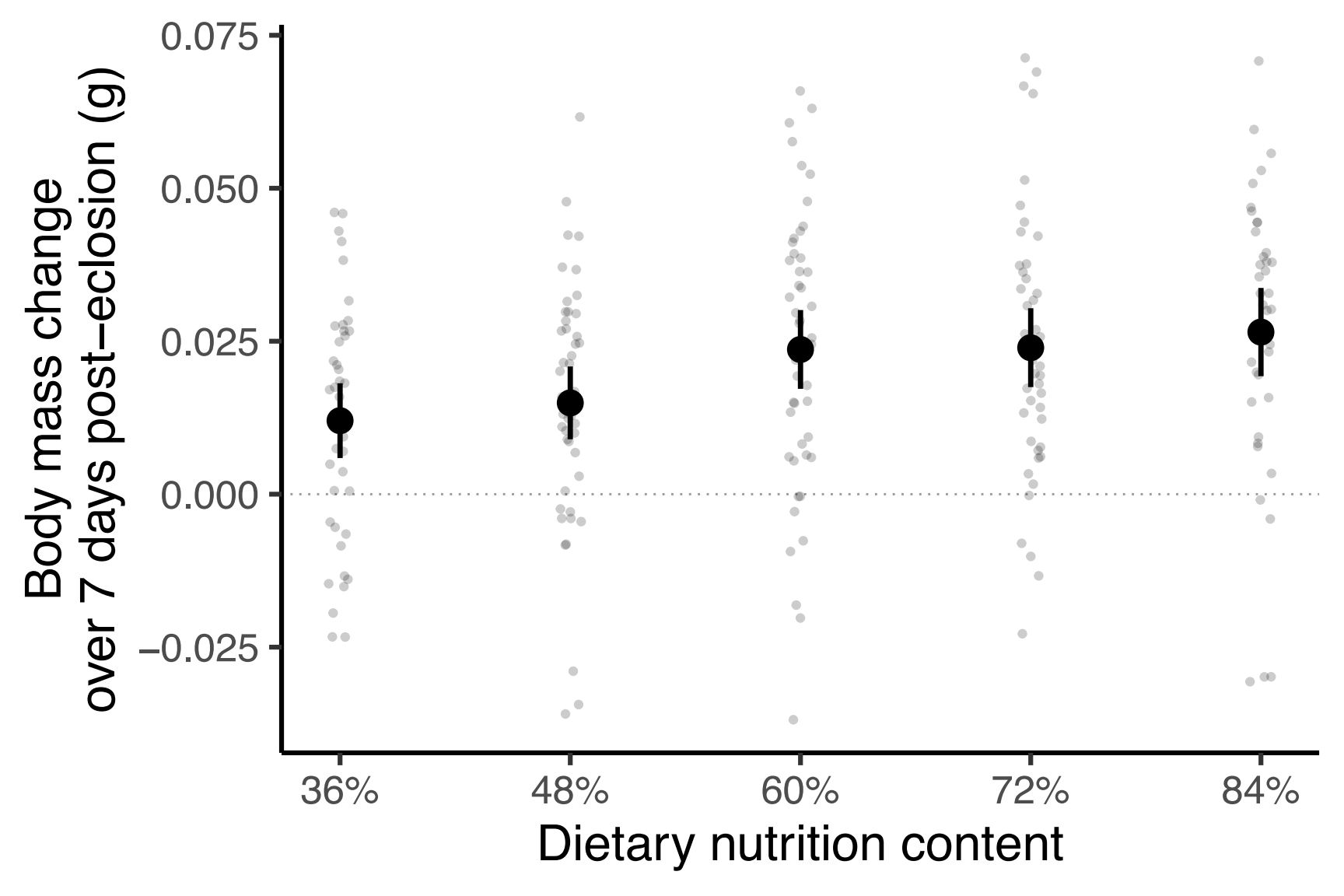
666 Figure 4: Males were only mounted after they started actively courting females,
667 shown by the positive relationship between (log-transformed) latency to male
668 calling (x-axis) and (log-transformed) total time to mating (i.e., calling latency +
669 mounting latency; y-axis). Open circles indicate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

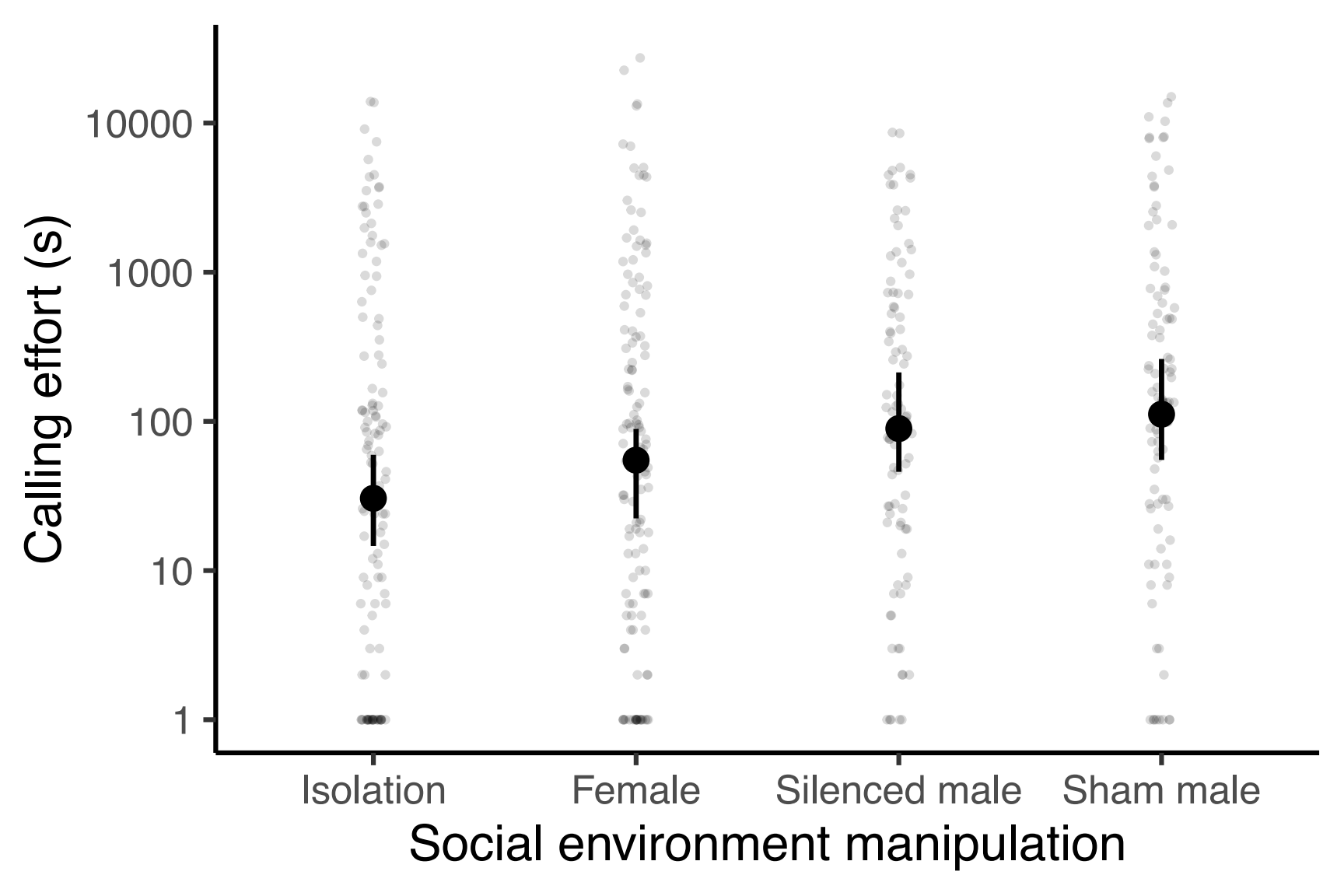
but were not mounted by the female (and were assigned a censored score of 1800 s for latency to mount). Dotted line shows the 1:1 relationship between latency to call and total time to mating. Males that did not call were not mounted, and are not shown.

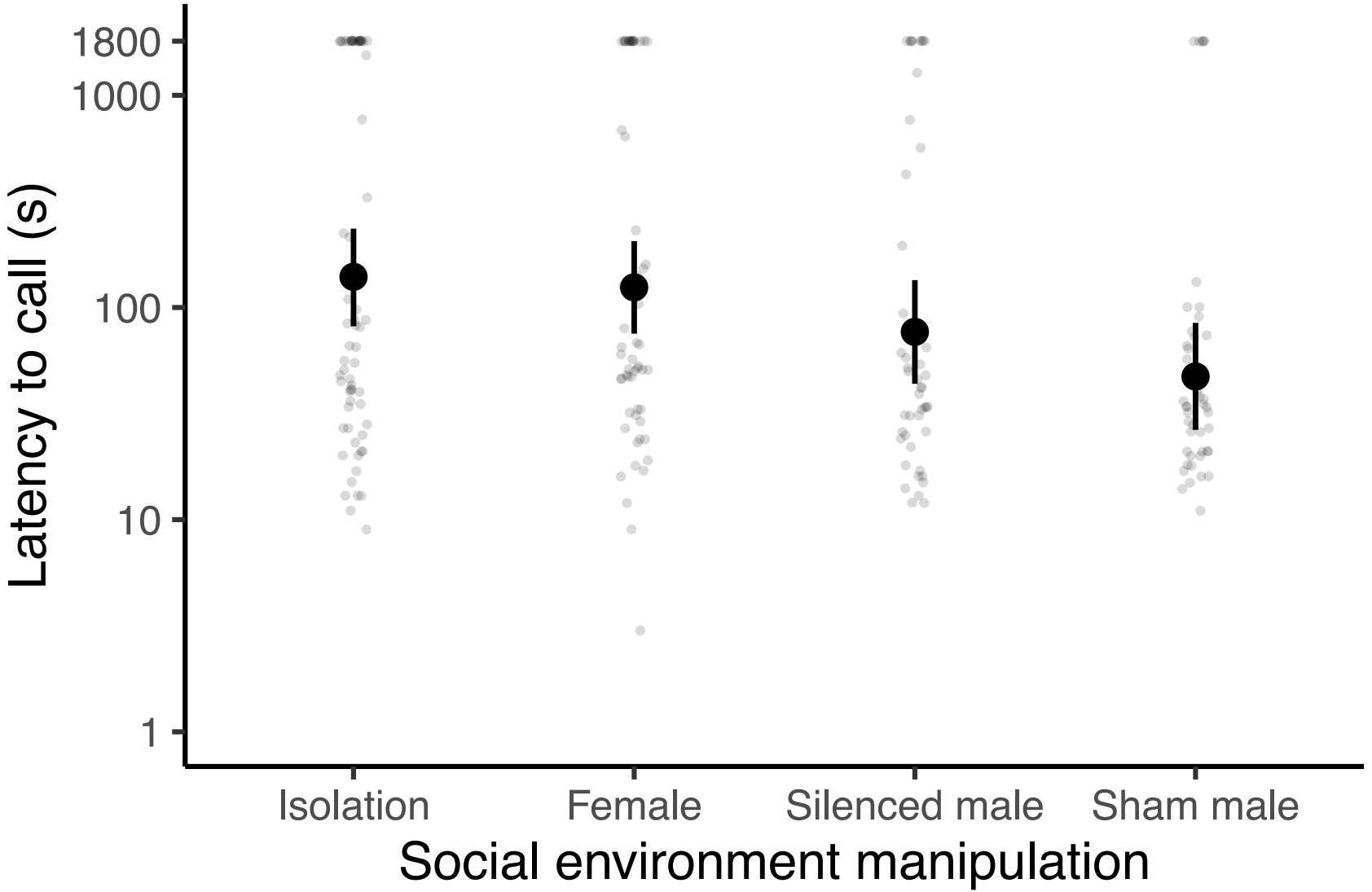
Supplementary material

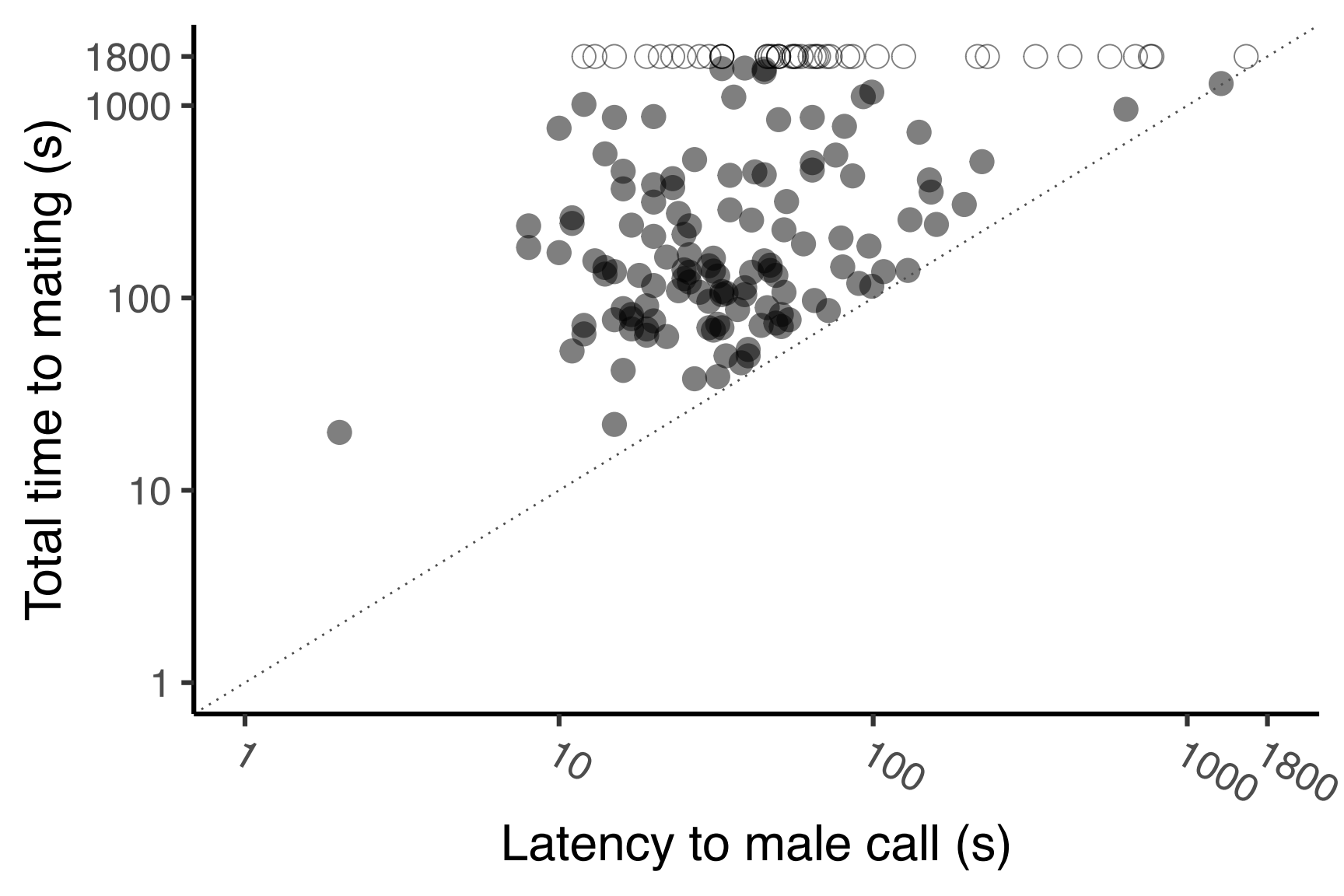
Table S1: Sample sizes of males completing the experiment across each combination of social manipulation, female mating status and dietary nutrition.

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


Body mass change
over 7 days post−eclosion (g)
0.075
0.050
0.025
0.000
−0.025
36%
48%
60%
72%
84%
Dietary nutrition content


Calling effort (s)
10000
1000
100
10
1
Isolation
Female
Silenced male
Sham male
Social environment manipulation


1800
1000
100
10
1
Latency to call (s)
Isolation
Female
Silenced male
Sham male
Social environment manipulation


1800
1000
100
10
1
Total time to mating (s)
1
10
100
1000
1800
Latency to male call (s)