Pre-maturation social experience affects female reproductive strategies and offspring fitness in a highly polyandrous insect

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Data availability The datasets of this study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Abstract

The pre-maturation social environment experienced by females may affect their post-maturation reproductive strategies, including mating preferences and investment in offspring. Whether the pre-maturation social environment also affects other aspects of females' reproductive strategies, such as the degree of polyandry and post-copulatory decisions, is still an open question. To address this question, we performed laboratory experiments using the cricket *Teleogryllus commodus*, a highly polyandrous species. Previous studies showed that juvenile females reared in an acoustic environment with recorded male calls with different rates (variable-quality, VQ) are more responsive to high-quality calls than females reared in an environment with recorded male calls with only high rates (high-quality, HQ). We exposed juvenile females to these same two acoustic environments and estimated their degree of polyandry, offspring fitness, and time of spermatophore retention. We found that the juvenile acoustic environment did not change a female's mating rate, indicating that the higher responsiveness shown in a previous study does not translate into higher degree of polyandry. An increased number of mates reduced offspring fitness, suggesting that there is an optimum number of mates for females. Finally, females from the VQ group retained spermatophores for shorter periods and produced higher quality offspring when mated with high-quality males, suggesting that the pre-maturation acoustic environment interacts with the quality of the males to determine post-copulatory female decisions and eventually offspring fitness. Taken together, our results indicate that both the pre- and post-mating strategies of females are subject to socially induced plasticity.

Keywords

Acoustic environment, Crickets, Offspring quality, Female choice, Polyandry, Socially induced plasticity.

Introduction

According to the Darwin-Bateman paradigm, only the reproductive success of males increases with mating number (Dewsbury 2005). However, there is growing evidence that polyandry can improve female reproductive success, independently if males provide nutritious gifts to the females during sexual interactions or if the benefits acquired by multiple mating are solely genetic (Arnqvist and Nilsson 2000; Slatyer et al. 2012). In fact, polyandry is widespread in nature, with great intra- and interspecific variation in the number and degree of polyandrous females (Taylor et al. 2014). This raises the questions of why variation in the frequency of polyandry exists, and which factors explain this variation. Studies focusing on proximate explanations for polyandry may improve the understanding of the ultimate causes and consequences of the variation in females' reproductive behavior. Such an understanding is important to be able to identify which factors modify females' remating decisions and offspring investment, and how polyandry modifies the strength of selection acting on individuals.

Like any phenotypic trait, polyandry itself can be determined by additive or interactive effects of genes and environmental conditions (Cornwallis and Uller 2010). Studies with invertebrates, for instance, demonstrated that the number of males accepted by females as mating partners may be heritable, but the interspecific variation in the heritability of this trait was quite high (from 0.41 to 0.73; e.g., Solymar and Cade 1990; Torres-Vila et al. 2001, 2002), indicating that environmental conditions still play an important role in a female's likelihood to remate. In fact, population density is regarded as one of the most important environmental conditions to predict the number of males a female will accept as mating partners (i.e., her degree of polyandry), irrespective of genetic tendencies (Taylor et al. 2014). A female' degree of polyandry can also be affected by both the attractiveness of potential mating partners (Rebar et al. 2011) and her previous social experience with conspecific males (Verzijden et al. 2014; Williams et al. 1992). The effects of social experience on females' reproductive behavior may start even in the pre-maturation period, as the

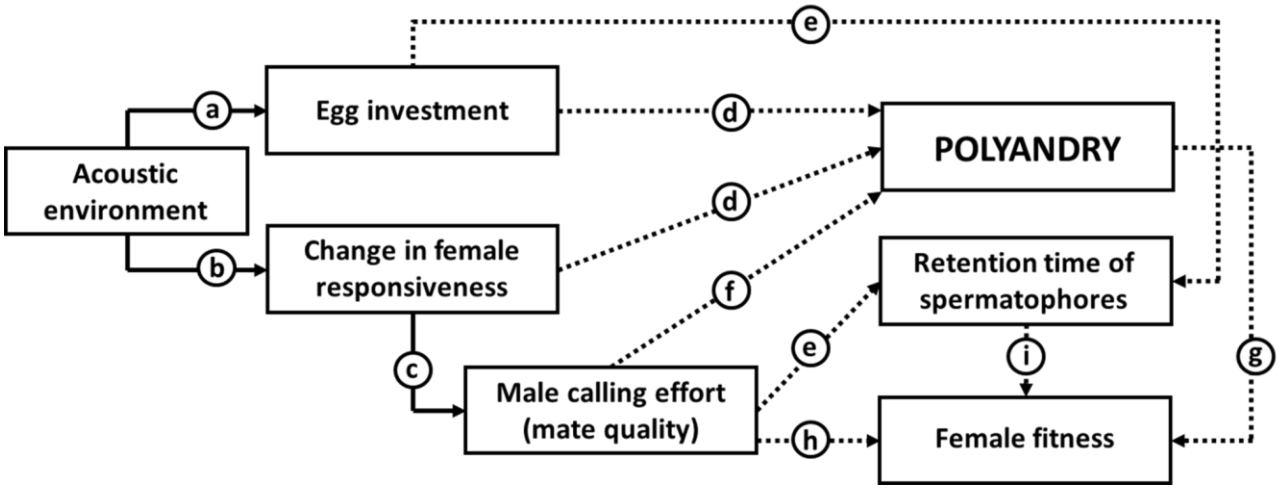
perceived quality and availability of males may affect females' sexual preferences (Hebets et al. 2007; Kasumovic et al. 2012) and their investment in offspring after maturity (Cunningham and Russell 2000; Kasumovic et al. 2011). Whether the pre-maturation social experience of females predicts polyandry, however, is still unknown.

The main goal of this study is to explore the role of both pre- and post-maturation experience on females' reproductive strategies, thereby shedding light on a new mechanism that may influence polyandry. To explore this question, we used the Australian black field cricket *Teleogryllus commodus*, a species in which females are highly polyandrous under both field (Evans,1988) and laboratory conditions (Hunt et al. 2005; Jennions et al. 2007). According previous experimental studies, there is no experimental evidence that polyandry in *T. commodus* increases offspring quality (Jennions et al. 2007) or offspring number (Loher and Edson 1973), but may be necessary to guarantee the fertilization of all eggs (Loher and Edson 1973). It is important to stress, though, that previous studies on the benefits of polyandry in *T. commodus* offered up to four mating partners to females (Jennions et al. 2007) and we know that females can copulate as much as 10 times in experiments that simulate the natural habitat of the species (Loher and Edson 1973). Thus, it is possible that the limited number of males offered to females in previous studies about polyandry may have underestimated the positive effects of multiple mating in *T. commodus*.

Despite being polyandrous, *T. commodus* females are choosy and strongly prefer males that have a high calling effort (Bentsen et al. 2006), probably because this trait is related with male quality, such as body condition (Hunt et al. 2004) and immunocompetence (Simmons et al. 2005). Moreover, male calls experienced by females in early life stages (i.e., pre-maturation) seem to be important for females' reproductive strategies as adults. Females reared in an acoustic environment with variable-quality male calls present a faster antepenultimate development rate, express genes associated with energy producing pathways, and have higher egg investment when compared with females reared in a silent environment (Kasumovic et al. 2011, 2016; Fig. 1A). These responses

indicate that the pre-maturation acoustic environment experienced by females affects their post-maturation reproductive strategies.

Previous studies with *T. commodus* also showed that females reared in an acoustic environment with variable-quality male calls are more responsive to high-quality male calls when adults compared with females reared in environments with only low- or only high-quality male calls (Kasumovic et al. 2012; Fig. 1B-C). As responsiveness to a courting male is a measure of motivation to mate (Edward 2015), an increase in responsiveness may promote an increase in the number of males accepted as mates. Thus, we predict that females reared in an acoustic environment with male calls of variable-quality will present higher degrees of polyandry as a result of their high responsiveness to males and/or to guarantee the fertilization of a larger number of eggs produced (Fig. 1D). We also predict that this effect will be stronger if adult females meet more males that express high-quality calls (Fig. 1F). Although the pre-maturation acoustic environment does not affect females' mating preference (Kasumovic et al. 2012), we explored whether a female's post-copulatory preference is a socially induced plastic trait. If so, we expect that females reared in different acoustic environments will show differences in the retention time of spermatophores (Fig. 1E), which is regarded as form of cryptic female choice in crickets (Bussière et al. 2006). Finally, given that males capable of expressing high-quality calls (i.e., attractive males) are in better condition (Hunt et al. 2004), we tested if the number and quality of the accepted males (Fig. 1H) or the interaction between the acoustic environment experienced by females and the number and quality of the accepted males determine offspring quality (Fig. 1G, I). Assuming that post-copulatory processes may also influence offspring quality, we tested whether male quality is positively related with the retention time of his spermatophore, and whether differences in this retention time affect offspring quality (Fig. 1E, I).



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125 **Fig. 1** Factors that may influence females' reproductive strategies in the cricket *Teleogryllus*
126 *commodus*. We know (solid lines) that when females are reared in an acoustic environment with
127 variable-quality male calls they show (A) higher egg investment and (B-C) higher responsiveness to
128 males with high calling effort than females reared in an acoustic environment in which the quality
129 of male calls is non-variable. Here we tested (dashed lines) six predictions. Females reared in an
130 acoustic environment with variable-quality male calls will show (D) higher degree of polyandry and
131 (E) higher retention time of spermatophores of high quality males (i.e., post-copulatory preference)
132 than females reared in an acoustic environment with non-variable male calls, as a result of their
133 high responsiveness to males and/or to guarantee the fertilization of the larger number of eggs
134 produced. (F) The effect of the acoustic environment will be stronger if adult females meet more
135 males that express high calling effort. (G) The number and (H) quality of males accepted as mates
136 will interact with the acoustic environment to determine offspring quality. Finally, (I) male quality
137 will be positively related with the retention time of his spermatophore, which in turn will affect
138 offspring quality.

139

Methods

Preparation of individuals

We used *T. commodus* individuals from a stock established in the laboratory from 200 individuals collected in March 2016 at Smiths Lake (32º22'S, 152º30'E), New South Wales, Australia, and consistently maintained at 200 or more breeding individuals in each generation. The individuals used in our study were reared in communal tubs with *ad libitum* food (Friskies Go-Cat senior) and water, separated in tubs according to life stage (i.e., early hatched nymphs, juveniles, and adults). Once a week, we checked the tub containing juveniles and removed all females in their penultimate nymphal instar (i.e., individuals with a small ovipositor visible) and all males in last nymphal instar (i.e., individuals with visible wing buds but without an ovipositor). We placed all individuals removed from the stock in individual plastic containers (5 x 5 x 3 cm) with food and water. The individual containers were checked daily to verify if males molted to maturity and if females molted to their last nymphal instar. All individuals were kept on a reverse 13:11 light cycle with night occurring between 11:00 and 21:00 hours to allow for mating experiments to occur during the day.

When females molted to their last nymphal instar, we randomly assigned them to one of two experimental groups (see below), each one located in a different room. We switched experimental treatments between rooms each day to minimize any possibility of room effects. We measured females' thorax width (mm) and body weight (mg) in two moments: when they molted to their last nymphal instar and when they molted to maturity. These measures were used to estimate female condition (see 'Statistical analyses' below) and to ensure that there was no initial difference in body size of females that would be later allocated to each experimental group.

When males became adults, they were placed in boxes with microphones connected in a computer system (hereafter 'call box') where males remained unless they were used in a mating experiment in that day. The call box recorded the number of seconds that each male spent calling between 11:00 and 21:00 hours. As male calling effort is a sexually selected trait under directional

female mate choice, more attractive males have more continuous calls and have a lower intercall interval (Brooks et al. 2005). As a result, we used a male's mean daily calling effort as a proxy for his quality or attractiveness. The days in which males were used in mating experiments were not included in this average. In total, the mean (± SD) number of days each male was recorded was 34 ± 17.2, which comprises more than 90% of the males' adult lifespan (pers. obs.).

We reared *T. commodus* females in their last nymphal instar in one of two acoustic environments (experimental groups): one composed only of high-quality (HQ) male calls and another composed of variable-quality (VQ) male calls. The sound track used in the HQ group was composed of calls from three different males with low intercall duration (three high-quality male calls), whereas the sound track used in the VQ group was composed of calls from one high-quality, one medium-quality, and one low-quality male call according the intercall duration of male calls (see Supplementary Material S1 for further details). In both experimental groups, each male soundtrack was repeated sequentially and broadcasted from 12 speakers placed in a 1-meter diameter circle with the individual plastic containers containing the experimental females placed in the center. The choice of the two experimental groups was based on previous results with *T. commodus*, according to which females reared either in a HQ or in a VQ calling environment showed the greatest differences in mating behavior as adults (Kasumovic et al. 2011).

Mating trials

To test our predictions, we conducted mating trials with mature virgin females (N = 50) of both experimental groups (HQ and VQ). To ensure that all individuals would be responsive to stimuli from the opposite sex, we only used adult males and females that were with at least 5 and 10 days of age, respectively. In a different room from that where females were reared, we presented a sequence of 10 different males to each female in two subsequent days (five males per day). The mean (± SD) age of experimental virgin females used in the mating trials was 23.7 ± 9.9 days as

adults. The 10 males used in the mating trials of each female were randomly sampled from experimental males previous placed in the calling boxes (N = 83), and the order and identity of male presentation to each female was also randomized. The mean (± SD) age of experimental males used in mating trials was 20.6 ± 12.3 days as adults.

As our goal was to explore mating frequency as a consequence of the juvenile acoustic environment and not mate attraction as a consequence of long-distance calls, in each trial a randomly sorted male was placed inside a female's individual plastic container where the pair was allowed to interact. The mating process of *T. commodus* usually consists of three main steps: (1) the male sees a female and starts the courtship calling, (2) the female approaches the calling male, and (3) the male turns his back to the female, allowing her to mount on him (Loher and Rence 1978; pers. obs.). Thus, we scored a trial as invalid when males remained silent for 5 min (N = 71 trials) because we assumed they were not willing to mate. In this case, we replaced the silent male by another randomly sorted male. In all trials in which the males called, we scored a mating as successful if: (a) the female remained motionless on top of the male for at least 5 seconds, and (b) the male successfully transferred his spermatophore to the female (following Bussière et al. 2006). We disregarded any mating trial in which a female successfully climbed on top of a male, but the male did not transfer a spermatophore. In such cases of mate failure (N = 22), a new male was offered to the female after 10 min. Finally, when the mating was successful, males were removed immediately after spermatophore transfer to avoid any male interference in female attempts to remove the spermatophore (Hall et al. 2010). If a male started to call but the female did not mount him until 5 min, we considered it a rejection. Due to the large number of males required for this study, we used males more than once, but never with the same female. However, each male was used only once per day to ensure that they had sufficient time (at least 24 h) to produce another spermatophore (Hall et al. 2008).

During pilot experiments, some females maintained spermatophores attached for more than 3 hours (pers. obs.). Thus, if mating was successful, we waited a maximum of 80 min until spermatophore removal by the female, since it takes an average of 68 min for all sperm within spermatophore to be transferred to female's reproductive tract (Loher and Rence 1978). If the female removed the spermatophore before 80 min, we waited 10 min after spermatophore removal to offer the next male. Otherwise, given that females remain receptive even with a spermatophore attached to them (pers. obs.), we still offered the next male after 80 min even if the spermatophore of the previous male was not removed. At the end of the mating trials, we recorded the final number of males each female accepted, the identity of these males, and the time that each female retained each spermatophore (hereafter 'retention time'). It was not possible to record data blind because our study involved focal animals in laboratory.

Offspring fitness

After the mating trials, females were maintained in their same individual plastic containers (5 x 5 x 3 cm) with food and a small Petri dish filled with moist cotton where they laid eggs (hereafter 'egg pads'). Once a week, each egg pad was replaced by a new one to avoid fungus proliferation. All egg pads removed from each individual female's container were placed in a plastic container (18.6 x 13.6 x 11.5 cm) (hereafter 'egg container'). Each egg container was checked once a week, when the number of hatched nymphs was counted, and the total weight of all nymphs was measured using an analytical balance (0.1 mg). We discarded the egg container once no new nymphs hatched for two weeks in a row.

To explore how females invested in offspring, we regressed total nymph mass on total number of nymphs produced by each female. Given that the relationship between these two variables is linear, we can use the residuals of the regression to explore whether females were shifting their investment in offspring number or offspring quality (i.e., individual offspring mass).

For the regression, we used total nymph number and total nymph mass produced during a female's lifetime. Positive residual values indicate that total nymph mass was higher than predicted by the total nymph number, and we interpreted it as increased investment into individual offspring (i.e., high offspring fitness). Negative residual values, in turn, indicate that the total nymph mass was lower than predicted by the total nymph number, and we interpreted it as decreased investment into individual offspring (i.e., low offspring fitness). We did not use the ratio between offspring mass and offspring number as a proxy of offspring quality because, in addition to other problems, ratios promote a substantial widening of the sampling variation compared to that of the original variables (Jasieński and Bazzaz 1999).

Statistical analyses

We first used linear models and generalized linear models (depending on the error distribution of the response variable) to test if the following female traits showed any difference between experimental groups (HQ and VQ): adult thorax width, adult total weight, weight increase (with Gaussian error distribution) and thorax width increase between the last nymphal instar and when they molted to maturity (with negative binomial error distribution), and time to maturity during calling treatment (with Poisson error distribution). We also used a linear model to test if a male's calling effort varied because of the treatment of the females they mated with. All tests were non-significant (see Supplementary Material S2), indicating that there was no initial bias in the experimental groups.

We fitted generalized linear models to test if females reared in the VQ acoustic environment presented higher degree of polyandry when compared with females reared the HQ acoustic environment (Fig. 1D), and if the effect of the juvenile acoustic environment was stronger when adult females met more males that expressed a high level of calling effort (Fig. 1F). We used the number of males accepted by each female (degree of polyandry) as response variable, with a

Poisson distribution of errors. As predictor variables, we used the interaction between the experimental group (categorical) and the average quality (mean daily calling effort, see above) of all males offered to each female during mating trials (continuous), regardless of whether they were accepted or rejected (i.e., quality of *actual* and *potential* mates).

We implemented generalized linear mixed-effects models to test if females reared in the VQ acoustic environment showed a higher retention time of spermatophores when compared with females reared in the HQ acoustic environment and if the quality of males accepted by females as mates was positively related with the retention time of their spermatophores (Fig. 1E). We used the retention time of spermatophores of each mating (in seconds) as the response variable (with a negative binomial distribution of errors), and the identity of each female as random factor. As predictor variables, we used the experimental groups, male quality, and order in which each male was offered to the females. In this model, we included the interaction between experimental group and the mean quality of the actual mates, and the additive effect of mating order.

We implemented two models to test if the acoustic environment interacts with the quality of the males accepted as mates, with the total retention time females spent with males' spermatophores, and with the degree of polyandry to determine female fitness (Fig. 1G-I). First, we fitted a generalized linear model with the total nymph number (i.e., offspring number) produced by each female as response variable (with a negative binomial distribution of errors). Second, we fitted a linear model with the residuals of the regression between total nymph number and total nymph mass of each female (i.e., offspring quality) as response variable (with a Gaussian distribution of errors). As predictor variables of both models, we used the experimental groups (i.e., acoustic environment) and its interaction with the average quality of the mates accepted by each female (i.e., average quality of *actual* mates), the total retention time of males' spermatophores, and the degree of polyandry presented by females. We also run two linear models in which offspring quality was the response variables and female body mass or female condition (i.e., the residuals of a regression

289 between female size and female mass) were the predictor variable. Given that offspring quality was
290 not influenced either by female body mass or female condition (Supplementary Material S3), we
291 did not include these two variables in the models on female fitness.

292 We used the *lme4* package of the software R version 3.4.3 (R Development Core Team 2017)
293 to implement all models. To make the coefficients of the predictor variables in each analyses
294 comparable, we standardized the values of the predictor variables using the function *scale*
295 (Schielzeth 2010).

296

297 **Results**

298 **Degree of polyandry**

299 The median number of males accepted as mates was 8 in the HQ group and 6 in the VQ group, with
300 great variation in both groups (Fig. 2). None of variables tested in the model explained the degree of
301 polyandry presented by females (Table 1).

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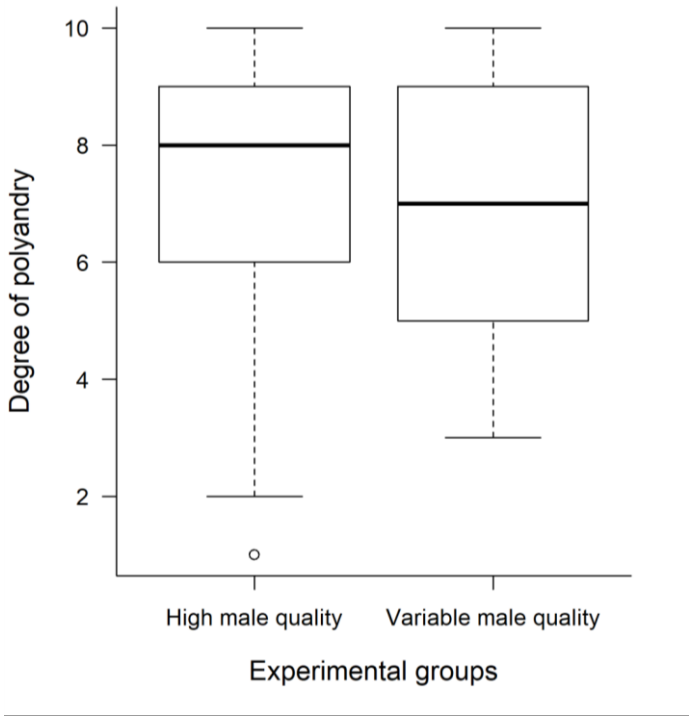

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304 **Fig. 2** Total number of males accepted by females (degree of polyandry) of the Australian black
305 field cricket *Teleogryllus commodus* reared in the two experimental groups (i.e., acoustic
306 environment): high-quality and variable-quality male calls. Boxplots represent medians, first and
307 third quartiles, whiskers indicate 1.5 times quartiles' values and the circle is an outlier.

308

Table 1 Results of the model that tested the effect of experimental group (i.e., acoustic environment) and mean quality of potential mates on the final number of males accepted by females (i.e., degree of polyandry). We present the estimated effects of each predictor, the standard deviation (SD), and the values of *z* and *p*.

Predictors	**Estimate**	**SD**	***z*-value**	***p*-value**
Mean male quality	-0.061	0.061	-1.012	0.311
Experimental group	-0.124	0.111	-1.117	0.264
Experimental group × Mean male quality	0.129	0.125	1.034	0.301

Retention time of spermatophores

The mean (± SD) time that females spent until spermatophore removal (i.e., retention time) was 1,308.8 ± 1,409.2 seconds in the HQ group and 999.5 ± 1,264.7 seconds in the VQ group. Both the mating order and experimental group significantly predicted spermatophore retention time (Table 2). There was a negative effect of mating order on the retention time for both groups, but the effect was consistently higher for females from the VQ group when compared with females from the HQ group (Fig. 3).

Table 2 Results of the model that tested the effect of experimental group (i.e., acoustic environment), male calling effort (i.e., male quality), and order in which each male was offered on the retention time of spermatophores by females. We present the estimated effects of each predictor, the standard deviation (SD), and the values of *z* and *p*. Significant *p*-values are highlighted in bold.

Predictors	Estimate	SD	*z*-value	*p*-value
Experimental group	-0.384	0.187	-2.051	**0.040**
Male quality	-0.010	0.098	-0.103	0.918
Mating order	-0.124	0.022	-5.668	**<0.001**
Experimental group × Male quality	0.206	0.132	1.555	0.120

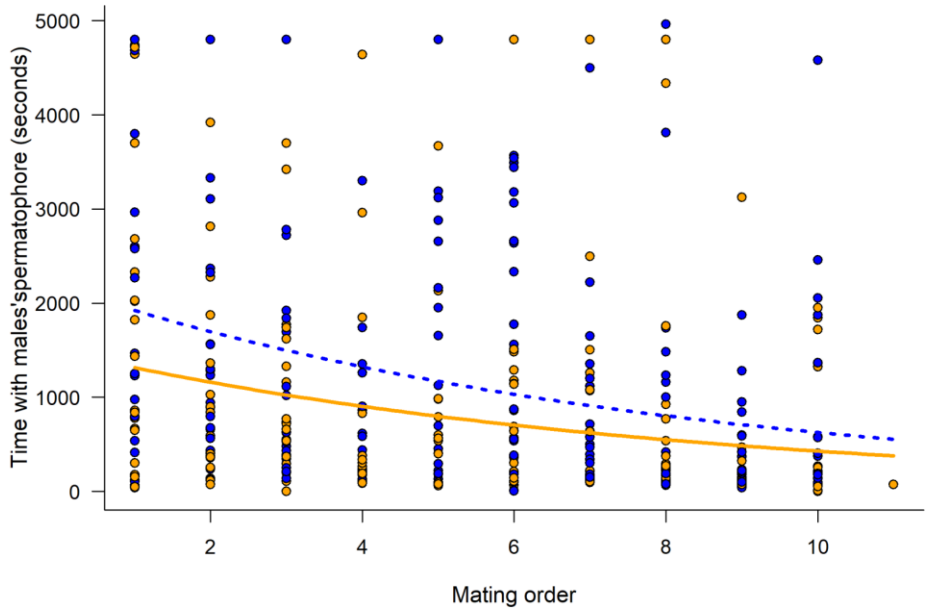


Fig. 3 Effect of mating order on the retention time of spermatophores by females of the Australian black field cricket *Teleogryllus commodus*. Coefficients used to build the graphic were extracted from the model (see Table 2). Blue circles and the blue-dashed line represent females from the high-quality group (intercept = 7.686); orange circles and the orange-solid line represent females from the variable-quality group (intercept = 7.306). The slope of both experimental groups is -0.124 (95%CI: -0.168, -0.081).

Female fitness

The median offspring number produced by females was 250.0 ± 197.6 nymphs in the HQ group and 298.9 ± 157.8 nymphs in the VQ group (Fig. 4). None of the variables used in the model explained the offspring number produced by females (Table 3). The mean residuals of the regression between total nymph number and total nymph mass produced by each female (i.e., offspring quality) was 0.267 ± 0.214 g in the HQ group and 0.316 ± 0.162 g in the VQ group. There was an additive effect of the degree of polyandry and total retention time of spermatophore on offspring quality (Table 4). Offspring quality decreased with an increase in polyandry (Fig. 5A), while it increased with an increase in spermatophore retention time (Fig. 5B). There was also an interaction between mean quality of the actual mates and the experimental group (Table 4). Offspring quality increased with male quality in the VQ group, while it decreased with male quality in the HQ group (Fig. 6).

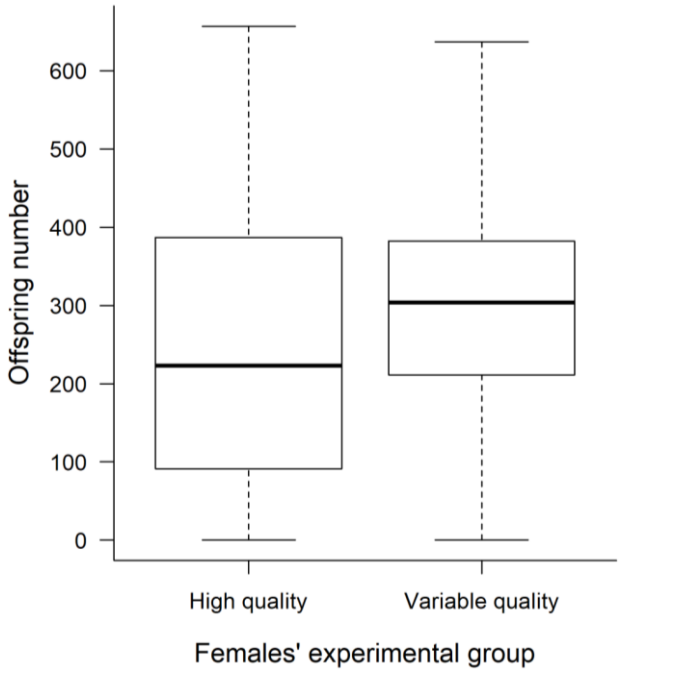


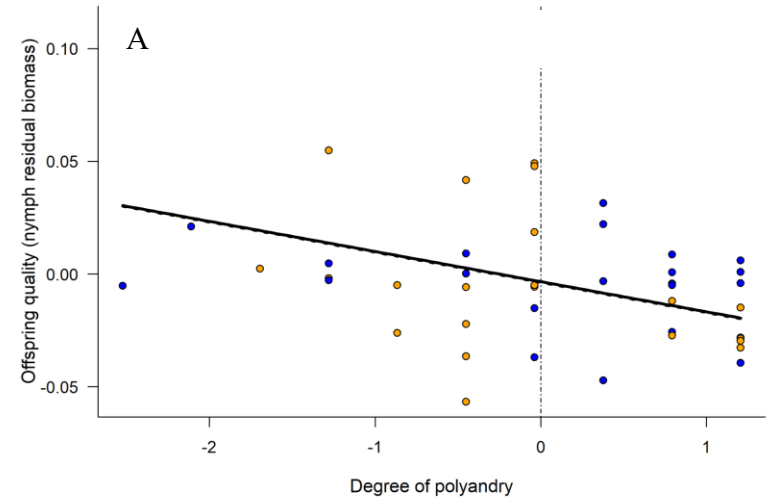
Fig. 4 Total number of nymphs produced by females of the Australian black field cricket *Teleogryllus commodus* during their lifetime (i.e., offspring number) in two experimental groups (i.e., acoustic environments): high-quality and variable-quality male calls. Boxplots represent medians, first and third quartiles and whiskers indicate 1.5 times quartiles' values.

Table 3 Results of the model that tested the effect of experimental group (i.e., acoustic environment), mean male calling effort (i.e., mean male quality), degree of polyandry, and total retention time of spermatophores on total nymph number produced by females (i.e., offspring number). We present the estimated effects of each predictor, the standard deviation (SD), and the values of *t* and *p*.

Predictors	**Estimate**	**SD**	***t*-value**	***p*-value**
Mean male quality	-0.041	0.177	-0.232	0.818
Experimental group	0.244	0.237	1.030	0.310
Degree of polyandry	0.239	0.135	1.765	0.086
Retention time	-0.051	0.134	-0.380	0.706
Male quality × Experimental group	-0.086	0.264	-0.326	0.747
Male quality × Degree of polyandry	-0.228	0.148	-1.544	0.131
Male quality × Retention time	0.072	0.129	0.557	0.581

Table 4 Results of the model that tested the effect of experimental group (i.e., acoustic environment), mean male calling effort (i.e., mean male quality), degree of polyandry, and the total retention time of spermatophores on the residuals of the regression between total nymph number and total nymph mass of each female (i.e., offspring quality). We present the estimated effects of each predictor, the standard deviation (SD) and the values of *t* and *p*. Significant *p*-values are highlighted in bold.

Predictors	**Estimate**	**SD**	***t*-value**	***p*-value**
Mean male quality	-0.012	0.007	-1.725	0.093
Experimental group	< 0.001	0.009	0.031	0.975
Degree of polyandry	-0.013	0.005	-2.513	**0.016**
Retention time	0.014	0.005	2.640	**0.012**
Male quality × Experimental group	0.025	0.010	2.417	**0.021**
Male quality × Degree of polyandry	-0.002	0.006	-0.422	0.676
Male quality × Retention time	0.007	0.005	1.346	0.186



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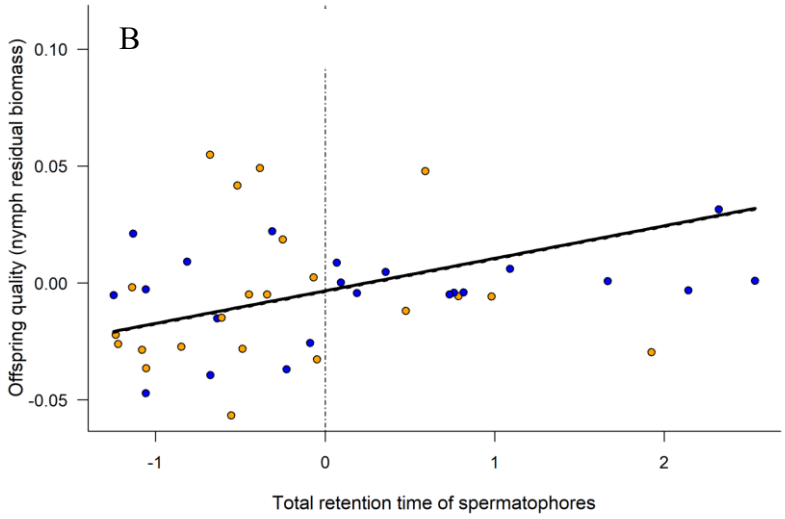

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372 **Fig. 5** Effect of (A) degree of polyandry and (B) total retention time of spermatophores (both
373 standardized) on offspring quality, measured as the residuals of a linear regression between nymph
374 number and nymph mass produced by females of the Australian black field cricket *Teleogryllus*
375 *commodus*. Blue circles represent females from the high-quality group and orange circles represent
376 females from the variable-quality group. The coefficients used to build the graphic were extracted
377 from the model (see Table 4). (A) Intercept = -0.004, slope [95%CI] = -0.013 [-0.024, -0.003], (B)
378 Intercept = -0.004, slope [95%CI] = 0.014 [0.003, 0.025].

379

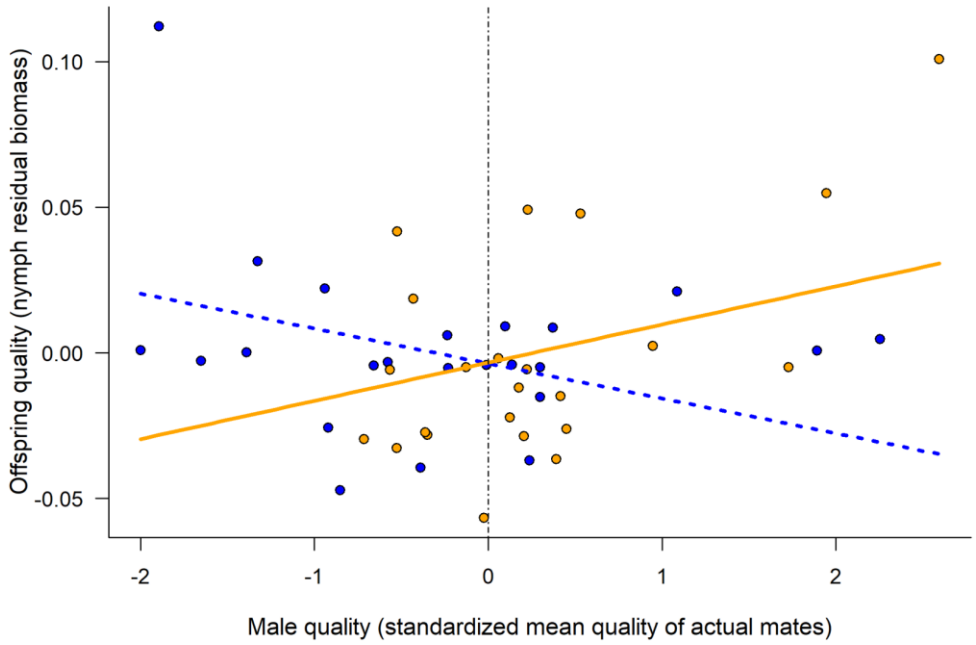

Fig. 6 Effect of standardized mean quality of actual mates and experimental group (i.e., acoustic environment) on offspring quality, measured as the residuals of a linear regression between nymph number and nymph mass produced by females of the Australian black field cricket *Teleogryllus commodus*. Coefficients used to build the graphic were extracted from the model (Table 4). Blue circles and blue-dashed line represent females from the high-quality group (intercept = -0.004, slope [95%CI] = -0.012 [-0.017, 0.009]); orange circles and orange-solid line represent females from the variable-quality group (intercept = -0.003, slope [95%CI] = 0.013 [0.004, 0.046]).

Discussion

Our results show that the influence of the acoustic environment on female reproductive strategies is more complex that we expected. Contrary to our predictions (Fig. 1D, F), females reared in the acoustic environment with variable-quality male calls did not show a higher degree of polyandry than females reared in an acoustic environment with only high-quality male calls, regardless of the mean quality of the mates they find as adults (Fig. 2). Also contrary to our predictions (Fig. 1E), the quality of the males accepted as mates did not influence the retention time of their spermatophores, and females reared in an acoustic environment with variable-quality male calls spent less time with males' spermatophores when compared with females reared in an acoustic environment with only high-quality male calls (Fig. 3). The more matings a female had, the lower her spermatophore retention time in both experimental groups (Fig. 3). The retention time of spermatophores had a positive effect on offspring quality, but it was not related with male quality, as we expected (Figs. 1E and 5B, Table 4). Polyandry, however, reduced offspring quality (Figs. 5A) for females from both experimental groups, which is not in accordance with our prediction (Fig. 1G). Finally, the offspring number produced by females from both groups did not differ (Fig. 4), but only females reared in an acoustic environment with variable-quality male calls produced higher quality offspring when mated with high-quality males (Fig. 6). This finding supports the prediction that the quality of males accepted as mates by females interacts with the acoustic environment experienced by these females to determine offspring quality (Fig. 1H).

Although *T. commodus* females are highly sensitive to acoustic stimuli from conspecific males during development, increasing their responsiveness to high-quality males (Kasumovic et al. 2012), the pre-maturation acoustic environment experienced by females did not influence their degree of polyandry. In several animal species, including guppies (Brooks and Endler 2001), fruit flies (Ritchie et al. 2005), and birds (McGlothlin et al. 2004), more responsive females are also the ones that exhibit stronger mating preferences (i.e., discrimination). However, a previous study with

414 the closely related cricket *T. oceanicus* showed that there is not necessarily a link between
415 responsiveness (i.e., the motivation to mate *sensu* Edward 2015) and a female's mating preferences
416 (Bailey 2008). In the case of *T. commodus*, females reared in a variable-quality acoustic
417 environment are known to be more responsive to high-quality males, but there is no change in their
418 mating preferences as adults (Kasumovic et al. 2012), which suggests that responsiveness and
419 discrimination are also not linked in this cricket species. Moreover, the results of our experiment
420 show that females reared in a variable-quality acoustic environment do not increase their degree of
421 polyandry when they find a high number of high-quality males as adults. Based on this finding, we
422 refute the notion that polyandry may be driven by increased responsiveness to high-quality males.
423 Thus, although an increase in responsiveness decreases the latency until copulation, it does not
424 translate into a higher number of mates accepted by females.

425 Previous studies on the role of polyandry in *T. commodus* have already shown that the number
426 of males a female accepts as mates does not increase offspring quality (Jennions et al. 2007) or
427 offspring number (Loher and Edson 1973). These studies, however, offered up to four males to each
428 female, raising the question of whether the limited number of males may have underestimated the
429 positive effects of polyandry in *T. commodus*. Considering that females can copulate as much as 10
430 times in captivity (Loher and Edson 1973), we offered females in our experiment a larger number of
431 males. In accordance to previous studies, we found that a higher degree of polyandry had no effect
432 on number of nymphs produced by females. Therefore, our finding provides additional evidence
433 against the suggestion that polyandry in *T. commodus* is a strategy to guarantee the fertilization of a
434 larger number of eggs (Loher and Edson 1973). Surprisingly, we also found that a large number of
435 males accepted as mates has a negative effect on offspring quality. This finding suggests that,
436 regardless of any possible benefit females may derive from polyandrous mating, there seems to be
437 an optimum mate number, above which offspring fitness is negatively affected (see discussion in
438 Arnqvist and Nilsson 2000).

Given the cost related to the high degree of polyandry reported here, the reason why *T. commodus* females accept several males as mates remains unanswered and deserves further investigation. One possible explanation may be the fact that *T. commodus* females need to receive, via males' spermatophore, a complex of compounds responsible for the synthesis of prostaglandins, which are necessary to stimulate mating-induced egg release (Loher et al. 1981). Although male calls are honest indicators of their body condition and immunocompetence (Hunt et al. 2004; Simmons et al. 2005), it is unknown whether male calls provide clues or are somehow related with the amount of prostaglandin-synthesizing compounds present in their spermatophores. A recently meta-analysis that encompassed 21 animal species showed no relationship between male traits under pre-copulatory female choice (e.g., ornaments and courtship displays) and traits related to ejaculate quality (Mautz et al. 2013). If males vary in the amount of prostaglandin-synthesizing compounds present in their spermatophores, and if females cannot access the composition of the spermatophores they acquire, polyandry can act as a bet-hedging strategy (Yasui and Garcia-Gonzalez 2016) to ensure the necessary amount of chemicals responsible for mating-induced egg release.

The retention time of the spermatophores was not related with male quality, a result that contrasts with a previous study with *T. commodus* (Bussière et al. 2006; Hall et al. 2010). Although females from both experimental groups reduced the retention time of the spermatophores over time, this effect was stronger in females reared in a variable-quality acoustic environment. The retention time of the spermatophores is interpreted as a mechanism of post-mating female choice in crickets (Bussière et al. 2006). Theoretically, the longer a females retains the spermatophore, the more sperm is transferred and the more eggs are sired by the spermatophore owner (e.g., Sakaluk and Eggert 1996). As females from the variable quality experimental group retain each spermatophore for a shorter period, they may be increasing sperm diversity in their spermatheca, and promoting more sperm competition. Fewer sperm from multiple males could result in greater genetic diversity

in the offspring because the chance that the sperm of a single male would outcompete rival males is lower. In fact, there is empirical evidence in other arthropod species showing that sperm diversity allows greater female control of paternity (Elgar et al. 2000), and also increases offspring fitness (Baer and Schmid-Hempel 1999). Therefore, although the pre-maturation acoustic environment does not affect pre-copulatory female preference (Kasumovic et al. 2012) and the degree of polyandry in *T. commodus* (Fig. 1), we suggest that the post-copulatory female decisions are affected by the pre-maturation social experience. Why pre-maturation social experience modulates mostly post-mating decisions in *T. commodus*, but pre-mating decisions in the sister species, *T. oceanicus* (Bailey and Zuk 2008), is a question that deserves future investigation.

The importance of socially induced post-mating female decisions is reinforced by our findings about offspring quality. Only females reared in an acoustic environment with variable-quality male calls showed higher offspring quality when mated with high-quality males. Differential female investment in offspring according to male quality is a widespread strategy, both before (e.g., Cunningham and Russell 2000) and after offspring birth (e.g., Robart and Sinervo 2019). Male traits perceived by females may influence female reproductive investment in egg number and size (e.g., Pischedda et al. 2011; Poisbleau et al. 2013), as well as in maternal care (e.g., Robart and Sinervo 2019). Changes in resource allocation to the offspring and also in the quality of parental behaviors in response to male quality are known mechanisms of cryptic female choice (reviewed in Ratikainen and Kokko 2010 and Firman et al. 2018). In the case of the cricket *T. commodus*, we showed that the change in resource allocation to the offspring as a form of cryptic female choice is dependent on the social acoustic environment experienced by females as juveniles. Although the precise mechanism underlying this pattern is unknown, previous studies with this species show that females reared in an acoustic environment with variable-quality male calls exhibit several plastic changes when compared with females reared in a silent environment. Some of these changes include the expression of genes associated with energy producing pathways and also higher egg

489 investment (Kasumovic et al. 2011, 2016), which could help to understand the results we found
490 here.

491 The social-induced plasticity of females has the potential to markedly modulate the
492 opportunity for sexual selection within a population. In a natural population where females mature
493 listening only high-quality male calls, females will invest equally in all offspring produced, and the
494 opportunity for post-mating sexual selection will be low. In a natural population where females
495 grow listening to variable-quality male calls, high-quality males would have higher fitness through
496 higher offspring quality, generating high variance in male reproductive success and high values of
497 opportunity for post-mating sexual selection. The hypothetical scenario proposed here is expected
498 to be found in multivoltine species in which there is overlap of generations — in particular, when
499 juvenile females coexist with adult males that attract receptive females using signals that stimulate
500 female choice as adults, such as many birds, insects and frogs.

501 In conclusion, when *T. commodus* females are reared in an acoustic environment with
502 variable-quality male calls, they increase their responsiveness to high-quality males (Kasumovic et
503 al. 2012), but it does not change the total number of males they accept as mates, which indicates
504 that higher responsiveness does not necessarily translate into a higher degree of polyandry. Contrary
505 to previous studies, we showed that a high number of mates reduces offspring fitness, suggesting
506 that there is an optimum number of mates for the females. Moreover, females reared in an acoustic
507 environment with variable-quality male calls showed two additional changes. First, they retained
508 the spermatophores for shorter periods, and second they produced higher quality offspring when
509 mated with high-quality males. These findings indicate that the pre-maturation acoustic
510 environment interacts with the quality of the males accepted as mates to determine post-copulatory
511 female strategies and eventually offspring fitness. Taken together, the results accumulated so far
512 clearly indicate that both the pre- and post-mating strategies of females may be subject to socially
513 induced plasticity.

References

Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. Anim Behav 60:145–164. https://doi.org/10.1006/anbe.2000.1446

Baer B, Schmid-Hempel P (1999) Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. Nature 397:151–154. https://doi.org/10.1038/16451

Bailey NW (2008) Love will tear you apart: different components of female choice exert contrasting selection pressures on male field crickets. Behav Ecol 19:960–966. https://doi.org/10.1093/beheco/arn054

Bailey NW, Zuk M (2008) Acoustic experience shapes female mate choice in field crickets. Proc R Soc London B 275:2645–2650. https://doi.org/10.1098/rspb.2008.0859

Bentsen CL, Hunt J, Jennions MD, Brooks R (2006) Complex multivariate sexual selection on male acoustic signaling in a wild population of *Teleogryllus commodus*. Am Nat 167:E102–E116. https://doi.org/10.1086/501376

Brooks R, Endler JA (2001) Female guppies agree to differ: phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. Evolution 55:1644–1655. https://doi.org/10.1111/j.0014-3820.2001.tb00684.x

Brooks R, Hunt J, Blows MW, Smith MJ, Bussière LF, Jennions MD (2005) Experimental evidence for multivariate stabilizing sexual selection. Evolution 59:871–880. https://doi.org/10.1111/j.0014-3820.2005.tb01760.x

Bussière LF, Hunt J, Jennions MD, Brooks R (2006) Sexual conflict and cryptic female choice in the black field cricket, *Teleogryllus commodus*. Evolution 60:792–800. https://doi.org/10.1111/j.0014-3820.2006.tb01157.x

Cornwallis CK, Uller T (2010) Towards an evolutionary ecology of sexual traits. Trends Ecol Evol, 25:145–152. https://doi.org/10.1016/j.tree.2009.09.008

539 Cunningham EJA, Russell AF (2000) Egg investment is influenced by male attractiveness in the
540 mallard. Nature 404:74–77. https://doi.org/10.1038/35003565
541 Dewsbury DA (2005) The Darwin-Bateman paradigm in historical context. Integr Comp Biol
542 45:831–837. https://doi.org/10.1093/icb/45.5.831
543 Edward DA (2015) The description of mate choice. Behav Ecol 26:301–310.
544 https://doi.org/10.1093/beheco/aru142
545 Elgar MA, Schneider JM, Herberstein ME (2000) Female control of paternity in the sexually
546 cannibalistic spider *Argiope keyserlingi*. Proc R Soc London B 267:2439–2443.
547 https://doi.org/10.1098/rspb.2000.1303
548 Evans AR (1988) Mating systems and reproductive strategies in three australian gryllid crickets:
549 *Bobilla victoriae* Otte, *Balamara gidya* Otte and *Teleogryllus commodus* Walker (Orthoptera:
550 Gryllidae: Nemobiinae; Trigonidiinae; Gryllinae). Ethology 78:21–52.
551 Firman C, Simmons LW, Andre I (2018) Phenotypic plasticity in genitalia : baculum shape
552 responds to sperm competition risk in house mice. Proc R Soc London B 285:20181086.
553 https://doi.org/10.1098/rspb.2018.1086
554 Hall MD, Bussière LF, Demont M, Ward PI, Brooks RC (2010) Competitive PCR reveals the
555 complexity of postcopulatory sexual selection in *Teleogryllus commodus*. Mol Ecol 19:610–619.
556 https://doi.org/10.1111/j.1365-294X.2009.04496.x
557 Hall MD, Bussière LF, Hunt J, Brooks R (2008) Experimental evidence that sexual conflict
558 influences the opportunity, form and intensity of sexual selection. Evolution 62:2305–2315.
559 https://doi.org/10.1111/j.1558-5646.2008.00436.x
560 Hebets EA, Vink CJ, Zealand N (2007) Experience leads to preference: experienced females prefer
561 brush-legged males in a population of syntopic wolf spiders. Behav Ecol 18:1010–1020.
562 https://doi.org/10.1093/beheco/arm070
563 Hunt J, Brooks R, Jennions MD (2005) Female mate choice as a condition-dependent life-history
564 trait. Am Nat 166:79–92. https://doi.org/10.1086/430672

565 Hunt J, Brooks R, Jennions MD, Smith MJ, Bentsen CL, Bussière LF (2004) High-quality male
566 field crickets invest heavily in sexual display but die young. Nature 432:1024–1027.
567 https://doi.org/10.1038/nature03084
568 Jasieński M, Bazzaz FA (1999) The fallacy of ratios and the testability of models in biology. Oikos
569 4:321–326. https://doi.org/10.2307/3546729
570 Jennions MD, Drayton JM, Brooks R, Hunt J (2007) Do female black field crickets *Teleogryllus*
571 *commodus* benefit from polyandry? J Evol Biol 20:1469–1477. https://doi.org/10.1111/j.1420-
572 9101.2007.01333.x
573 Kasumovic MM, Chen Z, Wilkins MR (2016) Australian black field crickets show changes in
574 neural gene expression associated with socially-induced morphological, life-history, and
575 behavioral plasticity. BMC Genomics 17:1–16. https://doi.org/10.1186/s12864-016-3119-y
576 Kasumovic MM, Hall MD, Brooks RC (2012) The juvenile social environment introduces variation
577 in the choice and expression of sexually selected traits. Ecol Evol 2:1036–1047.
578 https://doi.org/10.1002/ece3.230
579 Kasumovic MM, Hall MD, Try H, Brook RC (2011) The importance of listening: juvenile
580 allocation shifts in response to acoustic cues of the social environment. J Evol Biol 24:1325–
581 1334. https://doi.org/10.1111/j.1420-9101.2011.02267.x
582 Loher W, Edson K (1973) The effect of mating on egg production andrelease in the cricket
583 *Teleogryllus commodus*. Entomol Exp Appl 16:483–490.
584 Loher W, Ganjian I, Kubo I, Stanley-Samuelson D, Tobe SS (1981) Prostaglandins: their role in
585 egg-laying of the cricket *Teleogryllus commodus*. Proc Natl Acad Sci USA 78:7835–7838.
586 https://doi.org/10.1073/pnas.78.12.7835
587 Loher W, Rence B (1978) The mating behavior of *Teleogryllus commodus* (Walker) and its central
588 and peripheral control. Z Tierpsychol 46:225–259. https://doi.org/10.1111/j.1439-
589 0310.1978.tb01447.x

590 Mautz BS, Møller AP, Jennions MD (2013) Do male secondary sexual characters signal ejaculate
591 quality? A meta-analysis. Biol Rev 88:669–682. https://doi.org/10.1111/brv.12022
592 McGlothlin JW, Neudorf DLH, Casto JM, Nolan V, Ketterson ED (2004) Elevated testosterone
593 reduces choosiness in female dark-eyed juncos (*Junco hyemalis*): evidence for a hormonal
594 constraint on sexual selection? Proc R Soc London B 271:1377–1384.
595 https://doi.org/10.1098/rspb.2004.2741
596 Pischedda A, Stewart AD, Little MK, Rice WR (2011) Male genotype influences female
597 reproductive investment in *Drosophila melanogaster*. Proc R Soc London B 278:2165–2172.
598 https://doi.org/10.1098/rspb.2010.2272
599 Poisbleau M, Dehnhard N, Demongin L, Parenteau C, Quillfeldt P, Eens M (2013) Females paired
600 with new and heavy mates reduce intra-clutch differences in resource allocation. PLoS ONE,
601 8:e72136. https://doi.org/10.1371/journal.pone.0072136
602 Ratikainen II, Kokko H (2010) Differential allocation and compensation: who deserves the silver
603 spoon? Behav Ecol 21:195–200. https://doi.org/10.1093/beheco/arp168
604 Rebar D, Zuk M, Bailey NW (2011) Mating experience in field crickets modifies pre- and
605 postcopulatory female choice in parallel. Behav Ecol 22:303–309.
606 https://doi.org/10.1093/beheco/arq195
607 Ritchie MG, Saarikettu M, Hoikkala A (2005) Variation, but no covariance, in female preference
608 functions and male song in a natural population of *Drosophila montana*. Anim Behav 70:849–
609 854. https://doi.org/10.1016/j.anbehav.2005.01.018
610 Robart AR, Sinervo B (2019) Females increase parental care, but not fecundity, when mated to
611 high-quality males in a biparental fish. Anim Behav 148:9–18.
612 https://doi.org/10.1016/j.anbehav.2018.11.012
613 Sakaluk SK, Eggert A (1996) Female control of sperm transfer and intraspecific variation in sperm
614 precedence: antecedents to the evolution of a courtship food gift. Evolution 50:694–703.

615 Schielzeth H (2010) Simple means to improve the interpretability of regression coefficients.
616 Methods Ecol Evol 1:103–113. https://doi.org/10.1111/j.2041-210X.2010.00012.x
617 Simmons LW, Zuk M, Rotenberry JT (2005) Immune function reflected in calling song
618 characteristics in a natural population of the cricket *Teleogryllus commodus*. Anim Behav
619 69:1235–1241. https://doi.org/10.1016/j.anbehav.2004.09.011
620 Slatyer RA, Mautz BS, Backwell PRY, Jennions MD (2012) Estimating genetic benefits of
621 polyandry from experimental studies: a meta-analysis. Biol Rev 87:1–33.
622 https://doi.org/10.1111/j.1469-185X.2011.00182.x
623 Solymar BD, Cade WH (1990) Heritable variation for female mating frequency in field crickets,
624 *Gryllus integer*. Behav Ecol Sociobiol 26:73–76.
625 Taylor ML, Price TAR, Wedell N (2014) Polyandry in nature: a global analysis. Trends Ecol Evol
626 29:376–383. https://doi.org/10.1016/j.tree.2014.04.005
627 Torres-Vila LM, Gragera J, Rodríguez-Molina MC, Stockel J (2002) Heritable variation for female
628 remating in *Lobesia botrana*, a usually monandrous moth. Anim Behav 64:899–907.
629 https://doi.org/10.1006/anbe.2002.2000
630 Torres-Vila LM, Rodríguez-Molina MC, Gragera J, Bielza-Lino P (2001) Polyandry in
631 Lepidoptera: a heritable trait in *Spodoptera exigua* Hübner. Heredity 86:177–183.
632 https://doi.org/10.1046/j.1365-2540.2001.00821.x
633 Verzijden MN, Scobell SK, Svensson EI (2014) The effects of experience on the development of
634 sexual behaviour of males and females of the banded demoiselle (*Calopteryx splendens*). Behav
635 Proc 109:180–189. https://doi.org/10.1016/j.beproc.2014.08.023
636 Williams JR, Catania KC, Carter CS (1992) Development of partner preferences in female prairie
637 voles (*Microtus ochrogaster*): the role of social and sexual experience. Horm Behav 26:339–349.
638 https://doi.org/10.1016/0018-506X(92)90004-F
639 Yasui Y, Garcia-Gonzalez F (2016) Bet-hedging as a mechanism for the evolution of polyandry,
640 revisited. Evolution 70:385–397. https://doi.org/10.1111/evo.12847