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Prior mating success can affect allocation towards future sexual signalling in crickets

Fitness is often correlated with the expression level of a sexually selected trait. However, sexually selected traits are costly to express such that investment in their expression should be optimised to maximize their overall fitness gains. Social interactions, in the form of successful and unsuccessful matings, may offer males one type of feedback allowing them to gauge how to allocate their resources towards sexual signalling. Here we tested whether adult male black field crickets (*Teleogryllus commodus*) modify the extent of their calling effort (the sexually selected trait) in response to successful and unsuccessful matings with females. To examine the effect that mating interactions with females have on investment into sexual signalling, we monitored male calling effort after maturation and then provided males with a female at two points within their life, manipulating whether or not males were able to successfully mate each time. Our results demonstrate that males alter their investment towards sexual signalling in response to successful matings, but only if the experience occurs early their life. Males that mated early in their life decreased their calling effort sooner than males that were denied a mating. Our results demonstrate that social feedback in the form of successful and unsuccessful matings has the potential to alter the effort a male places in sexual signalling.
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**Introduction**

Sexually selected traits are costly to produce and their expression varies as a consequence of resource abundance (i.e. condition dependence; Rowe and Houle 1996; Cotton et al. 2004) (e.g., morphological traits: Bonduriansky and Rowe 2003; ejaculate traits: Fricke et al. 2008; behavioural traits: Lomborg and Toft 2009). This is most strongly reinforced by studies demonstrating longevity costs of extra investment in sexual signalling (Hunt et al. 2004), although questioned by studies that demonstrate the opposite (Kotiaho et al. 1999; Papadopoulos et al. 2004). Despite the well-established link between nutrient/resource abundance and trait expression (Bonduriansky 2007), resources are only one of the many factors that influence the expression of sexually selected traits. Decades of competition research suggests that the social environment is an important contributor that results in plasticity in the expression of sexually selected traits after maturity. For example, in many species of mammals, birds, and invertebrates, dominant individuals are able to moderate the behaviour of subordinate individuals by enforcing a lower level of sexual signalling (Ovarnström 1997; Bekoff and Dugatkin 2000; Tibbetts and Dale 2004).

As studies explore the effect of intra- and intersexual social interactions on individual behaviour, a secondary view that is gaining some traction in the literature is that plasticity in sexual signalling is a result of individual decisions resulting from conspecific feedback rather than enforcement. For example, studies manipulating the social environment during immaturity demonstrate that males alter how they allocate resources towards life-history (Gage 1995; Kasumovic and Andrade 2006; Walling et al. 2007; Kasumovic et al. 2011) metric (Rodd et al. 1997; Kasumovic and Andrade 2006; Kasumovic et al. 2011; DiRenzo et al. 2012), physiological (Stolz et al. 2012), sperm (Gray and Simmons 2013), and behavioural traits (Bailey et al. 2010; Kasumovic et al. 2012b) at maturity. Additionally, adult males show plastic responses when
exposed to rivals, enabling them to quickly respond to changes in the environment (Bretman et al. 2001) often with significant fitness benefits (Bretman et al. 2009). The feedback provided from the composition of the social environment can thus play an important role on individual perception of the intensity of competition, and therefore, the expression of sexually selected traits (Kasumovic and Brooks 2011).

The idea that changes in sexual signalling are a result of social feedback rather than social enforcement is strengthened by the theoretical and empirical understanding of winner and loser effects in the competition literature. Repeated competitions with rivals for access to resources provides individuals with feedback on their own performance relative to rivals within a population (Hsu et al. 2006; Fawcett and Johnstone 2010; Fuxjager and Marler 2010; Kasumovic et al. 2010). Individuals that win more contests are more likely to escalate future contests because they have a favourable perception of their fighting ability, while individuals that lose more contests have a lower perception of their own fighting ability (i.e. winner/loser effects; Hsu et al. 2006). In this sense, individuals are altering their behaviour and the expression of a sexually selected trait not as a consequence of condition, per se, but rather as a result of social interactions that indicate their relative fighting ability.

Fawcett and Bleay (2009) theoretically extended the idea of intra-sexual interactions modifying self-perception of fighting ability to inter-sexual interactions altering a male’s perception of his own attractiveness. Through their model, they demonstrate that experiences of rejection reduce choosiness and experiences of acceptance increase it, similar to winner and loser effects in direct male-male competitions (e.g., Kasumovic et al. 2009). Empirical evidence of mate rejection and acceptance in zebra finches (Taeniopygia guttata) supports this idea (Pariser et al. 2010; Royle and Pike 2010). Moreover, when Pariser et al. (2010) manipulated individual attractiveness through coloured leg rings, they found that zebra finch males subsequently changed
body mass, condition and courtship display rate, demonstrating the importance of social feedback in trait maintenance. These results illustrate that artificial ornamentation not only manipulated the female’s perception of the male but also changed the male’s perception of his own attractiveness, via the behavioural ‘mirror’ of female feedback. Whether the change in courtship rates (i.e., sexual signalling effort) was a result of direct social feedback or a consequence of reduced mass and condition, however, is difficult to determine.

Our goal in this study was to examine whether feedback from females in the form of successful or unsuccessful matings alters future male investment in sexually selected signals. We used the Australian black field cricket (Teleogryllus commodus) to investigate this question as males call throughout their adult lifetime in an attempt to attract mates. Males that call more attract significantly more mates (Bentsen et al. 2006), but calling is likely energetically costly (e.g., Hoback and Wagner Jr. 1997 on Gryllus lineaticeps) as it leads to a shorter lifespan (Hunt et al. 2004; Hunt et al. 2006); thereby demonstrating the trade-off between reproductive effort and lifespan. In addition, there is evidence in this and other closely related species that social interactions and feedback affect juvenile developmental tactics (Bailey et al. 2010; Kasumovic et al. 2011; DiRenzo et al. 2012; Kasumovic et al. 2012b), adult male behaviour (Judge et al. 2008), and lifetime calling effort (Zajitschek et al. 2012; Callandera et al. 2013). As a result, we hypothesized that males would differentially alter investment towards sexual signalling as a function of successful mating experiences. We also wanted to explore whether we could partition the effects of mating itself and interactions with females to better understand how males invest in sexual signalling.

We predicted that successful mating opportunities would result in positive feedback that would reinforce the current signalling effort as being sufficient to attract and successfully mate with females. In contrast, we hypothesized that unsuccessful mating opportunities would provide
negative feedback on reproductive effort and further suggest a choosier mating environment which should result in males increasing their calling effort. Since calling effort is costly, we predicted that these changes in calling effort should have subsequent effects on lifespan.

**Methods**

The crickets used for this experiment were first generation descendants of approximately 300 immature individuals collected at Smith’s Lake, New South Wales (32°22’S, 152°30’E) in 2012. We removed penultimate male and female crickets from the common population and reared them individually in a plastic container (5×5×3cm) with an egg carton for shelter, and *ad libitum* food (Friskies Go Cat Senior) and water. We kept all the individuals in a controlled temperature room of 28°C on a 14:10 light to dark cycle and replaced their water and food weekly. We checked crickets daily for adult eclosion and within 24 hours of eclosing, we weighed individuals using an electronic balance and measured pronotum width as a measurement of size. Adult females remained in individual containers within a controlled temperature room until required for matings.

Upon maturity, we placed males in a custom-built electronic monitoring device (*callbox; see Lailvaux et al. 2010*) overnight each day to determine daily calling effort. Males were kept in individual containers (5×5×3cm), which were then placed in plastic containers (14×6×6cm) surrounded by acoustic foam to keep males in acoustic isolation. We monitored male calling over a 12 hour period each night and used a male’s time spent calling each night in seconds as our estimate of calling effort. A male’s placement within the callbox was randomized every other day.

Although males were placed within the callbox upon maturity, we only monitored male calling effort from age 7-28. On day 14 and 21, we removed males from their individual cages and placed them in a 10×5×4cm mating arena. As males generally begin calling between days 5-
10, and usually reach their peak calling effort around day 20-22, we chose day 14 and 21 to
examine how social interactions affect calling effort after males have begun calling to before
reaching their peak. We chose two mating opportunities as males in a natural population of
another species of cricket (\textit{Gryllus campestris}) averaged two matings throughout their lifetime
\cite{Rodriguez-Munoz2010} and we wanted to examine whether single mating opportunities
affected age-specific calling effort. Once in the mating enclosure, a virgin female was placed
within the container for 24 hours along with her water and food containers and males were either
allowed to interact and mate with the female or allowed to interact but denied the opportunity to
mate. Previous studies demonstrate that males always mate when placed with a female over 24
hours \cite{Hall2010,Lailvaux2011,Zajitschek2012}. To ensure that selected males
were denied access to females, we placed females inside an overturned 100ml transparent plastic
cup. Each cup had approximately twenty holes evenly distributed throughout the cup and no
bigger than 4mm in diameter. These holes were large enough to allow males to pass the front of
their head and antenna through, but not their body. These holes thus allowed pheromonal,
acoustic and visual communication to occur between the males and females, but prevented any
matings from occurring.

As males were either granted or denied a mating at each mating opportunity (day 14 and 21),
we had a $2 \times 2$ design which resulted total of four experimental treatments: (i) an ‘early mating’
treatment where males were granted a mating on day 14 but denied a mating on day 21, (ii) a
‘late mating’ treatment where males were granted a mating on day 21 but denied a mating on day
14, (iii) a ‘multiple mating’ treatment where males were granted a mating on both day 14 and 21,
and (iv) a ‘denied mating treatment’ where males are prevented from mating on both day 14 and
21. We also had an isolation (control) treatment where males were placed in a mating arena but
no females were introduced. Since no female was introduced, we placed the male’s own egg
carton and water bottle in the mating arena to ensure he had food and water. All the males were
returned to the call box the day after their mating treatment and calling effort was monitored until
day 28. After 28 days, we removed males from the call box and monitored their survival.

**Statistical analysis**

In all our examinations, we used a mixed-model approach with individual identity as a
random factor using the nlme package ([Pinheiro et al. 2009](#)) in R 3.1.0 (R Core Team, 2014). We
log transformed calling effort and used the orthogonal quadratics to avoid issues with
multicollinearity. Since males began calling at different ages resulting in different rates of calling
increase early in their lifetime, it resulted in significantly different early portions of the calling
curve. As a result, we limited our analysis to calling effort between days 15 (the first mating) and
28 to ensure that calling effort prior to the treatment did not skew our results.

As the individuals in the isolation treatment never experienced any females throughout their
lifetime, there was no direct way to examine whether males in the isolation treatment behaved
differently to all the mating treatments without classifying each treatment separately, thus
decreasing our power. As such, we first examined whether age-specific calling effort of males in
the isolation treatment differed from the males in the denied mating treatment by adding a
treatment effect to a model containing only the linear and quadratic components of age. Since
adding treatment to the model did not improve our fit (see results), we combined the isolation and
denied treatments in our further analyses to maximize our sample size.

To examine whether early and late matings each individually or jointly affected age-specific
calling effort, we coded each mating (early and late) as a function of being granted or denied.
Following Zajitschek et al. ([2012](#)), we began with a full model consisting of the two-way
interactions between early and late mating crossed with the linear and quadratic effects of age as
well as all the lower order terms involved in this interaction. We then used the model selection function to reduce the model using the Akaike Information Criteria (AIC) (Akaike 1983) with models that decrease the AIC by at least 2 providing a significantly better fit (Burnham and Anderson 2002). We initially used maximum likelihood for model selection and then report statistics using REML. We visualized male age-specific calling effort using non-parametric splines generated with the general additive mixed model package (gamm4, Wood 2009).

We used an ANOVA to examine whether total calling effort (log transformed) differed as a consequence of either an early or late successful mating, and a GLM to examine whether lifespan was affected by a successful early or late mating with total calling effort as a covariate. These were performed in R 3.1.0.

Results

A total of 102 males were approximately equally distributed within each of the five treatments (Early: 22, Late: 22, Multiple: 19, Denied: 20, Isolation: 19). Each treatment began with 25 individuals and the difference in the sample size is a result of individuals that didn’t call at all within the first 14 days of their life being removed from the experiment. We removed these individuals since males that do not call by this time either do not call during their lifetime or call at a very reduced rate (Kasumovic pers. obs.), likely because they are following a different mating strategy (i.e. sneaking rather than calling; Bailey et al. 2010). As a result, keeping these non-calling males would skew results in the different treatments. In addition, although individuals may alter their behaviour, we cannot study changes in calling effort if males do not call. No individuals died before 28 days. As one individual escaped from the Late mating treatment prior to death, the lifespan analysis consists of 101 individuals.
In our initial comparison of the age-specific calling curves between individuals in the isolation and denied treatment, the addition of treatment in the model did not significantly improve the fit (log-likelihood difference = 0.66, P = 0.42). As a result, we combined the two treatments to improve our sample size.

The model that best described the variation in age-specific calling effort included the early mating term, the linear and quadratic effects of age, as well as the interaction between the early mating term and the linear and quadratic effects of age (Table 1). Only the quadratic terms were significant, suggesting that it is the non-linear component that is affected with an early mating. This is supported by the significant effect of the quadratic term in for males that mated early, while there was no significant effect of the quadratic term for males that were denied the early mating (Table 2).

There was no difference in total calling effort as a function of a successful early \((F_{1,99}=0.08, P=0.78)\) or late \((F_{1,99}=0.00, P=0.99)\) mating between treatments. Individual lifespan also was not affected by either a successful early \((t_{1,97}=-0.0.5, P=0.96)\) or late \((t_{1,97}=0.82, P=0.41)\) mating, or total calling effort \((t_{1,97}=0.95, P=0.34)\).

Discussion

Given the strong selection for high rates of acoustic calling in *Teleogryllus commodus* ([Bentsen et al. 2006](#)), there is likely to be an evolutionary benefit for males to optimise calling effort over their lifespan. Both diet ([Hunt et al. 2004](#); [Judge et al. 2008](#); [Bertram et al. 2011](#)) and the social environment encountered prior to maturity ([Kasumovic et al. 2012a](#); [Kasumovic et al. 2012b](#)) contribute to the variance in male age-specific calling effort trajectories. Here we investigated whether variation in age-specific calling effort may also be a response to social feedback in the form of successful and unsuccessful matings. Our results suggest that social
feedback from matings occurring early in a male’s lifetime have the potential to alter how they invest in sexual signalling throughout their life (Fig. 1). Despite this shift in calling effort, there was no difference in the total calling effort of males in the different treatments, and as a result, no effect on male lifespan.

A previous study examining the effect of diet and weekly successful matings in *T. commodus* found that males that were mated on a weekly basis had a lower lifetime calling effort than virgin males (Zajitschek et al. 2012). Our results broaden our understanding of sexual investment by males as we demonstrate that a shift in investment can occur as a consequence of a single successful mating. Our mating treatments were positioned such that they occur before a virgin male’s peak calling effort in an attempt to understand whether mating interactions affect how males reach their peak calling effort. We show that an unsuccessful mating prior to a male reaching his peak calling effort results in males maintaining their calling effort for a longer period of time relative to individuals that are successful early in their lifetime (Fig. 1). Our results thus provide evidence that males use social feedback from females to alter their investment towards sexual signalling as seen in other species (Pariser et al. 2010; Royle and Pike 2010; e.g., Callandera et al. 2013). Most importantly, we demonstrate that a single successful mating early in a male’s lifetime is enough to cause changes in signalling effort and that no social feedback from conspecific males in the form of social enforcement is necessary (Callandera et al. 2013).

Another possibility is, however, that mating itself was costly enough that it decreased male investment in sexual signalling. For example, in tree crickets (*Oecanthus nigricornis*), males provide a nuptial gift and what were considered high quality males decreased their investment in subsequent gifts in response to perceived female density (Bussière et al. 2005). Although *T. commodus* do not produce gifts as large as *O. nigricornis*, the spermatophores produced prior to courtship may be costly enough to result in a decrease in calling effort. Our mating design allows
us to further dissect this possibility. First, regardless of whether males successfully mated or not, all male *T. commodus* perform the costly courtship calling (*Hack 1998*) prior to mating and produce a spermatophore in preparation for mating (*Loher 1974*). This coupled with the fact that there was no difference in lifespan as a function of a successful mating suggests that similar contact costs were paid by all males in the mating treatments. Second, we did not see a change in age-specific calling effort as a function of a granted or denied second mating, further suggesting that decreased calling effort is not a consequence of a decreased energetic pool. Finally, males in the isolation (control) treatment where the extra-costly courtship calling and spermatophore transfer was presumably not triggered did not significantly differ in age-specific calling effort from males in the denied treatment. These factors suggest that a change in age-specific calling effort is a function of female feedback rather than spermatophore or mating costs, but further studies are necessary to rule this possibility out completely.

Despite this shift in calling effort, we saw no difference in lifetime calling effort or male lifespan between males as a consequence of successful matings in either the early or late period, in contrast to previous results demonstrating that repeated matings reduce calling effort (*Zajitschek et al. 2012*). This difference may be because males were continually mated throughout their life, rather than provided with only two matings well-spaced through the early period of their life in our experiment. Nevertheless, it may be beneficial for males to alter their signalling effort in response to female feedback early in their life because a male’s likelihood of survival under field conditions is significantly lower than a female’s (15.2 versus 21.4 days, respectively) (*Zajitschek et al. 2009*). Males that decrease their calling effort in response to their success may increase their natural lifespan by decreasing their mortality hazard rate from parasites and predators that exploit sexual signals (*Zuk and Kolluru 1998*). This idea, however, requires further testing in a field scenario.
Although all previous studies demonstrate that male *T. commodus* mate immediately with females when they are available (Hall et al. 2008), there is the possibility that some males did not mate or that some males mated more often than others. This, however, would add noise to the differences in age-specific calling effort between treatments making it less likely to see a significant effect of a mating rather than skewing it towards significance.

Our results add to the growing list of studies demonstrating that social feedback can influence individual reproductive effort in a myriad of ways ranging from experiencing them prior to maturity (Kasumovic and Brooks 2011), to direct (Fawcett and Johnstone 2003; Hsu et al. 2006) and indirect (Callandera et al. 2013) intrasexual feedback, and direct intersexual feedback (Fawcett and Bleay 2009; Pariser et al. 2010; Royle and Pike 2010). The competitive context is thus an important determinant of the level of trait expression, selection and therefore, how researchers view the concept of individual quality (Lailvaux and Kasumovic 2011). Our results, in combination with others, highlight the importance of examining and considering the social environment when exploring male mating strategies and investment towards sexual signalling.

**Acknowledgements**

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Bertram SM, Thomson IR, Auguste B, Dawson JW, Darveau CA (2011) Variation in cricket acoustic mate attraction signalling explained by body morphology and metabolic differences. Anim Behav 82:1255-1261


Table 1: The results of the best-fit model examining differences the effect of early mating on age-specific calling effort.
<table>
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<th>Factor</th>
<th>d.f.</th>
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<th>p-value</th>
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<td>Age$^2$</td>
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<td>Early mating</td>
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<tr>
<td>Age $^2$</td>
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<td>0.019</td>
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Table 2 (on next page)

Model regression coefficients

Table 2: The regression coefficients of the linear (age) and quadratic (age2) terms of age for the individuals denied and granted their first mating opportunity.
<table>
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<th>Early mating</th>
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<tr>
<td></td>
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<td>Quadrat</td>
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Figure 1

Age-specific curves of male calling effort

Figure 1: The age-specific calling curves of males that failed and succeeded in their early mating. The dotted lines are 95% confidence intervals.