



Behavioral Ecology (2018), 29(4), 876–884. doi:10.1093/beheco/ary059

Original Article

Are females in good condition better able to cope with costly males?

Maidier Iglesias-Carrasco,^{a,◉} Michael D. Jennions,^a Susanne R.K. Zajitschek,^{b,c,◉} and Megan L. Head^{a,◉}

^aDivision of Evolution, Ecology and Genetics, Australian National University, Research School of Biology, 134 Linnaeus Way, Acton ACT 2601, Canberra, Australia, ^bDoñana Biological Station – CSIC, Av. Americo Vespucio 26, 41092 Seville, Spain, and ^cSchool of Biological Sciences, Monash University, 25 Rainforest Walk, Clayton Campus, 3800 Melbourne, Australia

Received 15 August 2017; revised 11 February 2018; editorial decision 22 March 2018; accepted 4 April 2018; Advance Access publication 25 April 2018.

The costs of mating for a female might depend on both her phenotype and that of her mate. Sexually antagonistic male traits that negatively affect females are often condition dependent, so a male's rearing environment can affect the costs he imposes on his mate. Likewise, a female's ability to resist male-imposed costs might be condition dependent. We experimentally manipulated female and male body conditions in the seed beetle *Callosobruchus maculatus* by rearing larvae on a good or poor quality diet. We then tested whether the cost of mating for a female (measured as copulation behaviors associated with sexual conflict as well as her fecundity and survival) depended on her and/or her mate's body condition. As expected, females in better condition laid more eggs and lived longer, indicating higher fitness. More interestingly, females that mated with males in better condition had shorter copulations and started to kick sooner. Both results are potentially indicative of greater sexual conflict. We suggest that these changes in mating behavior might be driven by the higher toxicity of ejaculates of males that are in better condition. Crucially, however, the lack of any interaction between male and female conditions for the variables measured suggests that any increase in the costs of mating with a male in better condition is not ameliorated by the female's own condition.

Key words: *Callosobruchus maculatus*, condition, costs of mating, fitness, mating behavior.

INTRODUCTION

Whether to mate or not is an economic decision for females that comes with the obvious benefit of egg fertilization. Being choosy about whom to mate with can also confer additional benefits to females, such as the acquisition of material resources that increase fecundity (Møller and Jennions 2001), or genes that elevate the mean fitness of her offspring (Kuijper et al. 2012). In addition, mating can impose costs if it lowers other fitness components (review: Arnqvist and Nilsson 2000). For example, mating reduces resource availability for other fitness-enhancing activities like foraging (Griffiths 1996) or immune defense (Short and Lazzaro 2010), and the act of mating can increase the risk of predation (Magnhagen 1991), disease transmission (Hurst et al. 1995), and physical injury (Crudginton and Siva-Jothy 2000). For example, in the dung fly *Sepsis cynipsea*, mated females have lower survival than virgins, possibly due to damage caused by the armored genitalia of males (Blanckenhorn et al. 2002). Similarly, in the fruit fly *Drosophila*

melanogaster males transfer toxic seminal products that reduce a female's lifespan (Chapman et al. 1995). Costs of mating may also be “indirectly” mediated if females allocate resources away from eggs and parental care to ameliorate male-imposed costs on their own survival.

Both the benefits for and the costs imposed on females from mating are likely to vary depending on a male's phenotype. This should favor the evolution of female mate choice. For example, in the bush cricket *Poecilimon zimmeri*, females prefer heavier males that transfer larger nuptial gifts that elevate fecundity (Lehmann and Lehmann 2008). Similarly, a male's phenotype can affect his ability to coerce females to act in ways that benefit his reproductive success to the detriment of his mates (Chapman et al. 2003). For example, in *D. melanogaster* larger males have greater mating success (Hoffmann 1991), but mating with them reduces a female's lifespan, and hence her net fitness (Pitnick and Garcia-Gonzalez 2002). In some cases, despite lower fecundity, females might still benefit from mating with certain males if the sexually antagonistic male trait(s) that lower fecundity are heritable and elevate her sons' fitness (Head et al. 2005; Garcia-Gonzalez and Simmons 2010). However, much of the variation in male phenotypes is non-heritable, and instead

Address correspondence to M. Iglesias-Carrasco. E-mail: miglesias15@gmail.com.

results from variation in the males' rearing environment. The expression of sexually selected traits is often condition dependent, and partly reflects the availability of resources (Rowe and Houle 1996). Consequently, if sexually antagonistic male traits are condition dependent then a male's juvenile diet might predict how costly it is to mate with him. For example, costs of mating have been attributed to the amount of harmful seminal substances (Sakaluk et al. 2006), sperm number (Perry and Rowe 2010) or the extent of physical injury caused by males (Johnstone and Keller 2000), that can all be condition dependent (Perry and Rowe 2010; Crean et al. 2016). To date, however, relatively few studies have tested whether male condition (or early diet) influences his costliness as a mate (but see Aluja et al. 2009).

The cost of mating for a female might depend not only on her mate's but also on her own condition. Females in poorer condition could be more susceptible. For example, in the ladybird beetle, *Adalia bipunctata*, food-deprived females resisted mating more frequently and for longer periods than well-fed females to reduce the energetic costs of mating (Perry et al. 2009). This type of condition dependent female response to males might be even more pronounced when faced with the prospect of mating with a male that has a phenotype that is associated with high costs of mating. For instance, females mating with attractive males are more likely to become infected with sexually transmitted diseases (Lombardo 1998). If females in poorer condition have fewer resources to allocate to immune function, they are less likely to be able to bear the costs and might therefore be more reluctant to mate with such males (e.g. Wilgers and Hebets 2012). On the other hand, gaining male-delivered resources from ejaculates and nuptial gifts might be more valuable to females in poorer condition. In general, we expect females to adaptively adjust their mating behavior to balance these conflicting concerns (Edvardsson 2007).

There is a vast literature on the condition dependence of male sexual displays (e.g. Rowe and Houle 1996; Kotiaho 2000; Tomkins et al. 2004; Hill 2011; Iglesias-Carrasco et al. 2016) and a reasonably large literature on condition dependent female mating preferences (e.g. Cotton et al. 2004; Syriatowicz and Brooks 2004; Hunt et al. 2005). To date, however, very few studies have asked how male and female condition "interact" to influence mate choice, mating behavior and, ultimately, the costs and benefits of mating that determine a female's net fitness.

The seed beetle, *Callosobruchus maculatus*, is a good model species to investigate condition dependent mating. Body condition is easily altered by manipulating the rearing environment during larval development, which affects adult body size (Cayetano and Bonduriansky 2015). Moreover, this species exhibits high levels of sexual conflict. Males have genital spines that harm females during copulation and reduce female survival and lifetime fecundity (Crudginton and Siva-Jothy 2000). Larger males take longer to transfer their ejaculates (Edvardsson and Canal 2006), which reduces female longevity due to the link between genital damage and copulation duration (Crudginton and Siva-Jothy 2000; Edvardsson and Tregenza 2005). However, bigger (i.e. better condition) males also transfer larger ejaculates that elevate female fecundity (i.e. total number of eggs laid by a female, Edvardsson 2007; South and Lewis 2011). The net effect of these opposing effects on lifetime female fitness is unclear. As counter-adaptation to male harm, females perform a conspicuous kicking behavior during mating that might assist in terminating mating (Crudginton and Siva-Jothy 2000; Edvardsson and Tregenza 2005; but see Wilson and Tomkins 2014).

Here we tested how the quality of the rearing environment (i.e. larval food availability), which influences adult body condition, affects male and female mating behavior and if this has consequences for female fitness. We predicted that: 1) females mating with males in better condition will try to terminate copulations sooner if these males impose higher mating costs; 2) females mated to males in better condition will have elevated fitness if the net effect of the longevity costs they impose are less than the fecundity and fertility (egg hatching) benefits conferred; 3) females in better condition will be better able to dislodge males, leading to shorter kicking times and shorter copulations; 4) females in better condition will have higher fecundity and longer lifespans because they have more resources; 5) if females in better condition are better able to cope with the costs of mating then any decline in fitness associated with mating with males in better condition will be greater for females in poorer condition.

METHODS

Study organism

The seed beetle *Callosobruchus maculatus* is a pest species of stored legumes. Their life cycle begins with females attaching an individual egg to the surface of a host bean (Messina 1991). The larva hatches, burrows into the bean and remains there for 3–4 weeks until it emerges as an adult (Southgate 1979).

We used beetles from a stock maintained since 2005 at the University of Western Australia, where they were kept in a stock cultures of >500 adults in ambient laboratory conditions (approximately 22–24 °C) on black-eyed beans (*Vigna unguiculata*). We then maintained stock cultures of >500 beetles in a constant temperature room (27 ± 1 °C) with a 14:10 h light:dark cycle for 4 generations prior to our experiment. Stock larvae were raised on black-eyed beans. *C. maculatus* adults are facultatively aphagous (Messina and Slade 1997) so, as is standard, adult beetles had no access to food or water.

Experimental design

To investigate how male and female condition interact to influence mating behavior and the fitness consequences for females, we used a 2 × 2 factorial design where both sexes' conditions were manipulated. We had 4 experimental groups: 1) good condition females mated with good condition males (GG, $n = 88$); 2) good condition females mated with poor condition males (GP, $n = 91$); 3) Poor condition females mated with good condition males (PG, $n = 90$); 4) poor condition females mated with poor condition males (PP, $n = 95$). The sample size was constrained by how many beetles emerged from each rearing diet treatment.

Experimental procedure and diet treatment

Larval competition during development is an important determinant of environmental quality (Guedes et al. 2007), and female *C. maculatus* prefer to oviposit on unused beans (Messina and Mitchell 1989). To obtain poor condition individuals, we therefore ensured that females laid eggs on beans that had been previously used by a single larva to create a low-quality feeding environment (see Cayetano and Bonduriansky 2015). To obtain good condition individuals, we provided females with unused beans to create a high-quality larval feeding environment. In our experiment, we used mung beans rather than black-eyed beans because mung beans are smaller, and a used bean is therefore a more limited

food resource. Our stock performs equally well on unused mung or black-eyed beans (McCorquodale, personal communication).

To obtain previously used beans, 70 mated stock females were placed individually in petri dishes with 20 mung beans each (*Vigna radiata*). We monitored the females constantly, and each time they laid an egg on a bean, the bean was removed and placed individually in an Eppendorf tube with a pinhole in the cap for airflow. We repeated this procedure until we had 1000 beans each with a single egg. Around 21 days later adults started to emerge and we collected the used beans to act as our low-quality feeding environment. Used beans were stored in ambient lab conditions for ~2 days prior to being used in our experiment.

To generate good and poor condition beetles, we placed 110 mated stock females in individual petri dishes each with 5 previously used beans (total $n = 525$) and each of another 110 females with 5 non-used beans (total $n = 523$). Again, females were constantly monitored and beans with a single egg were immediately placed individually in an Eppendorf tube. The adults that emerged ~21 days later became the focal individuals for our experiment. All the Eppendorf tubes were individually labeled to avoid pairing siblings in subsequent mating trials.

Behavioral trials

From day 21, Eppendorfs were checked daily for emerging individuals. The eclosion date was recorded and individuals were given 24 h to attain sexual maturity (Fox et al. 1995). We then created female–male pairs (GG, GP, PG, or PP). Immediately before pairing, we weighed individuals using a Sartorius microbalance ($\pm 1 \mu\text{g}$). We checked that the rearing environment had a strong effect. Both sexes were heavier in the high-quality diet treatment (both $P < 0.001$; mean \pm SE: good condition females = 6.070 ± 0.569 , bad condition females = 4.106 ± 1.106 , good condition males = 3.539 ± 0.439 , bad condition males = 2.897 ± 0.603).

To test for condition dependence of mating behavior, we placed each pair in an Eppendorf tube (1.5 mL) and allowed them to mate. As soon as we closed the lid, we started timing the mating trial. We recorded three components of mating behavior:

- 1) Kicking latency: This is the time from when copulation started (i.e. male mounted female, inserted his aedeagus and remained stationary) to when the female began moving her legs.
- 2) Kicking duration: Time from when a female started kicking to the end of the copulation.
- 3) Total copulation duration: Time from when copulation started to when the pair separated.

We reweighed the males immediately after the copulation to estimate the transferred ejaculate size (Edvardsson and Tregenza 2005). The same observer (M.I.C.) collected all data blind to the female or male diet treatment.

Measured fitness traits

After females completed their mating trial, they were individually placed in small plastic containers (60 mL) with approximately 40 mung beans. Females were then transferred to a new container with approximately 40 new mung beans every day for 4 consecutive days. The females remained in the fourth container until death. This provisioning matches females' fecundity, which is highest during the first day, decreasing quickly (Credland and Wright 1989). This allowed us to measure both egg laying rate (i.e. the number of eggs laid in a 24 h period for each of the 3 days following mating) as well as the number of eggs a female laid over her lifetime.

We visually counted both fertile (opaque) and non-fertile (translucent) eggs. Female survival was monitored every 24 h and lifespan was recorded as the number of days a female survived after the copulation. Once counted, eggs were maintained at 27 °C until the emergence of offspring. From day 21, we made daily checks of containers with eggs for emerging adults. We recorded the eclosion date of the first emerging offspring in each container to estimate development time. Once offspring started to emerge, they were counted and removed each day for 10 days. We calculated the percentage of eggs laid that produced emergent offspring (hereafter referred to as "egg–adult survival").

Statistical analyses

For all the traits measured, we first ran General Linear Models with Gaussian error distribution and checked their residuals. In the cases in which the residuals did not meet the assumptions of normality, we analyzed our data using Generalized Linear Models with the package lme4 (Bates et al. 2015).

Analysis of mating behavior

We used Generalized Linear Models (GLM) to test how male and female condition influenced kicking latency, kicking duration and copulation duration. Female condition (good or poor) and male condition (good or poor), and their interaction, were fixed factors. We specified a Poisson error distribution for our models. We corrected for overdispersion in each model by specifying a randomly assigned identification number as a random effect (Harrison 2014). Following this correction, all our models were underdispersed. A comparison of AIC values showed that correcting for overdispersion provided a better fit to the data than the non-corrected models ($P < 0.001$).

Analysis of ejaculate size

To determine whether our treatment had an effect on ejaculate size and relative ejaculate size (ejaculate mass/body mass) transferred by males, and to test whether these differences were related to female and male condition, we ran 2 separate Linear Models with male and female condition and the 2-way interaction as fixed variables.

Analysis of fitness measures

We tested how female and male condition influenced egg laying in 2 ways. First, the total number of eggs laid over a female's entire life (i.e. in all 4 containers) was specified as the response variable in a GLM with Poisson error distribution (we corrected for overdispersion as explained above; fit of model comparison $P < 0.001$). Female and male condition and their interaction were specified as fixed factors. Second, we tested whether female fecundity changed over time depending on male and/or female condition. For this model, we included only egg counts from the first, second, and third day after the copulation because the time for egg laying was standardized at 24 h. We used a Generalized Linear Mixed Model (GLMM) with female condition, male condition, and day (first, second, or third) as fixed factors. We also included all 2-way and 3-way interactions. We treated female identity as a random effect to control for individual variation, and we specified a Poisson error distribution (we corrected for overdispersion as explained above; fit of model comparison $P < 0.001$).

To determine whether female condition, male condition, or the time since mating influenced egg–adult survival, we ran a GLMM with a binomial error distribution using the cbind function (number of adults eclosing; number of unhatched eggs). We treated female

and male condition, day the eggs were collected and all 2- and 3-way interactions as fixed factors. Female identity was treated as a random effect. We constructed a similar model to look at the effects of female condition, male condition, and time since mating on offspring development time, but in this case the best-fit model was a Linear Mixed Model with a Gaussian error structure.

We used Cox proportional hazard models (function `coxph`, R package “survival,” (Therneau and Grambsch 2000) to test for effects of female and male condition and the interaction between them on female survival.

In all models, we initially included 2- and 3-way interactions to test if male and female condition interacted to affect fitness traits. We then ran these models without the interactions. If removal of interactions did not reduce the model fit, we interpreted the main effects from the reduced model (see Tables in the [Supplementary Information](#)). In all cases, we ran a type III sums of square Anova from the “car” package (Fox and Weisberg 2011) to get the *P* values that we present in the results section. All analyses were conducted in R 3.2.2 (R Core Team 2016).

RESULTS

Model parameter estimates and test statistics for all analyses are provided in the [Supplementary Information](#) (behavior—[Supplementary Table S1](#), ejaculate size—[Supplementary Table S2](#), and fitness traits—[Supplementary Tables S3, S4, and S5](#)).

Behavioral trials

There was no effect of female condition on the latency to kicking ($X^2 = 0.23$, $df = 1$, $P = 0.631$), but females kicked sooner when mating with a good condition male ($X^2 = 23.14$, $df = 1$, $P < 0.001$, [Figure 1a](#)). For kicking duration there was no effect of male condition ($X^2 = 3.77$, $df = 1$, $P = 0.052$), but females in better condition kicked for a significantly shorter amount of time ($X^2 = 33.04$, $df = 1$, $P < 0.001$, [Figure 1b](#)). Total copulation duration was significantly shorter for both females ($X^2 = 26.70$, $df = 1$, $P < 0.001$) and males ($X^2 = 5.07$, $df = 1$, $P = 0.024$, [Figure 1c](#)) in better condition. Mating behavior was not dependent on an interaction between male and female condition (kicking latency: $P = 0.532$; kicking duration: $P = 0.670$, total copulation: $P = 0.366$).

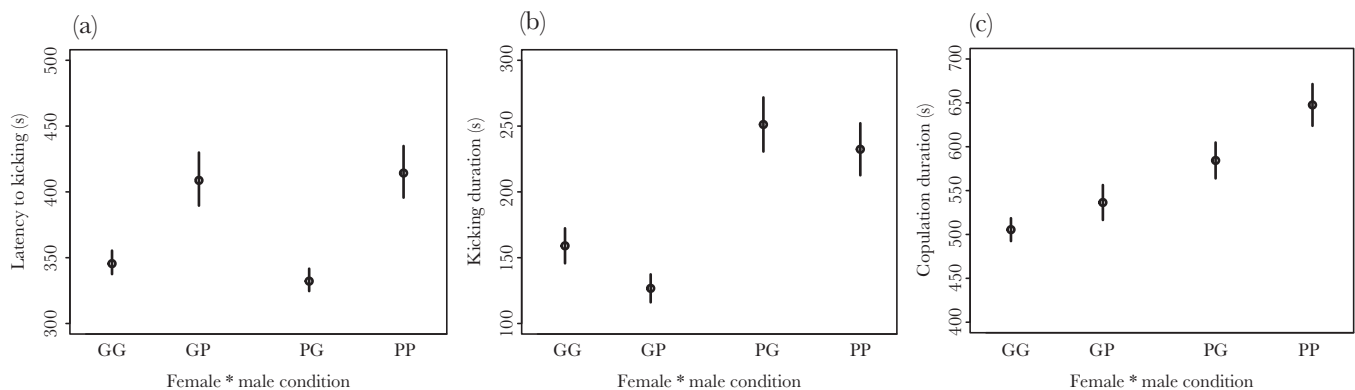


Figure 1 (a) Latency to kicking, (b) kicking duration, and (c) copulation duration (all mean \pm SE) in relation to female condition and the condition of the male she copulated with (GG: good condition female mated with good condition male; GP: good condition female mated with poor condition male; PG: poor condition female mated with good condition male; PP: poor condition female mated with poor condition male).

Male ejaculate size

Good condition males transferred larger ejaculates than poor condition males ($df = 1$, $F = 28.857$, $P < 0.001$), but ejaculate size did not depend on female condition ($df = 1$, $F = 0.303$, $P = 0.585$). There was also no 2-way interaction between male and female condition ($df = 1$, $F = 0.188$, $P = 0.620$). When controlled by body mass, we found that the relative ejaculate investment was not related to either male or female condition (male condition: $df = 1$, $F = 0.349$, $P = 0.555$; female condition: $df = 1$, $F = 0.197$, $P = 0.657$), or to the 2-way interaction between them ($df = 1$, $F = 0.011$, $P = 0.918$).

Fitness traits and female survival

Females in better condition laid significantly more eggs in total ($X^2 = 179.84$, $df = 1$, $P < 0.001$), but male condition had no effect ($X^2 = 0.018$, $df = 1$, $P = 0.892$, [Figure 2a](#)). There was no interaction between female and male condition ($X^2 = 0.313$, $df = 1$, $P = 0.077$). Females in better condition also laid significantly more eggs per day for the first 3 days ($X^2 = 146.34$, $df = 1$, $P < 0.001$), but there was no comparable effect of male condition ($X^2 = 0.00$, $df = 1$, $P = 0.998$). The number of eggs laid per day decreased significantly over time ($X^2 = 1608.31$, $df = 1$, $P < 0.001$, [Figure 2b](#)). There were no significant 2-way interactions that affected daily egg laying (male*female condition: $P = 0.059$; female condition*day: $P = 0.050$; Male condition*day: $P = 0.604$), nor was there a 3-way interaction ($P = 0.233$).

Egg-adult survival was significantly higher for females in good condition ($X^2 = 7.661$, $df = 1$, $P = 0.005$), and eggs that were laid on later days by females were less likely to survive to adulthood ($X^2 = 852.414$, $df = 3$, $P < 0.001$). This is due to a reduction in survival of offspring laid on day 4 compared to the previous 3 days (see [Figure 3b](#)). Male condition had no effect on patterns of egg-adult survival ($X^2 = 3.566$, $df = 1$, $P = 0.058$) and there were no significant 2-way interactions (female*male condition: $P = 0.187$; female condition*day: $P = 0.952$; male condition*day: $P = 0.979$), nor was there a significant 3-way interaction ($P = 0.397$).

For larval development time, females in better condition had offspring that developed significantly faster ($X^2 = 54.927$, $df = 1$, $P < 0.001$), but male condition had no effect ($X^2 = 2.691$, $df = 1$, $P = 0.101$). The longer the time since mating the longer it took for offspring to emerge ($X^2 = 535.17$, $df = 1$, $P < 0.001$, [Figure 4](#)). There was little evidence for 2- or 3-way interactions (male*female

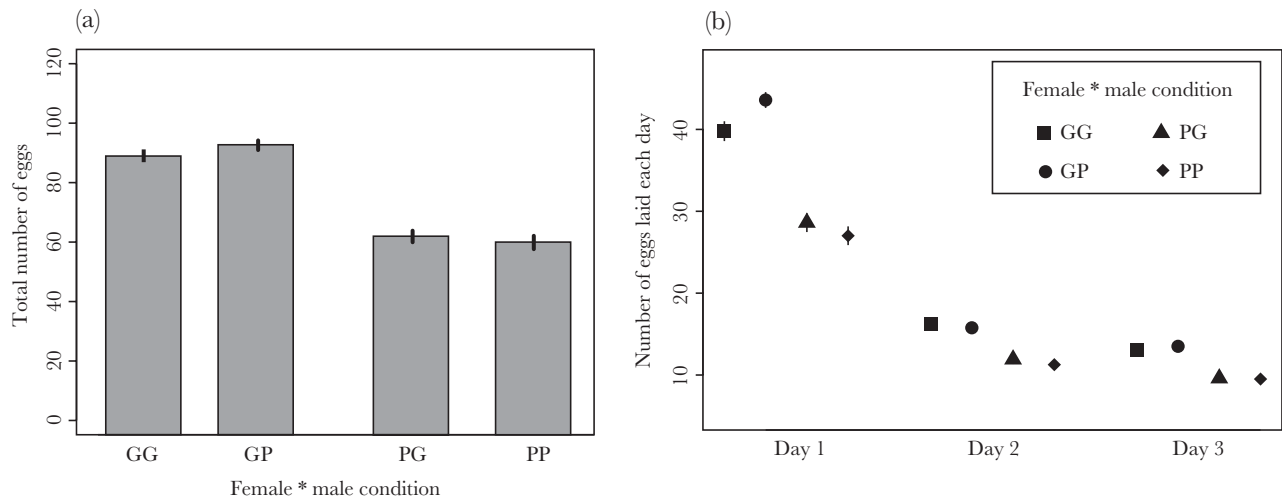


Figure 2

(a) Total number of eggs, and (b) number of eggs laid on each of the 3 days after copulation (all mean \pm SE) in relation to female condition and the condition of the male she copulated with (GG: good condition female mated with good condition male; GP: good condition female mated with poor condition male; PG: poor condition female mated with good condition male; PP: poor condition female mated with poor condition male).

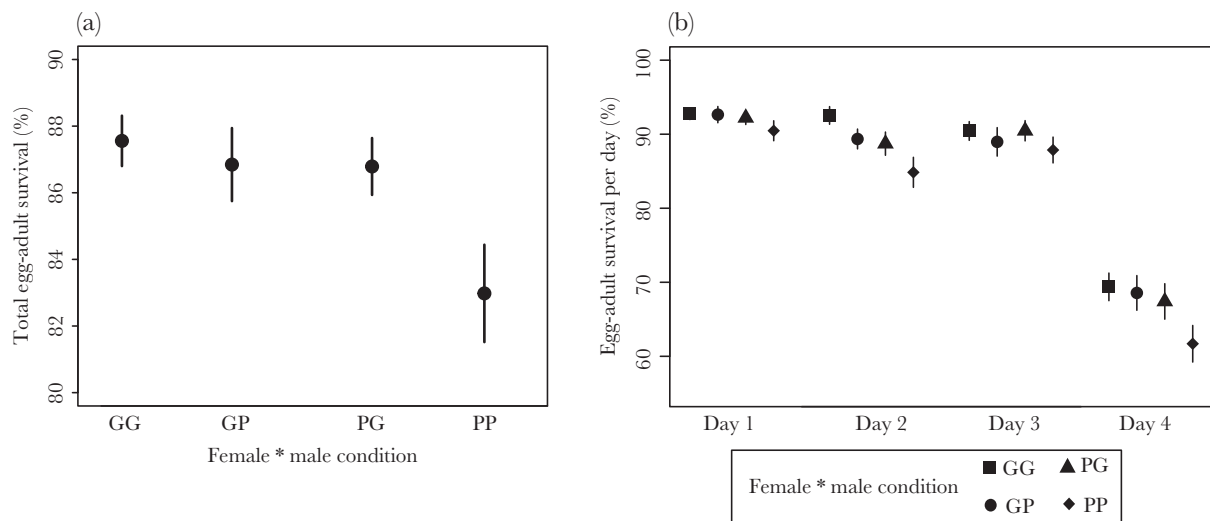


Figure 3

Percentage of (a) the total emerging adults (total egg-adult survival) and (b) the emerging adults for eggs laid on each of the 4 days after female copulation (all mean \pm SE) in relation to female and male condition (GG: good condition female mated with good condition male; GP: good condition female mated with poor condition male; PG: poor condition female mated with good condition male; PP: poor condition female mated with poor condition male). Note: Day 4 includes eggs laid on the fourth day or thereafter.

condition: $P = 0.857$; male condition*day: $P = 0.143$; 3-way: $P = 0.557$), although there was a weak interaction between female condition and day ($X^2 = 3.995$, $df = 1$, $P = 0.046$). Crucially, however, removing interactions did not reduce the model fit ($\chi^2 = 7.647$, $P = 0.105$).

Females in better condition lived significantly longer ($X^2 = 6.038$, $df = 1$, $P = 0.014$ Figure 5), but there was no effect of male condition on female survival ($X^2 = 0.024$, $df = 1$, $P = 0.876$). There was also no 2-way interaction between male and female condition ($X^2 = 0.465$, $df = 1$, $P = 0.495$).

DISCUSSION

We predicted that the cost of mating for females would depend on male and female body condition. To test this claim, we manipulated male and female body condition in *C. maculatus* by altering

their diet during larval development. As predicted we found that: 1) mating with a male in better condition led to the earlier onset of kicking and a shorter copulation duration; 2) females in better condition did not initiate kicking sooner, but they had a shorter copulation duration; and 3) females in better condition had higher fitness (higher fecundity, egg laying rate, longer longevity, higher egg-adult survival, and shorter development time of their offspring). Contrary to our predictions, however, females mated to males in better condition had no fitness advantages. Moreover, there was little evidence that female condition interacts with the magnitude of male-imposed mating costs to affect mating behavior or fitness. There were no significant interactions between the body conditions of mating pairs for any of our focal traits. This finding is not attributable to the ineffectiveness of our dietary treatments, nor to low statistical power, given the strong and significant main effects of male and female body condition.

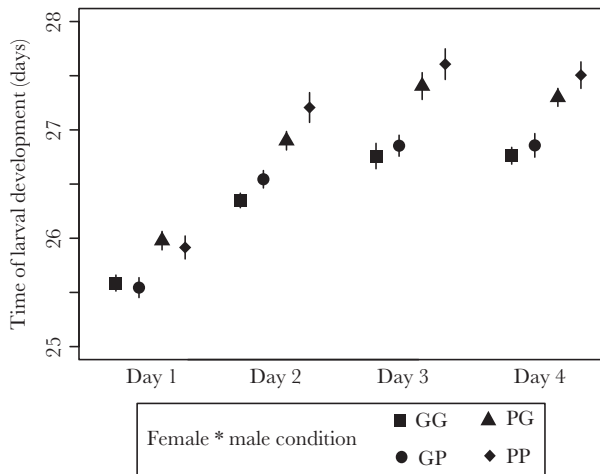


Figure 4

Offspring development time for eggs laid on each of the 4 days after female copulation (all mean \pm SE) in relation to female and male condition (GG: good condition female mated with good condition male; GP: good condition female mated with poor condition male; PG: poor condition female mated with good condition male; PP: poor condition female mated with poor condition male). Note: Day 4 includes eggs laid on the fourth day or thereafter

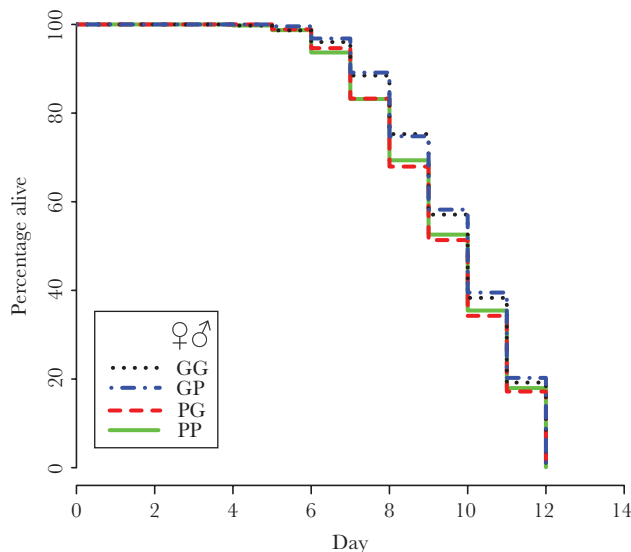


Figure 5

Kaplan-Meier survival plots for *C. maculatus* females in good and bad condition mated with good and poor condition males. Results showed no difference in female survival in relation to male condition.

Behavioral trials

The duration of copulation in *C. maculatus* is known to be associated with both gains and losses. For example, females receive hydration benefits from ejaculates (Ursprung et al. 2009), which might be critical in determining their propensity to remate (Edvardsson 2007). It is well known that ejaculate size, hence the benefits of hydration, increases with copulation duration (Edvardsson and Canal 2006). In contrast, prolonged copulation duration is associated with a cost due to increased genital damage (Crudgington and Siva-Jothy 2000; Edvardsson and Tregenza 2005). The effect of these conflicting consequences of copulation duration on net female fitness is

unclear. Even so, if we assume that females respond adaptively to males, we expect them to start kicking males sooner, to hasten the termination of copulation (Edvardsson and Canal 2006), when the net cost of being in prolonged copulation is higher. The duration of copulation should, however, reflect a balance between the optimal duration for males and females, which might depend on the condition of each sex.

If receiving a large ejaculate confers a net benefit, from a female perspective, we expect a longer delay to the onset of female kicking and prolonged copulation when mating with a male in good condition. We found, however, that females kicked “sooner” when the male was in good condition, which resulted in a “shorter” copulation duration. This finding could be related to male ability to transfer sperm to females. Previous studies have found a positive correlation between ejaculate size and female fecundity (Savalli and Fox 1999; Rönn et al. 2011). Thus, it might be expected that if poor condition males are less efficient at transferring sperm, females mating with these males might allow a longer copulation duration to ensure they acquire enough sperm to fertilize all their eggs. Our data show that poor condition males did indeed transfer smaller ejaculates, even with longer copulation durations, but that male condition did not influence female fecundity. Why females mate longer with males in poor condition remains unclear.

With respect to female condition, if receiving a large ejaculate confers a relatively larger net benefit for females in poor condition, we might expect them to delay the onset of kicking and engage in prolonged copulation. For example, female ladybirds *Adalia bipunctata* in poor condition exhibit increased mating resistance, since the costs of mating are greater for them than for females in good condition (Perry et al. 2009). Although there was no significant effect of female condition on the onset of kicking in *C. maculatus*, females in worse condition did copulate for significantly longer. If female behavior drives this pattern, it can be explained if: 1) females in poorer condition are less capable of resisting male attempts to prolong copulation, 2) females in poorer condition are not able to perform an effective kicking behavior, prolonging copulation duration, 3) gaining resources from male ejaculates are most valuable to females in poor condition, and 4) poor condition females are less choosy due to reduced opportunities to copulate in the future.

Alternatively, the longer copulation of poor condition females could be driven by males, for instance, if they adjust ejaculate size depending on female condition (i.e. males engage in strategic ejaculation; Kelly and Jennions 2011). Males of several insect species are known to adjust the quantity and/or composition of ejaculates in response to female size/fecundity (Thomas and Simmons 2007; Harley et al. 2013). Moreover, we might expect between-male differences in the allocation of ejaculates. For example, if poor condition males are less able to obtain copulations (e.g. less attractive), they should be less selective and make smaller ejaculate adjustments (Tazzyman et al. 2009). However, we did not find an effect of male or female body condition on the relative ejaculate size transferred by males. This suggests that males do not adjust ejaculation in relation to their own condition or that of their mate. This indicates that a more plausible explanation for our results is that there is adaptive plasticity in mating behavior, and copulating with males in better condition imposes greater net costs to females.

One explanation as to why mating with males in better condition might be more costly is related to semen toxicity and/or genital injury to females (Simmons 2001). In *C. maculatus*, male genital spines can wound females during copulation and this facilitates the circulation of seminal fluids into the body cavity (Hotzy et al.

2012). Males with longer spines are known to be more costly to females due to an increased genital damage, but they are more successful in gaining fertilizations and in sperm competition (Hotzy and Arnqvist 2009; Hotzy et al. 2012). However, increased genital damage when mating with males in better condition seems implausible, since the quality of the larval rearing environment has no effect on male genital traits, such as aedeagus spines, that damage females (Cayetano and Bonduriansky 2015). In contrast, in several insect species seminal fluids contain toxins that reduce female longevity and net fitness (e.g. Chapman et al. 1995; Yamane et al. 2008), and increasing the amount of ejaculate transferred increases the negative effect on female fitness (e.g. Chapman et al. 1995). We found that males in better condition transferred bigger ejaculates, which might increase the costs of copulating with good condition males. Similarly, bigger body size in male *Drosophila melanogaster* increases male mating success, but reduces female lifespan and egg-production rate (Pitnick and Garcia-Gonzalez 2002). There does not appear to be a compensatory fecundity benefit of mating with larger males in *C. maculatus*. Bigger ejaculates did not increase female fecundity in our experiment (there was no correlation between ejaculate size and fecundity, $R^2 = 0.061$, $P = 0.248$). The fact that female *C. maculatus* in better condition terminated copulations sooner can be parsimoniously attributed to their greater physical ability to repel males.

Female fitness

There was a strong positive effect of the larval rearing environment on female fitness. Females in better condition lived longer, had a higher rate of egg laying and laid more eggs. These eggs were more likely to result in adult offspring, and the speed with which their offspring matured was higher. These findings are unsurprising given that female body size was greater when reared on a high-quality diet, which indicates that these females had more resources that they could allocate both to reproduction and survival (see also Savalli and Fox 1999). In addition, it has been suggested that behaviors to avoid males, such as kicking during copulation, could be energetically costly in animals, like *C. maculatus*, that usually do not feed during the adult stage. Females in poorer condition spent longer kicking to remove a male, so the energetic costs of mating might also reduce their survival. Alternatively, the costs of genital damage might be higher for females in poorer condition if they need to allocate the limited resources to combat infections, reducing the resources used for self-maintenance.

Unexpectedly, beneficial effects of male condition on components of female fitness were not detected. For example, unlike Savalli and Fox (1999), we did not find that female *C. maculatus* mated to larger males had higher fecundity than those mated to smaller males (although in their study females were mated three times with the same male). Previous studies have found that males reared in high larval densities invest more in competitive traits in anticipation of more frequent mate encounters and greater sperm competition (Gage 1995; Stockley and Seal 2001). In such cases, poor condition males would end up investing more in their ejaculates, potentially transferring a similar amount of ejaculate as good condition males. This might explain the lack of a difference in the fecundity of females that copulated with poor and good condition males. However, we found that relative ejaculate size was similar for poor and good condition males and that poor condition males transferred smaller absolute ejaculates. Unlike previous studies which have found greater fertilization success (Tigreros 2013; Zikovitz and Agrawal 2013; Kahrl and Cox 2015), or increased egg-adult

survival (Polak et al. 2017) when mating with males in good condition, we found no benefits of mating with good condition males. Further, previous research on another species of seed beetle (*Stator limbatus*) has shown that females mated to larger males have larger eggs (Savalli and Fox 1998), whereas research on *C. maculatus* has shown that females mated to multiple males or females multiply mated with the same male lay larger eggs and have offspring with higher larval survival (Fox 1993; Eady et al. 2000). In sum, results from previous studies suggest that females gain resources from male ejaculates that females then allocate toward offspring (Fox 1993; Savalli and Fox 1998). Similarly, based on previous studies suggesting that the production of beneficial seminal fluids is condition dependent (Polak et al. 2017), we might have expected a paternal influence on early development in offspring in relation to male condition.

In general, we found little evidence for differences in the costs and benefits of mating with males in poor and good condition. This could be because, in a polyandrous species such as *C. maculatus*, a single copulation is insufficient to provoke detectable changes. For example, Savalli and Fox (1999) found a fecundity advantage for females mated with larger males, but only after several copulations with the same male. This suggests that resource depletion of poor condition males might only become apparent after several copulations, if their ability to produce sizeable ejaculates declines with the number of copulations. We found, however, that even in their first copulation, good condition males transferred larger ejaculates than poor condition males. This suggests that any effects of mating with good condition males could be detectable after a single copulation.

On the other hand, the costs of mating in *C. maculatus* are related not only to copulation itself, but also with the mating attempts of harassing males (e.g. Rönn et al. 2006). One limitation of our study is that we did not allow males to harass females, which might reduce the costliness of exposure to males in good condition. Likewise, we did not directly test for the costs of copulation for females (i.e. measuring scars in female bursa, Rönn et al. 2007; Dougherty and Simmons 2017). Even so, we found that a subset of excess females that were not mated in our experiment ($n = 23$) had longer lifespans than our experimental females (mean \pm SE: experiment females = 7.33 ± 1.42 ; not mated females = 18.39 ± 2.15). This suggests that there is a cost of mating (and egg laying) after a single copulation. We did not allow females to mate multiply or males to harass females because our aim was to examine the costs and benefits of copulation events, to understand the complex balance between male-imposed costs (i.e. genital spines and toxic ejaculate substances) and the benefits females gain from direct material benefits (i.e. larger ejaculates). Future studies to better understand the consequences and benefits of polyandry might benefit from exploring how male phenotype influences his costliness to females after multiple mating and harassing events.

Interactive effects of male and female conditions

If male traits involved in sexual conflict are condition dependent, then females mated to males in better condition might be expected to have lower fitness. However, females in better condition should be better able to cope with or reduce any such male-imposed costs. This suggests that there will be an interaction between male and female condition for fitness and for behavioral traits predicted to affect fitness (i.e. copulation duration). Contrary to this prediction, there were no detectable interactions between female and male condition for the eight behavioral traits and fitness components that we measured. One simple explanation for this is that there

was no evidence that male condition affects female fitness. Given the results of earlier studies (Savalli and Fox 1998; Savalli and Fox 1999), this suggests that any difference in costs imposed by the 2 types of males we created are, after a single mating, insufficient to induce large changes in female fitness in the traits measured. Moreover, in our experiment, the effect of the treatment on condition was stronger for females than for males. It is possible that the average reduction of 0.7 μg in the condition of males reared in previously used beans is insufficient to provoke detectable changes in female behavior and fitness. Alternatively, it is possible that the potential effects are masked by the stronger effect of our treatment on female condition (2 μg difference between good and poor condition females). As such, there is little scope to expect female and male condition to interact to affect mating behavior.

CONCLUSIONS

There was little evidence for a difference in the relative fitness of females that mated with males in poor or good condition in *C. maculatus*. In contrast, the quality of the female larval rearing environment has a strong effect on her fitness. Additionally, females in good condition were able to end copulations sooner than bad condition females, irrespective of the males' condition. It is possible that the costs and benefits of copulating with males in different condition were balanced for the fitness components we examined. Our results could indicate a tug-of-war between sexual conflict (i.e. potentially higher toxicity in good condition seminal fluids) and the benefits of mating with such males (i.e. larger water gifts). Our results indicate that any increase in the costs of mating with a male in better condition is not ameliorated by the female's own condition. Future studies may benefit from exploring the potential indirect costs and benefits of male phenotype in female offspring after multiple matings.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

FUNDING

This work was supported by Australian Research Council (M.J.), the European Society for Evolutionary Biology (Hewitt Mobility Award 2017 to M.I.C.), and a TalentHub grant from the Agencia Andaluza del Conocimiento (S.Z.).

We thank 3 anonymous reviewers for their helpful comments improving the manuscript, Sam Brookes and David Duchene for assistance in the lab and Joe Tomkins for providing beetles and advising how to look after them.

Data accessibility: Analyses reported in this article can be reproduced using the data provided by Iglesias-Carrasco et al (2018).

Handling editor: Dan Papaj

REFERENCES

Aluja M, Rull J, Sivinski J, Trujillo G, Pérez-Staples D. 2009. Male and female condition influence mating performance and sexual receptivity in two tropical fruit flies (Diptera: *Tephritidae*) with contrasting life histories. *J Insect Physiol.* 55:1091–1098.

Arnqvist G, Nilsson T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim Behav.* 60:145–164.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw.* 67:1–48.

Blanckenhorn WU, Hosken DJ, Martin OY, Reim C, Teuschl Y, Ward PI. 2002. The costs of copulating in the dung fly *Sepsis cynipsea*. *Behav Ecol.* 13:353–358.

Cayetano L, Bonduriansky R. 2015. Condition dependence of male and female genital structures in the seed beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae). *J Evol Biol.* 28:1364–1372.

Chapman T, Arnqvist G, Bangham J, Rowe L. 2003. Sexual conflict. *Trends Ecol Evol.* 18:41–47.

Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature.* 373:241–244.

Cotton S, Fowler K, Pomiankowski A. 2004. Condition dependence of sexual ornament size and variation in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). *Evolution.* 58:1038–1046.

Crean AJ, Adler MI, Bonduriansky R. 2016. Seminal fluid and mate choice: new predictions. *Trends Ecol Evol.* 31:253–255.

Credland PF, Wright AW. 1989. Factors affecting female fecundity in the cowpea seed beetle, *Callosobruchus maculatus* (Coleoptera, Bruchidae). *J Stored Prod Res.* 25:125–136.

Crudgington HS, Siva-Jothy MT. 2000. Genital damage, kicking and early death. *Nature.* 407:855–856.

Dougherty LR, Simmons LW. 2017. X-ray micro-CT scanning reveals temporal separation of male harm and female kicking during traumatic mating in seed beetles. *Proc R Soc London B Biol Sci.* 284:20170550.

Eady PE, Wilson N, Jackson M. 2000. Copulating with multiple mates enhances female fecundity but not egg-to-adult survival in the bruchid beetle *Callosobruchus maculatus*. *Evolution.* 54:2161–2165.

Edvardsson M. 2007. Female *Callosobruchus maculatus* mate when they are thirsty: resource-rich ejaculates as mating effort in a beetle. *Anim Behav.* 74:183–188.

Edvardsson M, Canal D. 2006. The effects of copulation duration in the bruchid beetle *Callosobruchus maculatus*. *Behav Ecol.* 17:430–434.

Edvardsson M, Tregenza T. 2005. Why do male *Callosobruchus maculatus* harm their mates? *Behav Ecol.* 16:788–793.

Fox CW. 1993. The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Oecologia.* 96:139–146.

Fox CW, Hickman DL, Raleigh L, Mousseau TA. 1995. Paternal investment in a seed beetle (Coleoptera: Bruchidae): influence of male size, age and mating history. *Entomol Soc Am.* 88:100–103.

Fox J, Weisberg S. 2011. An (R) companion to applied regression, 2nd edn. Thousand Oaks (CA): Sage. Available from: <http://socserv.socsci.mcmaster.ca/~jfox/Books/Companion>.

Gage MJG. 1995. Continuous variation in reproductive strategy as an adaptive response to population-density in the moth *Plodia interpunctella*. *Proc R Soc B Biol Sci.* 261:25–30.

García-González F, Simmons LW. 2010. Male-induced costs of mating for females compensated by offspring viability benefits in an insect. *J Evol Biol.* 23:2066–2075.

Grafen A. 1990. Biological signals as handicaps. *J Theor Biol.* 144:517–546.

Griffiths S. 1996. Sex differences in the trade-off between feeding and mating. *J Fish Biol.* 48:891–898.

Guedes RNC, Guedes NMP, Smith RH. 2007. Larval competition within seeds: from the behaviour process to the ecological outcome in the seed beetle *Callosobruchus maculatus*. *Austral Ecol.* 32:697–707.

Harley E, Birge LM, Small J, Tazzyman SJ, Pomiankowski A, Fowler K. 2013. Ejaculate investment and attractiveness in the stalk-eyed fly, *Diasemopsis meigenii*. *Ecol Evol.* 3:1529–1538.

Harrison XA. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ.* 2:e616.

Head ML, Hunt J, Jennions MD, Brooks R. 2005. The indirect benefits of mating with attractive males outweigh the direct costs. *PLoS Biol.* 3:e33.

Hill GE. 2011. Condition-dependent traits as signals of the functionality of vital cellular processes. *Ecol Lett.* 14:625–634.

Hoffmann AA. 1991. Heritable variation for territorial success in field-collected *Drosophila melanogaster*. *Am Nat.* 138:668–679.

Hotzy C, Arnqvist G. 2009. Sperm competition favors harmful males in seed beetles. *Curr Biol.* 19:404–407.

Hotzy C, Polak M, Rönn JL, Arnqvist G. 2012. Phenotypic engineering unveils the function of genital morphology. *Curr Biol.* 22:2258–2261.

Hunt J, Brooks R, Jennions MD. 2005. Female mate choice as a condition-dependent life-history trait. *Am Nat.* 166:79–92.

- Hurst GDD, Sharpe RG, Broomfield AH, Walker LE, Majerus TMO, Zakharov IA, Majerus MEN. 1995. Sexually transmitted disease in a promiscuous insect, *Adalia bipunctata*. *Ecol Entomol.* 20:230–236.
- Iglesias-Carrasco M, Head ML, Jennions MD, Cabido C. 2016. Condition-dependent trade-offs between sexual traits, body condition and immunity: the effect of novel habitats. *BMC Evol Biol.* 16:135.
- Iglesias-Carrasco M, Jennions MD, Zajitschek SRK, Head ML. 2018. Data from: are females in good condition better able to cope with costly males? Dryad Digital Repository. <http://dx.doi.org/10.5061/dryad.7kh2sh1>.
- Johnstone RA, Keller L. 2000. How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. *Am Nat.* 156:368–377.
- Kahl A, Cox RM. 2015. Diet affects ejaculate traits in a lizard with condition-dependent fertilization success. *Behav Ecol.* 26:1502–1511.
- Kelly CD, Jennions MD. 2011. Sexual selection and sperm quantity: meta-analyses of strategic ejaculation. *Biol Rev.* 86:863–884.
- Kotiaho JS. 2000. Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. *Behav Ecol Sociobiol.* 48:188–194.
- Kuijper B, Pen I, Weissing FJ. 2012. A guide to sexual selection theory. *Annu Rev Ecol Syst.* 43:287–311.
- Lehmann GUC, Lehmann AW. 2008. Bushcricket song as a clue for spermatophore size? *Behav Ecol Sociobiol.* 62:569–578.
- Lombardo MP. 1998. Evolution of sexually transmitted diseases in birds. *J Avian Biol.* 29:314–321.
- Magnhagen C. 1991. Predation risk as a cost of reproduction. *Trends Ecol Evol.* 6:183–186.
- Messina FJ. 1991. Life-history variation in a seed beetle: adult egg-laying vs. larval competitive ability. *Oecologia.* 85:447–455.
- Messina FJ, Mitchell R. 1989. Intraspecific variation in the egg-spacing behavior of the seed beetle *Callosobruchus maculatus*. *J Insect Behav.* 2:727–742.
- Messina F, Slade A. 1997. Inheritance of host-plant choice in the seed beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Ann Entomol Soc Am.* 90:848–855.
- Møller AP, Jennions MD. 2001. How important are direct fitness benefits of sexual selection? *Naturwissenschaften.* 88:401–415.
- Perry JC, Rowe L. 2010. Condition-dependent ejaculate size and composition in a ladybird beetle. *Proc R Soc B Biol Sci.* 277:3639–3647.
- Perry JC, Sharpe DMT, Rowe L. 2009. Condition-dependent female remating resistance generates sexual selection on male size in a ladybird beetle. *Anim Behav.* 77:743–748.
- Pitnick S, García-González F. 2002. Harm to females increases with male body size in *Drosophila melanogaster*. *Proc R Soc B Biol Sci.* 269:1821–1828.
- Polak M, Simmons LW, Benoit JB, Ruohonen K, Simpson SJ, Solon-Biet SM. 2017. Nutritional geometry of paternal effects on embryo mortality. *Proc R Soc B Biol Sci.* 284:20171492.
- R Core Team. 2016. R: A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. Available from: <https://www.R-project.org/>.
- Rönn J, Katvala M, Arnqvist G. 2006. The costs of mating and egg production in *Callosobruchus* seed beetles. *Anim Behav.* 72:335–342.
- Rönn J, Katvala M, Arnqvist G. 2007. Coevolution between harmful male genitalia and female resistance in seed beetles. *Proc Natl Acad Sci USA.* 104:10921–10925.
- Rönn JL, Katvala M, Arnqvist G. 2011. Correlated evolution between male and female primary reproductive characters in seed beetles. *Funct Ecol.* 25:634–640.
- Rowe L, Houle D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc R Soc B Biol Sci.* 263:1415–1421.
- Sakaluk SK, Avery RL, Weddle CB. 2006. Cryptic sexual conflict in gift-giving insects: chasing the chase-away. *Am Nat.* 167:94–104.
- Savalli UM, Fox CW. 1998. Sexual selection and the fitness consequences of male body size in the seed beetle *Stator limbatus*. *Anim Behav.* 55:473–483.
- Savalli UM, Fox CW. 1999. The effect of male size, age, and mating behavior on sexual selection in the seed beetle *Callosobruchus maculatus*. *Ethology Ecol Evol.* 11:49–60.
- Short SM, Lazzaro BP. 2010. Female and male genetic contributions to post-mating immune defence in female *Drosophila melanogaster*. *Proc R Soc B Biol Sci.* 277:3649–3657.
- Simmons LW. 2001. Sperm competition and its evolutionary consequences in the insects. Princeton (NJ): Princeton University Press.
- South A, Lewis SM. 2011. The influence of male ejaculate quantity on female fitness: a meta-analysis. *Biol Rev Camb Philos Soc.* 86:299–309.
- Southgate BJ. 1979. Biology of the Bruchidae. *Annu Rev Entomol.* 24:449–473.
- Stockley P, Seal NJ. 2001. Plasticity in reproductive effort of male dung flies (*Scatophaga stercoraria*) as a response to larval density. *Funct Ecol.* 15:96–102.
- Syriatowicz A, Brooks R. 2004. Sexual responsiveness is condition-dependent in female guppies, but preference functions are not. *BMC Ecol.* 4:5.
- Tazzyman SJ, Pizzari T, Seymour RM, Pomiankowski A. 2009. The evolution of continuous variation in ejaculate expenditure strategy. *Am Nat.* 174:E71–E82.
- Therneau T, Grambsch P. 2000. Modeling survival data: extending the Cox model. New York (USA): Springer.
- Thomas ML, Simmons LW. 2007. Male crickets adjust the viability of their sperm in response to female mating status. *Am Nat.* 170:190–195.
- Tigreros N. 2013. Linking nutrition and sexual selection across life stages in a model butterfly fly system. *Funct Ecol.* 27:145–154.
- Tomkins JL, Radwan J, Kotiaho JS, Tregenza T. 2004. Genic capture and resolving the lek paradox. *Trends Ecol Evol.* 19:323–328.
- Ursprung C, Den Hollander M, Gwynne DT. 2009. Female seed beetles, *Callosobruchus maculatus*, remate for male-supplied water rather than ejaculate nutrition. *Behav Ecol Sociobiol.* 63:781–788.
- Wilgers DJ, Hebets EA. 2012. Age-related female mating decisions are condition dependent in wolf spiders. *Behav Ecol Sociobiol.* 66:29–38.
- Wilson CJ, Tomkins JL. 2014. Countering counteradaptations: males hijack control of female kicking behavior. *Behav Ecol.* 25:470–476.
- Yamane T, Miyatake T, Kimura Y. 2008. Female mating receptivity after injection of male-derived extracts in *Callosobruchus maculatus*. *J Insect Physiol.* 54:1522–1527.
- Zikovitz AE, Agrawal AF. 2013. The condition dependency of fitness in males and females: the fitness consequences of juvenile diet assessed in environments differing in key adult resources. *Evolution.* 67:2849–2860.