



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

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Abstract

Pre-maturation social experiences may affect post-maturation reproductive strategies of females, including mating preferences and investment in offspring. Whether pre-maturation social experiences affect other aspects of females' reproductive strategies, including the number of accepted males and post-copulatory decisions, is still an open question. To address this question, we performed laboratory experiments using the highly polyandrous cricket *Teleogryllus commodus*. Previous studies showed that juvenile females reared in an acoustic environment with male calls of different rates (MIX) are more responsive to highly attractive calls than females reared in an environment with male calls of only high calling rates (HIGH). We exposed juvenile females to the same two acoustic environments (MIX and HIGH) and estimated the number of accepted males, time of spermatophore retention, and offspring quality. We found that the acoustic environment did not change the number of accepted males, indicating that the higher responsiveness shown in a previous study does not translate into high degree of polyandry. An increased number of mates reduced offspring quality, suggesting that polyandry is costly to females. Finally, when compared with females of the HIGH group, females of the MIX group retained spermatophores for shorter periods and produced higher quality offspring if mated with highly attractive males. This finding suggests that both the pre-maturation acoustic environment and the quality of the mates found by the adult females determine their post-copulatory decisions and eventually offspring quality. Taken together, our results indicate that both the pre- and post-mating strategies of females are subject to socially cued plasticity.

Significance statement

There is increasing evidence that pre-maturation experiences affect mating decisions of females after maturity, making them more or less selective. Both the pre-maturation social environment and the quality of potential mates found by an adult female may determine the number and quality of accepted males, which in turn may influence offspring quality. We experimentally tested this hypothesis using a highly polyandrous cricket species and showed that the pre-maturation acoustic environment does not affect the number and attractiveness of males accepted by the females. However, offspring quality was determined by an interaction between the pre-

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maturity acoustic environment and attractiveness of males accepted by the females. This result provides a link between the pre- and post-maturation social experience of a female and the quality of her offspring.

Keywords Acoustic environment · Female choice · Male attractiveness · Offspring quality · Polyandry · Socially cued plasticity

Introduction

Polyandry is a widespread pattern in nature. It can occur in species where males provide nutritious gifts to the females, where females have a direct benefit of multiple mating, and even in

species where the benefits acquired by multiple mating are solely genetic (Arnqvist and Nilsson 2000; Slatyer et al. 2012). Another widespread pattern is that both the frequency of polyandrous females and the number of males accepted as mates (i.e., degree of polyandry) show great intra- and interspecific variation (reviewed in Taylor et al. 2014). This raises the questions of why variation in the frequency and degree of polyandry exists and which factors explain this variation.

Polyandry is determined by genes and environmental conditions. Studies with invertebrates show that the number of males accepted by females may be heritable, but environmental conditions still play an important role in the degree of polyandry (e.g., Solymar and Cade 1990; Torres-Vila et al. 2001). Population density, for instance, is regarded as one of the most important environmental conditions to predict the degree of polyandry, irrespective of genetic tendencies (Taylor et al. 2014). A female's 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). In fact, the effects of social experience on females' reproductive behavior may start even in the pre-maturation period, as the perceived attractiveness and availability of males may affect females' sexual preferences (e.g., Hebert et al. 2007; Kasumovic et al. 2012) and their investment in offspring after maturity (e.g., Cunningham and Russell 2000; Kasumovic et al. 2011). Whether the pre-maturation social experience of females affects polyandry, however, is still unknown.

The main goal of this study is to explore the role of pre- and post-maturation experience on females' reproductive strategies. To explore this question, we used the Australian black field cricket *Teleogryllus commodus*, in which females are highly polyandrous under both field (Evans 1988) and laboratory conditions (Hunt et al. 2005; Jennions et al. 2007). Previous experimental studies showed that polyandry in *T. commodus* does not increase offspring quality (Jennions et al. 2007) nor offspring number (Loher and Edson 1973), but it may be necessary to guarantee the fertilization of all eggs (Loher and Edson 1973). However, it is important to stress 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 with different males 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 may have underestimated any possible positive effect of polyandry in *T. commodus*.

Despite being polyandrous, *T. commodus* females are choosy and strongly prefer males with high calling effort (Bentsen et al. 2006), hereafter referred to as *highly attractive males*. Female preference for males with high calling effort probably occurs because this trait is related with male 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 calls of males showing different levels of calling effort 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. 1(a)). Moreover, females reared in an acoustic environment with calls of males showing different levels of calling effort are also more responsive when adults to highly attractive male calls compared with females reared in environments with male calls of only low or only high calling effort (Kasumovic et al. 2012; Fig. 1(b, c)). These responses indicate that the pre-maturation acoustic environment experienced by females affects their post-maturation reproductive strategies.

Given that 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 of *T. commodus* reared in an acoustic environment with calls of males showing different levels of calling effort will be more polyandrous as a result of their high responsiveness to males and/or to guarantee the fertilization of a larger number of eggs produced (Fig. 1(d)). We also predict that this effect will be stronger if adult females meet more males that express highly attractive calls (Fig. 1(f)). Although pre-maturation acoustic cues, which provide reliable information on the social environment (Kasumovic and Brooks 2001), do not affect females' mating preference (Kasumovic et al. 2012), we explore whether a female's post-copulatory preference is a socially cued plastic trait. If so, we expect that females reared in different acoustic environments will show differences in the retention time of spermatophores (Fig. 1(e)), which is regarded as a form of cryptic female choice in crickets (Bussière et al. 2006). Finally, given that males capable of expressing highly attractive calls are in better condition (Hunt et al. 2004), we tested if the number and attractiveness of the accepted males (Fig. 1(h)) or the interaction between the acoustic environment experienced by females and the number and attractiveness of the accepted males determine offspring quality (Fig. 1(g, i)). Assuming that post-copulatory processes may also influence offspring quality, we tested whether male attractiveness is positively related with the retention time of his spermatophore and whether differences in this retention time affect offspring quality (Fig. 1(e, i)).

Methods

Preparation of individuals

We used *T. commodus* individuals from a stock established in the laboratory from 200 individuals collected in

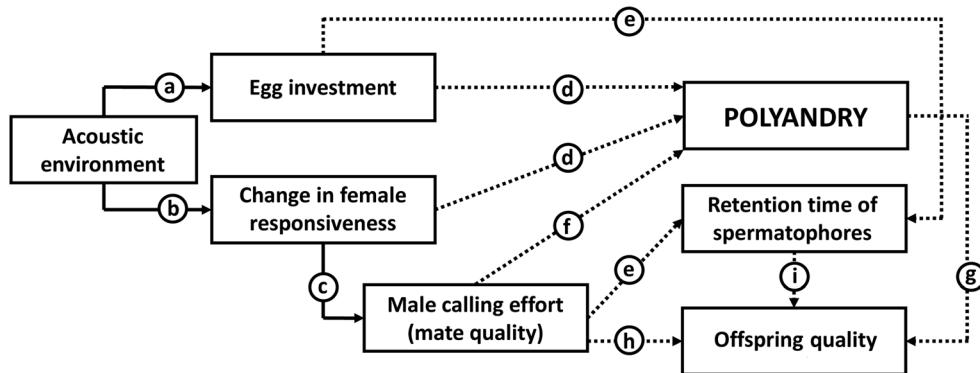


Fig. 1 Factors that may influence females' reproductive strategies in the cricket *Teleogryllus commodus*. We know (solid lines) that when females are reared in an acoustic environment composed of calls showing different levels of calling effort, they show (a) higher egg investment and (b, c) higher responsiveness to males with high calling effort than females reared in an acoustic environment in which the calling effort of the males is high and non-variable. Here we tested (dashed lines) six predictions. Females reared in an acoustic environment with different levels of calling effort will show (d) higher degree of polyandry and (e) higher retention time of spermatophores (i.e., post-copulatory preference)

of highly attractive males than females reared in an acoustic environment with high and non-variable calling effort, as a result of their high responsiveness to males and/or to guarantee the fertilization of the larger number of eggs produced. (f) The effect of the acoustic environment will be stronger if adult females meet more males that express high calling effort (i.e., attractive males). (g) The number and (h) attractiveness of males accepted as mates will interact with the acoustic environment to determine offspring quality. Finally, (i) male attractiveness will be positively related with the retention time of his spermatophore, which in turn will affect offspring quality

March 2016 at Smiths Lake ($32^{\circ} 22' S$, $152^{\circ} 30' E$), 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 developed wing buds but without an ovipositor). We placed all individuals removed from the stock in individual plastic containers ($5 \times 5 \times 3$ 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 h 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 acoustic treatments (see below), each one located in a different room. We switched acoustic treatments between rooms each day to minimize any possibility of room effects. We measured (i) females' thorax width using a stereomicroscope equipped with a millimeter ruler and (ii) body mass using an analytical balance (to the nearest 0.1 mg). These measures were taken at two points: when individuals molted to their last nymphal instar and when they molted to maturity. These measures were used to estimate female condition and to ensure that there was no initial difference in body size of females that would be later allocated to each acoustic treatment.

When males became adults, they were placed individually in boxes with microphones connected in a computer system (hereafter "call box") where males remained unless they were used in a mating experiment on that day. The call box recorded a male's calling effort, which is the number of seconds that each male spent calling between 11:00 and 21:00 h. 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 attractiveness. The days in which males were used in mating experiments were not included in this average. In total, the mean (\pm SD) number of days each male was recorded was 34.0 ± 17.2 ($N = 83$ males), which comprises nearly 90% of the males' adult lifespan (pers. obs.).

We reared *T. commodus* females in their last nymphal instar in one of two acoustic treatments: one composed only of calls with high calling effort (hereafter "HIGH") and another composed of calls showing different levels of calling effort (hereafter "MIX"). The sound track used in the HIGH group was composed of calls from three different males with low intercall duration (i.e., three highly attractive male calls), whereas the sound track used in the MIX group was composed of calls from one high calling effort, one mean calling effort, and one low calling effort according the intercall duration of male calls (see Supplementary Material S1 for further details). In both acoustic treatments, each male soundtrack was repeated sequentially and broadcasted from 12 speakers placed in a 1-m-diameter circle with the individual plastic containers containing the experimental females placed in the center. The choice of the two acoustic treatments was based on previous

results with *T. commodus*, according to which females reared either in a HIGH or in a MIX calling treatment showed the greatest differences in mating behavior as adults (Kasumovic et al. 2011).

We used linear models and generalized linear models (GLMs) to test if the following female traits showed any difference between acoustic treatments: adult thorax width, adult total mass, mass increase (with Gaussian error distribution), thorax width increase between the last nymphal instar and the adult stage (with negative binomial error distribution), and how long (in days) it took for them to molt to adulthood (with Poisson error distribution). We also used a linear model to test if the calling effort (i.e., attractiveness) of males presented to females of each group during the mating trials is equivalent. All tests were non-significant (see Supplementary Material S2), indicating that there was no unexpected and undesired initial difference between acoustic treatments.

Mating trials

We conducted mating trials with mature virgin females ($N=25$ in each acoustic treatment). 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 (\pm SD) age of experimental virgin females used in the mating trials was 24.2 ± 10.3 days ($N=50$), which corresponds to nearly 50% of their adult lifespan (pers. obs.). The 10 males used in the mating trials of each female were randomly sampled from experimental males previously placed in the individual calling boxes. The order and identity of male presentation to each female was also randomized. Due to the large number of males required for this study, we used males more than once, but never with the same female. The mean (\pm SD) age of experimental males used in mating trials was 20.6 ± 12.3 days as adults ($N=83$).

As our goal was to explore mating frequency as a consequence of females' 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* 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 produced courtship call, we scored a mating as successful if 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 his 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. 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 h. 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 the 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 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 *spermatophore retention time*).

To test if females reared in the MIX acoustic treatment accepted more males than females reared in the HIGH acoustic treatment (Fig. 1(d)), and if the effect of the pre-maturation acoustic treatment was stronger when adult females met more males that expressed a high level of calling effort (Fig. 1(f)), we fitted GLMs. We used the number of males accepted by each female as response variable, with a Poisson distribution of errors. As predictor variables, we used the interaction between the acoustic treatment (categorical) and the average calling effort of all males offered to each female during mating trials (continuous), regardless of whether they were accepted or rejected (see also Supplementary Material S3 for additional exploration of the data). Given that our prediction postulates that the number of males accepted as mates will be modulated by the calling effort (i.e., attractiveness) of the males a female meets as adults, high values of average calling effort indicate that she interacted with several highly attractive males, whereas low values of average calling effort indicates that she interacted with several poorly attractive males.

To test if females reared in the MIX acoustic treatment showed a higher retention time of spermatophores when compared with females reared in the HIGH acoustic treatment and if the calling effort of the males accepted by females was positively related with the retention time of their

spermatophores (Fig. 1(e)), we implemented generalized linear mixed effects models. We used the retention time of spermatophores of each male (in seconds) as the response variable (with a negative binomial distribution of errors) and the identity of each female and male as random factors. As predictor variables, we used the acoustic treatments, male mean calling effort, and the order (i.e., 1st, 2nd, 3rd, 4th...) in which each male was offered to the females. The full model included the interaction between acoustic treatment and mean calling effort of the actual mates and the additive effect of mating order (included in the model as a factor rather than a continuous variable).

Offspring quality

After the mating trials, females were maintained in their same individual plastic containers ($5 \times 5 \times 3$ 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 ($18.6 \times 13.6 \times 11.5$ cm) (hereafter “egg container”). Each egg container was checked once a week, when the number of hatched nymphs was counted, and the total mass of all nymphs was measured using an analytical balance (to the nearest 0.1 mg). We discarded the egg container once no new nymphs hatched for 2 weeks in a row.

To explore how females invested in offspring, we regressed the total nymph mass on the total number of nymphs produced by each female. For the regression, we used the total nymph number and the total nymph mass produced during a female’s lifetime. Given that the relationship between these two variables is linear ($R^2 = 0.967$; $df = 44$; p value < 0.001), we used the residuals of the regression as a proxy of *offspring quality*. 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 quality). 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 quality).

To test if the acoustic treatment interacts with the calling effort of the males accepted as mates, the total retention time females spent with males’ spermatophores, and the number of males accepted to determine female fecundity and residual nymph weight (Fig. 1(g–i)), we implemented two models. First, we fitted a GLM with the total nymph number produced by each female as response variable (with a negative binomial distribution of errors). Second, we fitted a linear model with residual nymph weight as response variable (with a Gaussian distribution of errors). As predictor variables of both models, we used the acoustic treatments and its interaction with the average calling effort of the mates accepted by each female

(i.e., average attractiveness of the mates that may have sired some offspring), the total retention time of males’ spermatophores, and the number of males accepted as mates. In both models, we included the interaction between average male calling effort and all other predictor variables. Finally, we also ran a linear model with residual nymph weight as response variable, and female body mass, female condition (i.e., the residuals of a regression between female size and mass), and the interaction between them as the predictor variables. Given that residual nymph weight was not influenced by female body mass, female condition, or the interaction between them (Supplementary Material S4), we did not include these variables in the model.

Statistical analyses

We used the *lme4* package (Bates et al. 2015) to implement all models and the *lmerTest* package (Kuznetsova et al. 2020) to obtain p values. To increase the biological interpretation of model results and to make the effect sizes comparable with other studies, we mean centered predictor variables using the *scale* function (Schielzeth 2010). All analyses were performed in the software R version 3.6.2 (R Development Core Team 2019). Because each model we built addresses a specific prediction presented in Fig. 1 and includes only the variables necessary to test these predictions, we opted for reporting the full models instead of reducing them.

Results

Degree of polyandry

The median number of males accepted as mates was 8 in the HIGH group and 6 in the MIX group, with great variation in both groups ($SE_{HIGH} = 0.51$ males; $SE_{MIX} = 0.46$ males). None of variables tested in the model explained the number of males accepted by the females (Table 1).

Retention time of spermatophores

Females from the HIGH group retained male spermatophores for longer after mating. The mean (\pm SE) time that females spent until spermatophore removal was 21.81 ± 6.90 min in the HIGH group ($N = 25$) and 16.66 ± 4.22 min in the MIX group ($N = 25$). Both the mating order and acoustic treatment significantly predicted spermatophore retention time (Table 2). There was a negative effect of mating order on the spermatophore retention time for both groups, but the effect was consistently higher for females of the HIGH group when compared with females of the MIX group (Fig. 2).

Table 1 Results of the model that tested the effect of acoustic treatment and average male calling effort of potential mates on the final number of males accepted by females. We present the estimated effects of each

Predictors	Estimate	SD	<i>z</i> value	<i>p</i> value
Intercept (HIGH group)	2.011	0.074	27.105	< 0.001
Acoustic treatment	-0.131	0.110	-1.194	0.233
Average male calling effort	-0.058	0.060	-0.969	0.332
Acoustic treatment × average male calling effort	0.130	0.129	1.013	0.311

Female fecundity

The mean (\pm SE) offspring number produced by females was 250.0 ± 41.2 nymphs in the HIGH group ($N=23$) and 298.9 ± 32.9 nymphs in the MIX group ($N=23$). None of the variables used in the model explained the offspring number produced by females (Table 3).

Offspring quality

The mean (\pm SE) residuals of the regression between total nymph number and total nymph mass produced by each female was 0.001 ± 0.006 g in the HIGH group ($N=23$) and -0.001 ± 0.008 g in the MIX group ($N=23$). There was an effect of the number of males accepted as mates and total retention time of spermatophore on residual nymph weight (Table 4). The residual nymph weight decreased with an increase in the number of accepted males (Fig. 3a), while it increased with an increase in spermatophore retention time (Fig. 3b). There was also an interaction between average calling effort of the males and the acoustic treatment (Table 4). The residual nymph weight increased with male calling effort in the MIX group, while it is not predicted by male calling effort in the HIGH group (Fig. 4).

predictor, the standard deviation (SD), and the values of *z* and *p*. Each female in both acoustic treatments (MIX: $N=25$; HIGH: $N=25$) was exposed to 10 different males, totaling 500 mating trials

Discussion

In this study, we investigated the role of pre- and post-maturation experience on females' reproductive strategies. Contrary to our predictions (Fig. 1(d, f)), females reared in the acoustic treatment with males' calls with different levels of calling effort (i.e., attractiveness) did not accept a higher number of males than females reared in an acoustic treatment with only calls of high effort, regardless of the attractiveness of the mates they find as adults. Also contrary to our predictions (Fig. 1(e)), the attractiveness of males accepted as mates did not influence the retention time of their spermatophores, but females reared in an acoustic treatment with males' calls with different levels of calling effort spent less time with males' spermatophores than females reared in an acoustic treatment with only calls of high effort. The more matings a female had, the lower was her spermatophore retention time in both acoustic treatments. As we expected, the retention time of spermatophores had a positive effect on residual nymph weight (i.e., offspring quality), but it was not related with male attractiveness (Figs. 1(e) and 3(b)). Polyandry, however, reduced offspring quality for females from both acoustic treatments, which is not in accordance with our prediction (Fig. 1(g)). Finally, the offspring number produced by females from both groups did not differ, but only females reared in an

Table 2 Results of the model that tested the effect of acoustic treatment, male calling effort, 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 italics. Each female in both

acoustic treatments (MIX: $N=25$; HIGH: $N=25$) was exposed to 10 different males, totaling 500 mating trials. The variance (\pm SD) explained by the random variables was as follows: females ID = 0.245 ± 0.495 ; male ID = 0.008 ± 0.089

Predictors	Estimate	SD	<i>z</i> value	<i>p</i> value
Intercept (HIGH group)	7.672	0.183	42.029	< 0.001
Acoustic treatment	-0.369	0.188	-1.964	0.049
Male calling effort	-0.004	0.106	-0.042	0.966
Mating order	-0.124	0.022	-5.579	< 0.001
Acoustic treatment × male calling effort	0.135	0.138	0.976	0.329

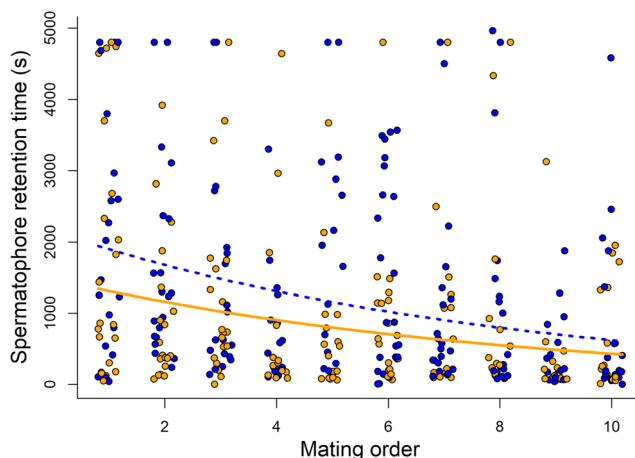


Fig. 2 Effect of mating order on the retention time of spermatophores by females of the Australian black field cricket *Teleogryllus commodus*. Coefficients used to build the graphic were extracted from the model (see Table 2). Blue circles and the blue-dashed line represent females from the acoustic treatment composed of only calls with high calling effort (HIGH) (intercept = 7.686); orange circles and the orange-solid line represent females from the acoustic treatment composed of calls showing different levels of calling effort (MIX) (intercept = 7.306). The slope of both acoustic treatments is -0.124 (95% CI $-0.168, -0.081$). The points were jittered to minimize overlapping

acoustic treatment with different levels of calling effort produced higher quality offspring when mated with highly attractive males. This finding supports the prediction that the attractiveness of males accepted as mates interacts with the pre-maturation acoustic environment of the females to determine offspring quality (Fig. 1(h)).

Although *T. commodus* females are sensitive to acoustic stimuli from conspecific males during development (Kasumovic et al. 2012), 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 (McGlothin et al. 2004), more responsive females are also the ones that exhibit stronger mating preferences (i.e., discrimination). However, a previous study with the closely related cricket *T. oceanicus* showed that there is not necessarily a link

between responsiveness (i.e., the motivation to mate sensu Edward 2015) and a female's mating preferences (Bailey 2008). In the case of *T. commodus*, females reared in an acoustic environment with calls of males showing different levels of calling effort are known to be more responsive to highly attractive males, but there is no change in their mating preferences as adults (Kasumovic et al. 2012), which suggests that responsiveness and discrimination are also not linked in this cricket species. Moreover, the results of our experiment show that females reared in an acoustic environment with calls of males showing different levels of calling effort do not increase their degree of polyandry when they find a high number of highly attractive males as adults. Based on this finding, we argue that polyandry is not driven by increased responsiveness to highly attractive males. Thus, despite the fact that an increase in responsiveness decreases the latency until copulation, it does not necessarily translate into a higher number of mates accepted by females.

A previous study on the role of polyandry in *T. commodus* has already shown that the number of males a female accepts as a mate does not increase offspring quality (Jennions et al. 2007). The aforementioned study, however, offered up to four males to each female, raising the question of whether the limited number of males may have underestimated any possible positive effect of polyandry in *T. commodus*. Considering that females can copulate with as much as 10 different males in captivity (Loher and Edson 1973), we offered females a larger number of males in our experiment. We found that a higher degree of polyandry had no effect on the number of nymphs produced by females, which confirms the results by Jennions et al. (2007) and provides further evidence against the suggestion that polyandry in *T. commodus* is a strategy to guarantee the fertilization of a larger number of eggs (Loher and Edson 1973). Although an increase in offspring number in response to polyandry has already been reported for other insect species, most of these cases occur in species in which males provide some kind of nutritious nuptial gift (reviewed in Arnqvist and Nilsson 2000), which is not the case of any *Teleogryllus* species. Interestingly, we found that a large

Table 3 Results of the model that tested the effect of acoustic treatment, average male calling effort, number of males accepted as mates, and total retention time of spermatophores on total nymph number produced by females. We present the estimated effects of each predictor, the standard deviation (SD), and the values of *t* and *p*. Each female in both acoustic treatments (MIX: $N=23$; HIGH: $N=23$) was exposed to 10 different males, totaling 500 mating trials

Predictors	Estimate	SD	<i>t</i> value	<i>p</i> value
Intercept (HIGH group)	5.418	0.161	33.752	0.001
Acoustic treatment	0.267	0.225	1.185	0.243
Average male calling effort	-0.049	0.171	-0.289	0.774
Number of males accepted as mates	0.195	0.132	1.476	0.148
Retention time	-0.030	0.129	-0.239	0.812
Average male calling effort \times acoustic treatment	-0.091	0.251	-0.363	0.718
Average male calling effort \times number of males accepted as mates	-0.174	0.142	-1.227	0.227
Average male calling effort \times spermatophore retention time	0.085	0.128	0.665	0.510

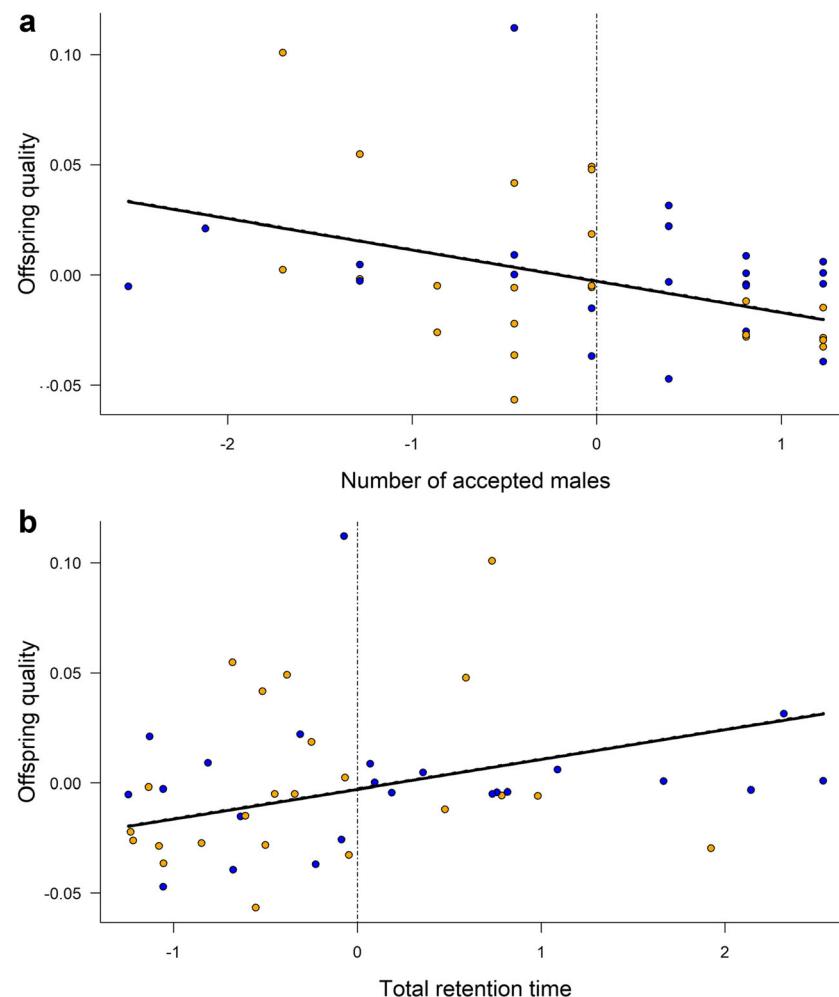
Table 4 Results of the model that tested the effect of acoustic treatment, average male calling effort, number of males accepted as mates by females, and the total retention time of spermatophores on the residuals of the regression between total nymph number and total nymph mass of each female (i.e., offspring quality). We present the estimated effects of

Predictors	Estimate	SD	t value	p value
Intercept (HIGH group)	-0.003	0.006	-0.413	0.682
Acoustic treatment	-0.001	0.009	-0.026	0.980
Average male calling effort	-0.012	0.007	-1.743	0.090
Number of males accepted as mates	-0.014	0.005	-2.681	<i>0.011</i>
Spermatophore retention time	0.013	0.005	2.610	<i>0.013</i>
Average male calling effort × acoustic treatment	0.022	0.010	2.201	<i>0.034</i>
Average male calling effort × number of males accepted as mates	-0.005	0.006	-0.808	0.424
Average male calling effort × spermatophore retention time	0.007	0.005	1.447	0.156

number of males accepted as mates have a negative effect on offspring quality. This finding suggests that highly polyandrous females do not receive indirect genetic benefits and that polyandry is costly in terms of offspring quality.

each predictor, the standard deviation (SD) and the values of *t* and *p*. Significant *p* values are highlighted in italics. Each female in both acoustic treatments (MIX: *N*=23; HIGH: *N*=23) was exposed to 10 different males, totaling 500 mating trials

Fig. 3 Effect of **a** number of males accepted as mates and **b** total retention time of spermatophores (both mean centered) on offspring quality estimated as the residuals of a linear regression between nymph number and nymph mass produced by females of the Australian black field cricket *Teleogryllus commodus*. Blue circles represent females from the acoustic treatment composed of only calls with high calling effort (HIGH) and orange circles represent females from acoustic treatment composed of calls showing different levels of calling effort (MIX). The coefficients used to build the graphic were extracted from the model (see Table 4). **a** Intercept = -0.004, slope = -0.013 (95% CI -0.024, -0.003). **b** Intercept = -0.004, slope = 0.014 (95% CI 0.003, 0.025)



Given the cost related to the high degree of polyandry reported here, why do *T. commodus* females accept several males as mates? One possible explanation may be the fact that females need to receive, via males' spermatophore, a complex

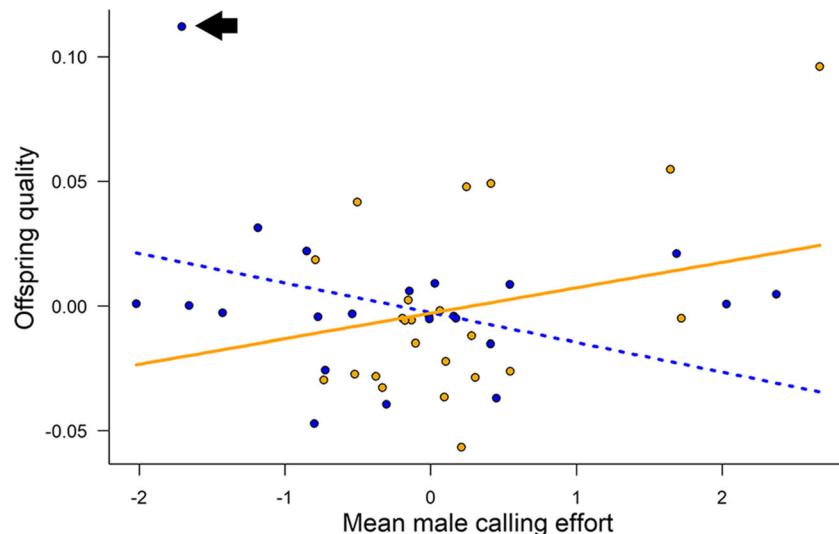


Fig. 4 Effect of standardized mean calling effort of actual mates and acoustic treatment on offspring quality estimated as the residuals of a linear regression between nymph number and nymph mass produced by females of the Australian black field cricket *Teleogryllus commodus*. Coefficients used to build the graphic were extracted from the model (Table 4). Blue circles and blue-dashed line represent females from the acoustic treatment composed of only calls with high calling effort

(HIGH), intercept = −0.004, slope = −0.012 (95% CI −0.017, 0.009); orange circles and orange-solid line represent females from the acoustic treatment composed of calls showing different levels of calling effort (MIX), intercept = −0.003, slope = 0.013 (95% CI 0.004, 0.046). The blue dot indicated by an arrow is an outlier and the removal of this value from the analysis makes the results marginally significant (t value = 1.925; p value = 0.061)

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 indicators of their body condition and immunocompetence (Hunt et al. 2004; Simmons et al. 2005), it is unknown whether male calls are related with the amount of prostaglandin-synthesizing compounds in their spermatophores. If males vary in the amount of prostaglandin-synthesizing compounds present in their spermatophores, and if females cannot access the composition of the spermatophores, 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. In accordance with this hypothesis, females of the bean weevil *Callosobruchus maculatus* that mate polyandrously and receive sperm from different males lay more eggs than monandrous females and receive sperm from a single—even though females of both experimental groups have the same numbers of matings (Eady et al. 2000).

The retention time of the spermatophores was not related with male attractiveness, a result that contrasts with a previous study with *T. commodus* (Bussière et al. 2006; Hall et al. 2010). Although females from both acoustic treatments reduced the retention time of the spermatophores over time, this effect was stronger in females reared in the acoustic treatment with males' calls showing different levels of calling effort. 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 female 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 MIX group retained 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 offspring because the chance that the sperm of a single male would outcompete rival males is lower. Moreover, 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 quality (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*, we suggest that the post-copulatory female decisions are affected by the pre-maturation social experience. An acoustic environment with calls of males showing different levels of calling effort could provide a cue to immature females that the adult male population is heterogeneous, both phenotypically and genetically. In this scenario, a female could increase genetic diversity in her offspring by combining polyandry with post-copulatory selection via a low retention time of spermatophores.

The importance of socially cued post-mating female decisions is reinforced by our findings about offspring quality. Only females reared in an acoustic treatment with different levels of calling effort (MIX) showed higher offspring quality when mated with highly attractive males. In the bush cricket *Ephippiger diurnus*, females reared in an acoustic treatment with highly attractive males invested more in the offspring when mated with highly attractive males (Rebar et al. 2019). Although this pattern is not the same found in our study, it also

indicates that females are able to adjust post-mating decisions based on both male attractiveness and their pre-maturation social acoustic experience. Differential female investment in offspring according to male attractiveness 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). In the case of the cricket *T. commodus*, female investment into individual offspring seems to be modulated by the pre-maturation social acoustic environment experienced by females. Although the precise mechanism underlying this pattern is unknown, previous studies with this species show that females reared in an acoustic environment with calls of males showing different levels of calling effort exhibit several socially cued 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 higher egg investment (Kasumovic et al. 2011, 2016), which could help to understand the results we found here.

In conclusion, when *T. commodus* females are reared in an acoustic environment with calls of males showing different levels of calling effort (i.e., attractiveness), they increase their responsiveness to highly attractive males (Kasumovic et al. 2012), but it does not change the total number of males they accept as mates. This result indicates that higher responsiveness does not necessarily translate into a higher degree of polyandry. Contrary to previous studies, we showed that a high number of mates reduce offspring quality, suggesting that polyandry may be costly in terms of offspring quality. Moreover, females reared in an acoustic environment with calls of males showing different levels of calling effort showed two additional changes: (1) they retained the spermatophores for shorter periods, and (2) they produced higher quality offspring when mated with highly attractive males. These findings indicate that the pre-maturation acoustic environment interacts with the attractiveness of the males accepted as mates to determine post-copulatory female strategies and eventually offspring quality. Taken together, the results accumulated so far clearly indicate that both the pre- and post-mating strategies of females are subject to socially cued plasticity.

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Data availability The datasets generated and analyzed during the current study are available in the Mendeley repository (<https://doi.org/10.17632/exgfzg3kcs.1>).

Compliance with ethical standards

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

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