



Original Article

Does developmental environment affect sexual conflict? An experimental test in the seed beetle

Zhuzhi Zhang^o and Megan L. Head^o

Division of Ecology and Evolution, Research School of Biology, Australian National University, Canberra, ACT, 2601, Australia

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Sexual conflict and sexually antagonistic coevolution are driven by differences in reproductive interests between the sexes. There have been numerous studies focused on how both the social and physical environment that individuals experience as adults, or where mating occurs, mediate the intensity of sexual conflict. However, how the physical environment that juveniles experience, mediates their later mating interactions, is still poorly understood. In seed beetles, *Callosobruchus maculatus*, water is an important resource that can impact fitness and reproduction. Here, we manipulated the water content of beans that beetles were reared in and explored how this environmental variation affects mating interactions and subsequent male and female fitness. We measured the mass of ejaculate transferred, mating behavior, female fecundity, and offspring production as well as male and female lifespan. We found that males reared in wet environments transferred a larger ejaculate to females, but only when females were reared in dry environments. We also found that females mated to males reared in dry environments laid more eggs than those mated to males from wet environments. Additionally, eggs laid by females reared in dry conditions had greater survival when they had mated to males reared in dry than wet environments. Overall, however, there were no treatment effects on the number of adult offspring females produced nor male or female adult lifespan, thus it is difficult to determine the evolutionary implications of these results. Our research provides evidence for the importance of developmental environment for determining the expression of adult mating and fitness traits.

Key words: *Callosobruchus maculatus*, costs of mating, ejaculate size, environment, fitness, sexual conflict, water.

INTRODUCTION

Sexual conflict is widespread and common in sexually reproducing organisms and is driven by different evolutionary interests and mating strategies of males and females (Arnqvist and Nilsson 2000; Chapman et al. 2003). For instance, in some species males elevate fitness through longer, and repeated copulations (Chapman et al. 2003) which result in greater ejaculate transfer (Edvardsson and Canal 2006) and higher fertilization success (Simmons 2001). However, for females, extended mating duration and higher copulation frequency can reduce fitness as a result of increased costs associated with intense male harassment, transfer of toxic substances in ejaculates, disease transmission, and genital injury (Stockley 1997; Watson et al. 1998; Simmons 2001; Takahashi and Watanabe 2010). In such situations, sex-specific selection to maximize the benefits and reduce the costs of mating, can lead to sexually antagonistic coevolution and drive an evolutionary arms race between the sexes.

How sexual conflict influences the behavior of males and females depends on the environment (Fricke et al. 2009; Vincent et al. 2020). The net costs and benefits of mating are dependent on the immediate environment in which mating occurs (Fricke et al. 2009). In addition, the conditions that individuals experience during development can affect traits that influence how males and females perform as adults and as a consequence may influence mating interactions (Perry and Rowe 2010; Iglesias-Carrasco et al. 2018b). For example, small male fruit flies, *Drosophila melanogaster*, reared in high population densities transfer ejaculates with a higher percentage of seminal proteins, that promote egg production and inhibit remating, than those reared at low densities (Wigby et al. 2016). Likewise, male cockroaches, *Nauphoeta cinerea*, reared with and without exposure to conspecific odor alter investment in sperm number and spermatophore size depending on the sex of the individual that produced the odor (Harris and Moore 2004). These examples show that individuals adjust their investment in reproductive traits depending on the social environment they experienced during development.

Address correspondence to Z. Zhang. E-mail: zhuzhi.zhang@anu.edu.au.

In addition to the effects of the social environment, resource availability experienced during development can play an important role in determining how adults invest in reproduction. Numerous studies across a broad range of taxa have demonstrated that developmental diet influences adult male and female reproductive traits. For instance, the effects of diet quality have been shown to affect reproductive traits in a range of species, like sperm quality and fecundity in flies (Macartney et al. 2018; Klepsatel et al. 2020), remating frequency and resistance in ladybirds Perry et al. 2009, sexually responsive and mate preferences in crickets (Hunt et al. 2005), mate preferences in spiders (Hebets et al. 2008), sperm reserves and sperm replenishment rate in fish (Vega-Trejo et al. 2016) and sexual signals in birds (Naguib and Nemitz 2007). However, all of these studies only manipulate diet in one sex or did not focus on the sexual interaction, and few studies explore how resource availability influences mating interactions and the resulting fitness of females involved in these matings.

The seed beetle (*Callosobruchus maculatus*) is an ideal species to explore how early life environments influence adult reproductive behavior and sexual conflict. In seed beetles there is sexual conflict over mating duration (Edvardsson and Canal 2006)—longer copulations allow males to transfer larger ejaculates (Van Lieshout et al. 2014), but also increase genital damage in females (Crudgington and Siva-Jothy 2000). As a result of this conflict, it is believed that females perform a behavioral adaptation (kicking) to end mating sooner (Edvardsson and Tregenza 2005; Van Lieshout et al. 2014). Further, evidence suggests that reproductive behavior in seed beetles may be mediated by their developmental environment. For instance, previous research has shown female kicking behavior is mediated by access to larval food resources, and that this may be important for regulating the benefits and costs of mating (Iglesias-Carrasco et al. 2018b).

Most previous research investigating how access to resources during development influences adult mating behavior has focused on manipulating juvenile food intake (Vega-Trejo et al. 2016, but see Iglesias-Carrasco et al. 2018b). However, water is also an important resource that could influence adult phenotypes—particularly in species like seed beetles which inhabit dry environments. Previous studies have manipulated water availability in adult seed beetles and found that females that have had access to water have lower remating rates than females who have not had access to water and that females with access to water also live longer (Edvardsson 2007; Ursprung et al. 2009; Iglesias-Carrasco et al. 2018a; Vincent et al. 2020) and sometimes have greater lifetime fecundity (Edvardsson 2007; Ursprung et al. 2009). These results have led to the suggestion that water is a limiting resource that females obtain during mating and that males may transfer water-rich ejaculates to females in order to prevent female remating (Edvardsson 2007). Subsequently, studies have looked at how access to water during adulthood affects mating interactions and their fitness consequences in an attempt to elucidate the potential for water availability to mediate sexual conflict (Iglesias-Carrasco et al. 2018a; Vincent et al. 2020). These studies found no evidence that male or female access to water affects ejaculate size, but that water availability does affect mating behavior. Specifically, pairs containing males that have had access to water copulate for longer and spend more time kicking, and pairs in which both males and females have had access to water had the longest kicking durations (Vincent et al. 2020). One interpretation of this result is that water availability affects the escalation of male persistence and female resistance through effects on condition. However, it is currently unknown how water availability

during development influences subsequent mating interactions between adults or the fitness consequences of these interactions.

In this study, we raise seed beetles in beans with low or high moisture content and then pair males and females emerging from these beans (using a 2×2 factorial design) to look at the effects of developmental water availability on ejaculate size, mating behavior, and fitness. Given the findings of previous studies (described above) we hypothesize that water availability during development could mediate sexual conflict via two pathways. It could affect the condition of males and females and hence lead to escalated persistence and resistance in pairs (i.e. longer kicking durations) where both sexes have had access to water, like seen when manipulating adult access to water. Such an effect would be indicative of increased sexual conflict. Alternatively, having greater access to water during development could increase the amount of water males are able to transfer to females in their ejaculates and/or decrease female need for water, subsequently leading to changes in ejaculation size and reproductive performance. If, as has been previously suggested, water is an important resource that females acquire during mating (Edvardsson 2007), then this could lead to divergence in optimal male and female expression of mating traits (e.g. wet males prefer to mate for longer/transfer larger ejaculates, but wet females prefer to mate for shorter/receive smaller ejaculates) which could subsequently reduce fitness of pairs where both sexes are reared in wet environments.

METHODS

Study species

Callosobruchus maculatus is a pest of stored legumes. Females lay eggs on the surface of host beans into which larvae burrow after hatching. Larvae feed on the endosperm of the bean, pupate, and then emerge as adults 21 to 29 days later (depending on the ambient temperature and humidity) (Fox 1993). Beetles used in our study were from a large stock population maintained at The Australian National University, on mung beans, *Vigna radiata*, for 24 generations and incubated at $25 \pm 1^\circ\text{C}$.

Generating “wet” and “dry” beans

To generate wet and dry beans, 10 to 20 beans were placed into individual 70 ml plastic vials. Vials for generating wet beans were stored with the lid open in an enclosed box with a wet towel on the bottom for one day at $23.5 \pm 0.5^\circ\text{C}$. Vials for generating dry beans were stored in exactly the same way, except the box contained no wet towel. All vials of beans were weighed (to 0.1 mg, using an electronic balance, Mettler Toledo AG135) before and after incubating to measure their water gain, which was used to evaluate the moisture content of the beans. Beans in the wet treatment gained $1.57 \pm 0.78\%$ (mean \pm SE) of their original weight and beans in the dry treatment gained $0.11\% \pm 0.10\%$ (mean \pm SE) of their original weight.

Generating experimental beetles

To generate our experimental beetles, we randomly picked 100 mated female beetles from our lab stock and placed each of them with 60 beans (30 wet and 30 dry) over a 2 day period. Egg laying was divided into two phases. In the first phase (i.e. day 1) each female was provided with 10 wet and 10 dry beans at the same time. Beetles were given 2hrs to lay on these beans. After that, each female was provided with 20 wet or 20 dry beans over two consecutive 24 hour periods. In this second phase, the order that wet and dry beans were presented to the female was decided by coin toss (i.e. random). After laying eggs, females were removed and the

beans were checked—if there was more than one egg on a bean, the extra eggs were scraped off to ensure each bean had only one egg. Each bean was then placed in an individual Eppendorf tube with a small hole in the lid for ventilation, and all Eppendorf tubes were allocated with a unique ID to keep track of each beetle. We kept a maximum of 5 beans from each female for each water treatment. Eppendorf tubes with beans were then incubated at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for around 28 days, following which virgin adults started to emerge. When beetles emerged, they were sexed and left for one day without food or water, prior to being used in mating trials.

Mating trials and measurement of fitness traits

To test how the rearing environment of males and females influenced mating behavior we paired one-day old adult beetles emerging from wet and dry beans over a 30 day period. We used a 2×2 factorial (wet and dry environment versus two sexes) design so that we could look at the effects of both male and female rearing environment independently, as well as their interaction. This resulted in four pair combinations: dry male with dry female ($\sigma\text{D} \times \varphi\text{D}$, $n = 90$); dry male with wet female ($\sigma\text{D} \times \varphi\text{W}$, $n = 86$); wet male with dry female ($\sigma\text{W} \times \varphi\text{D}$, $n = 84$); and wet male with wet female ($\sigma\text{W} \times \varphi\text{W}$, $n = 82$). On any given day males and females were paired randomly except that sibling beetles were never paired to avoid potential effects of inbreeding. All beetles were weighed to 0.001 mg, using a Sartorius Cubis microbalance, before mating.

To begin a mating trial, a male was added to a female's Eppendorf tube and both beetles were knocked to the bottom of the tube to ensure they made contact with each other. We then observed the pairs' mating behavior. The latency to mating was recorded as the time from when the male and female first made contact to when the male climbed on the female's back and inserted his aedeagus into her reproductive tract. Latency to kicking was recorded as the time from when the mating started to when the female started kicking the male, this measure reflects the optimum mating duration of females (Edvardsson and Canal 2006). Kicking duration was the time from when the female started kicking to when the copulation ended and total copulation duration was the time from when mating started to when it ended. If beetles did not start mating within 10 minutes, they were separated and the pair was recorded as not mating and excluded from subsequent analyses. There were 5 pairs that did not mate (0 of $\sigma\text{D} \times \varphi\text{D}$, 3 of $\sigma\text{D} \times \varphi\text{W}$, 1 of $\sigma\text{W} \times \varphi\text{D}$ and 1 of $\sigma\text{W} \times \varphi\text{W}$). After mating, males were weighed again so that we could calculate their ejaculate weight (weight before mating—weight after mating), and then they were transferred back to their original Eppendorf tube; females were placed in individual vials with 20 untreated beans (i.e. beans stored in the freezer that we use to rear stock beetles) to lay eggs for 24 hours.

To look at remating behavior and introduce different levels of multiple mating, two-thirds of pairs were placed together again for a second mating trial. Although this species is unlikely to remate with the same partner, our experimental beetles were remated with the same partner for the following reasons: first, it allowed us to control for variation in male traits (e.g. the male size) in our analyses; second, it removed sperm competition between males, which could mask effects of the male treatment; and third, it prevented confounding the number of matings with the number of mates when comparing our remating status treatments. We set up two-thirds of the pairs to remate because we estimated based on previous work, that only around half of the remating pairs would actually mate (Savalli and Fox 1998). This resulted in

three types of pairs with different remating status: pairs that were placed together and remated (remated group, $N = 112$), pairs that were placed together for 10 min to remate but they did not (failed mating group, $N = 115$), and pairs that were not allowed to remate (not re-paired group, $N = 115$). This second trial was conducted in the same way as outlined above for the first mating trial and the same behaviors were recorded. After the second mating trials were completed, all females were transferred into new vials, containing 40 untreated beans, to lay eggs until they died. After the mating trials both males and females were monitored daily to determine their adult lifespan. Once females died, we counted eggs in both of their vials. Once eggs were counted, these were placed back in the incubator, left until the tenth day after the first offspring emerged from each vial, and then frozen to allow us to count the number of offspring emerging as adults in each vial. From these data, we also calculated the proportion of eggs surviving to adulthood (hereafter termed egg-to-adult survival). This gave us two measures of female fecundity, number of adult offspring, and egg-to-adult survival: those measured following the first mating (referred to as “Day 1”) where eggs were laid over a 24-hour period prior to the second mating trial and thus where females had only mated once; and those measured following the second mating trial (referred to as “Rest of life”).

Statistical analysis

To test how bean moisture affects mating traits and subsequent female/offspring fitness traits, we analyzed our data using linear mixed models (LMM) and generalized linear mixed models (GLMM) with the “glmmTMB” package (Brooks et al. 2017) in R (version 3.6.2), including mother ID (of both males and females as appropriate) as a random effect to control for potential non-independence of siblings (number of siblings per family ranged from 1 to 15: mean = 7.857). For all linear models (i.e. those with a Gaussian error distribution) we checked model residuals to ensure they met the assumptions of normality and heteroscedasticity. We used the “summary” function to obtain parameter estimates and the “Anova” function to determine statistical significance. In all models, when two-way interactions were significant, we conducted post hoc pairwise comparisons using the “lsmeans” function in the emmeans package (Searle et al. 1980) to determine which treatments differed. When two-way interactions were not significant, we dropped them from the models so that we could interpret the main effects (Engqvist 2005). For transparency, in our model output tables (provided in the Appendix) we show results for both full models and main effects models. Figures were plotted using the “ggplot2” package (Wickham 2016). Further model details are provided below. Sample sizes analyzed for all traits are shown in Supplementary Tables A1–A3.

Body weight

Because the adult body weight of seed beetles can affect their mating behavior and fitness traits, we looked at whether males and females reared in wet and dry beans differed in body size prior to conducting further analyses. We modeled body weight separately for males and females because large size differences between the sexes are known in *C. maculatus*, and we did not want this variation to swamp potential within sex variation. Male and female body weights were both analyzed using LMM. In this model, we included the bean water treatment (wet or dry) as a fixed effect.

Mating traits

In order to analyze the effect of water availability during development, we ran separate models for male ejaculate weight, mating behavior (i.e. behavioral data collected during a pair's first mating assay—mating duration, latency to kick and kicking duration), and remating behavior (behavioral data collected during a pair's second mating assay—mating duration, latency to kick and kicking duration) were all analyzed using LMM. We included the water treatment of both males and females as well as their interaction as fixed effects. In addition, we included male and female body weight as covariates, as body size of both sexes is known from previous studies to influence these traits (Savalli and Fox 1998; Edvardsson and Canal 2006). The likelihood of remating was analyzed using GLMM with a binomial error distribution. Here, we included water treatment of both males and females as well as their interaction as fixed effects, and male and female body weight as covariates.

Fitness traits

The fecundity of females on day 1 and for the rest of their life (i.e. before and after the second mating trial) were analyzed separately using GLMMs with a Poisson distribution and an observation level random effect to reduce overdispersion. This was done so that we could tease apart short-term and long-term treatment effects. For the number of eggs laid on day 1, we included the water treatment of both males and females, as well as their interaction as fixed effects. In addition, we included the body weight of both males and females as covariates, because body weight of both sexes is known from previous studies to affect fecundity (Savalli and Fox 1999). For the number of eggs laid during the rest of life, we additionally included the remating status (i.e. remated, failed mating, and not re-paired) as well as its interactions with male and female rearing treatment as fixed effects.

Egg-to-adult survival of eggs laid by experimental females on day 1 and during the rest of life were also analyzed separately. These models were the same as those outlined for the number of eggs, except that we used a binomial error distribution to analyze the proportion of beans with an egg that had beetles emerge from them as adults. Our response variable was created using the “cbind” function (number of emerged adults; number of unhatched eggs) and can be interpreted as egg to adult survival, weighting for the total number of eggs laid by each female. The number of adult offspring emerging was analyzed in the same way as fecundity.

Male and female lifespan were modeled separately as the sexes are known to differ in life span, and we were not interested in comparing them. Lifespan was analyzed using a Cox proportional hazard model (function `coxph`, R package “survival,” Therneau and Grambsch 2000). In these models, we included the water treatment (wet or dry) of the focal beetle and their partner, as well as the remating status and all their two-way interactions as fixed effects. In addition, the focal beetle's body weight was included as a covariate, as this is known to influence lifespan (Fox et al. 2003).

RESULTS

Body weight

The water content of beans that beetles were reared in did not significantly affect the adult body weight of males or females (Table 1 and Supplementary Table A4).

Ejaculate weight

The rearing treatment of males and females interacted to affect male ejaculate weight (Table 1 and Supplementary Table A5). Males reared in wet beans transferred larger ejaculates to females reared in dry beans, compared to all other male—female pair combinations (all pairwise: $P < 0.001$, Supplementary Table A6, Figure 1). In addition, larger males had larger ejaculates (Table 1 and Supplementary Table A5).

Mating behavior

None of the mating behaviors recorded during the first mating trial were significantly affected by the male's or female's rearing treatment (Table 1, Supplementary Tables A7–A9). Latency to kicking and the kicking duration were, however, associated with both male and female body weight (Table 1, Supplementary Tables A7 and A9). Females started kicking sooner, and kicked for longer, when they were smaller (Table 1, Supplementary Tables A7 and A9) and when the male they were mating with was larger (Table 1, Supplementary Tables A7 and A9).

Remating behavior

Whether a pair remated or not was unaffected by the rearing treatment of males or females, nor the interaction between them (Table 1 and Supplementary Table A10). The likelihood of remating was however affected by male body weight, with smaller males being more likely to remate than larger males (Table 1 and Supplementary Table A10). When looking at mating behavior during a pair's second mating (for those that mated), we found that none of the mating behaviors were significantly affected by either the male's or the female's rearing treatment (Table 1, Supplementary Tables A11–A13). Latency to kicking and mating duration were, however, associated with male body weight. Females kicked sooner when they were mating with larger males (Table 1 and Supplementary Table A11) and mating ended sooner when the male involved was larger (Table 1 and Supplementary Table A12).

Fitness traits

In the first 24 hours after their first mating females mated with males reared in dry beans laid more eggs than those mated with males reared in wet beans (Figure 2), but there was no effect of the females own rearing treatment, nor an interaction between male and female rearing treatment (Table 1 and Supplementary Table A14). In contrast, the likelihood that eggs laid after the first mating survived to adulthood was affected by the interaction between male and female rearing treatment (Table 1 and Supplementary Table A15, Figure 3). When we look more closely at this result, we see that eggs laid by wet females mated to dry males were less likely to survive until emergence than eggs laid by dry females mated to dry males (t ratio = 2.295, $df = 325$, $P = 0.022$, Supplementary Table A16). Further, the number of adult offspring that a female produced in the 24 hours following the first mating was not affected by the rearing treatment of males, females nor their interaction (Table 1 and Supplementary Table A17). We also found that larger females laid more eggs and had more adult offspring emerging after the first mating (Table 1 and Supplementary Table A17).

After the second mating assay, female fecundity was not affected by the rearing treatment of males, females nor their interaction. It was also not affected by the remating status or the two-way interactions between remating status and either the male or female

Table 1

Summary of the ANOVA results showing the effect of male and female developmental water treatment on all traits measured in our experiment, degrees of freedom were shown in brackets after Chi-square. Significance of the main effects comes from models after nonsignificant interactions were removed “–” indicates the effect was not included in the model (or in the case of main effects that the interaction was significant and so could not be interpreted). Significant effects are highlighted in bold.

Trait	Male water	Female water	Male Water * Female Water	Male body weight	Female body weight	Remating status	Remating status * Male Water	Remating status * Female Water
Male body weight at emergence	$\chi^2_{(1)} = 0.387, P = 0.534$	–	–	–	–	–	–	–
Female body weight at emergence	–	$\chi^2_{(1)} = 0.263, P = 0.608$	–	–	–	–	–	–
Male ejaculate weight (first mating)	–	–	$\chi^2_{(1)} = 6.797, P = \mathbf{0.009}$	$\chi^2_{(1)} = 43.276, P < \mathbf{0.001}$	$\chi^2_{(1)} = 3.084, P = 0.079$	–	–	–
Kicking latency (first mating)	$\chi^2_{(1)} = 0.299, P = 0.584$	$\chi^2_{(1)} = 1.270, P = 0.260$	$\chi^2_{(1)} = 0.133, P = 0.715$	$\chi^2_{(1)} = 10.786, P = \mathbf{0.001}$	$\chi^2_{(1)} = 5.779, P = \mathbf{0.016}$	–	–	–
Mating duration (first mating)	$\chi^2_{(1)} = 0.462, P = 0.497$	$\chi^2_{(1)} = 0.103, P = 0.748$	$\chi^2_{(1)} = 0.259, P = 0.611$	$\chi^2_{(1)} = 0.992, P = 0.319$	$\chi^2_{(1)} = 0.509, P = 0.476$	–	–	–
Kicking duration (first mating)	$\chi^2_{(1)} = 0.162, P = 0.687$	$\chi^2_{(1)} = 0.243, P = 0.622$	$\chi^2_{(1)} = 0.012, P = 0.911$	$\chi^2_{(1)} = 8.835, P = \mathbf{0.003}$	$\chi^2_{(1)} = 5.970, P = \mathbf{0.015}$	–	–	–
Likelihood of remating	$\chi^2_{(1)} = 0.150, P = 0.698$	$\chi^2_{(1)} = 0.192, P = 0.661$	$\chi^2_{(1)} = 0.033, P = 0.856$	$\chi^2_{(1)} = 9.427, P = \mathbf{0.002}$	$\chi^2_{(1)} = 3.072, P = 0.080$	–	–	–
Kicking latency (second mating)	$\chi^2_{(1)} = 1.015, P = 0.314$	$\chi^2_{(1)} = 0.002, P = 0.961$	$\chi^2_{(1)} = 1.896, P = 0.168$	$\chi^2_{(1)} = 8.176, P = \mathbf{0.002}$	$\chi^2_{(1)} = 0.061, P = 0.805$	–	–	–
Mating duration (second mating)	$\chi^2_{(1)} = 3.630, P = 0.057$	$\chi^2_{(1)} = 0.242, P = 0.623$	$\chi^2_{(1)} = 1.773, P = 0.183$	$\chi^2_{(1)} = 6.102, P = \mathbf{0.013}$	$\chi^2_{(1)} = 0.114, P = 0.736$	–	–	–
Kicking duration (second mating)	$\chi^2_{(1)} = 3.736, P = 0.053$	$\chi^2_{(1)} = 1.160, P = 0.281$	$\chi^2_{(1)} = 0.007, P = 0.934$	$\chi^2_{(1)} = 0.176, P = 0.675$	$\chi^2_{(1)} = 1.302, P = 0.254$	–	–	–
Female fecundity after the first mating	$\chi^2_{(1)} = 5.154, P = \mathbf{0.023}$	$\chi^2_{(1)} = 1.674, P = 0.196$	$\chi^2_{(1)} = 1.768, P = 0.184$	$\chi^2_{(1)} = 3.812, P = 0.051$	$\chi^2_{(1)} = 21.584, P < \mathbf{0.001}$	–	–	–
Egg-to-adult survival after the first mating	–	–	$\chi^2_{(1)} = 8.231, P = \mathbf{0.004}$	$\chi^2_{(1)} = 1.975, P = 0.160$	$\chi^2_{(1)} = 2.538, P = 0.111$	–	–	–
Number of adult offspring after the first mating	$\chi^2_{(1)} = 2.793, P = 0.095$	$\chi^2_{(1)} = 3.380, P = 0.066$	$\chi^2_{(1)} = 0.035, P = 0.851$	$\chi^2_{(1)} = 2.015, P = 0.156$	$\chi^2_{(1)} = 8.104, P = \mathbf{0.004}$	–	–	–
Female fecundity after second mating	$\chi^2_{(1)} = 2.566, P = 0.109$	$\chi^2_{(1)} = 0.045, P = 0.832$	$\chi^2_{(1)} = 0.396, P = 0.529$	$\chi^2_{(1)} = 2.710, P = 0.100$	$\chi^2_{(1)} = 127.166, P < \mathbf{0.001}$	$\chi^2_{(2)} = 0.585, P = 0.747$	$\chi^2_{(2)} = 0.894, P = 0.640$	$\chi^2_{(2)} = 3.260, P = 0.196$
Egg-to-adult survival after second mating	$\chi^2_{(1)} = 0.189, P = 0.664$	$\chi^2_{(1)} = 1.113, P = 0.291$	$\chi^2_{(1)} = 0.050, P = 0.823$	$\chi^2_{(1)} = 0.960, P = 0.327$	$\chi^2_{(1)} = 9.974, P = \mathbf{0.002}$	$\chi^2_{(2)} = 1.094, P = 0.869$	$\chi^2_{(2)} = 12.143, P = \mathbf{0.002}$	$\chi^2_{(2)} = 2.355, P = 0.308$
Number of adult offspring after second mating	$\chi^2_{(1)} = 0.579, P = 0.447$	$\chi^2_{(1)} = 0.004, P = 0.947$	$\chi^2_{(1)} = 1.283, P = 0.257$	$\chi^2_{(1)} = 0.175, P = 0.676$	$\chi^2_{(1)} = 69.846, P < \mathbf{0.001}$	$\chi^2_{(2)} = 0.800, P = 0.670$	$\chi^2_{(2)} = 1.663, P = 0.435$	$\chi^2_{(2)} = 3.501, P = 0.174$
Male lifespan	$\chi^2_{(1)} = 0.179, P = 0.673$	$\chi^2_{(1)} = 1.023, P = 0.312$	$\chi^2_{(1)} = 0.028, P = 0.868$	$\chi^2_{(1)} = 98.429, P < \mathbf{0.001}$	$\chi^2_{(1)} = 1.513, P = 0.219$	$\chi^2_{(2)} = 6.058, P = \mathbf{0.048}$	–	–
Female lifespan	$\chi^2_{(1)} = 0.344, P = 0.558$	$\chi^2_{(1)} = 0.588, P = 0.443$	$\chi^2_{(1)} = 0.728, P = 0.393$	$\chi^2_{(1)} = 0.495, P = 0.482$	$\chi^2_{(1)} = 26.292, P < \mathbf{0.001}$	$\chi^2_{(2)} = 3.061, P = 0.216$	–	–

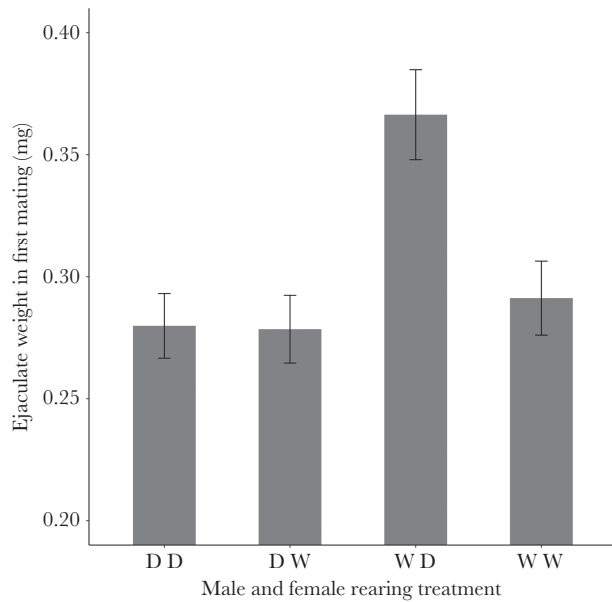


Figure 1
Effect of male and female rearing treatment on ejaculate weight (male body weight before mating—male body weight after mating) in the first copulation. DD: dry males mating with dry females mating group; DW: dry males mating with wet females mating group; WD: wet males mating with dry females mating group; WW: wet males mating with wet females mating group). Raw means \pm SE are presented.

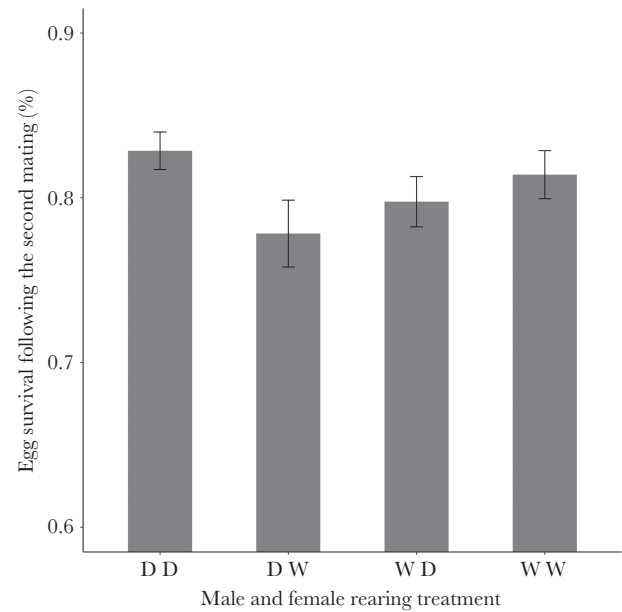


Figure 3
Effect of male and female rearing treatment on egg-to-adult survival following the first mating. DD: dry males mating with dry females mating group; DW: dry males mating with wet females mating group; WD: wet males mating with dry females mating group; WW: wet males mating with wet females mating group. Raw means \pm SE are presented.

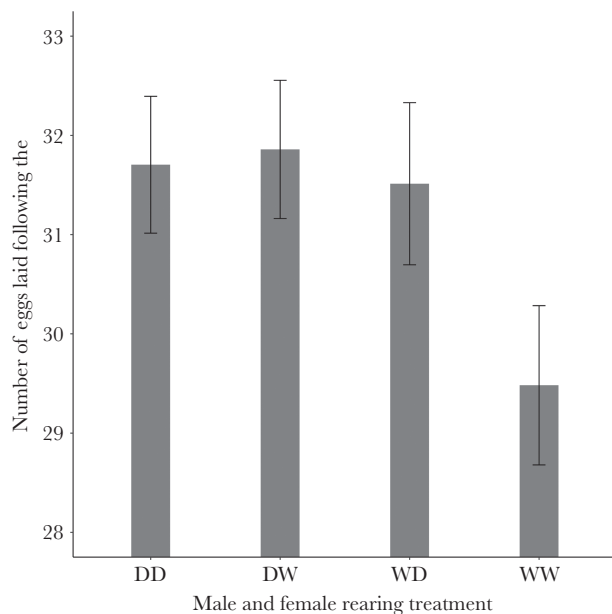


Figure 2
Effect of male and female rearing treatment on the number of eggs females laid following the first mating. Raw means \pm SE are presented.

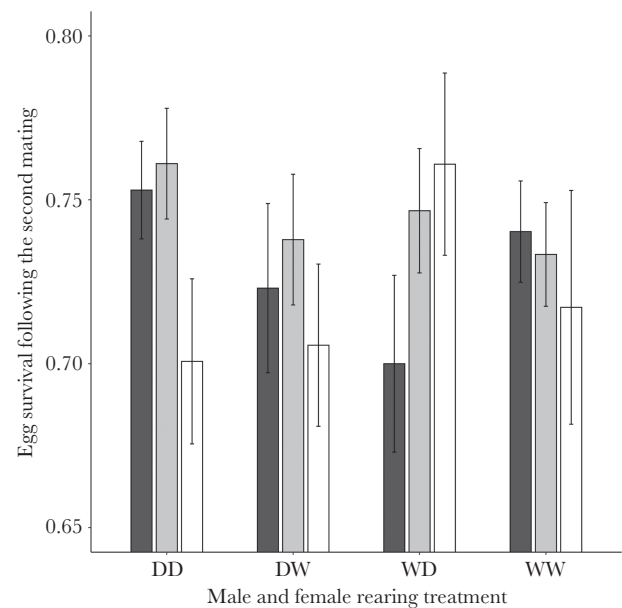


Figure 4
Effect of male and female rearing treatment and remating status on egg-to-adult survival following the second mating (failed mating group in black; not re-paired group in grey; remated group in white). Raw means \pm SE are presented.

water treatment (Table 1 and Supplementary Table A18). However, the likelihood that these eggs survived until adulthood was affected by the interaction between male water treatment and remating status (Table 1 and Supplementary Table A19, Figure 4), but was not affected by the rearing treatment of males or females, their remating status, or their interactions (Table 1 and Supplementary

Table A19). When we looked more closely, our analysis showed that egg-to-adult survival of dry males is lower when they successfully remate compared to when they have the opportunity to remate, but failed (dry males which successfully remated vs. dry males which failed to remate: t ratio = 2.993, Df = 298, P = 0.035, Supplementary Table A20). The number of adult offspring shared

the same pattern as for number of eggs laid. It was not affected by either the male or female rearing treatment, the remating status, or any of their interactions, and larger females had more adult offspring (Table 1 and Supplementary Table A21).

Lifespan of neither sex was affected by their own rearing treatment, the rearing treatment of their partner, nor the interaction between them (Table 1, Supplementary Tables A22 and A23). Female lifespan was also not affected by the remating status (Table 1 and Supplementary Table A23), but male lifespan was (Table 1 and Supplementary Table A22). Here, males that successfully remated lived shorter than those that were not provided the opportunity to remate (i.e. not re-paired group, t ratio = -2.387 , $Df = 294$, $P = 0.045$, Supplementary Table A24). Finally, for both males and females, larger beetles lived longer (Table 1, Supplementary Tables A22 and A23).

DISCUSSION

The degree of sexual conflict between males and females over reproduction is expected to vary depending on the environment (Fricke et al. 2009; Yun et al. 2017; Vincent et al. 2020). In seed beetles, water is thought to be a limiting resource that could change the economics of mating and thus the degree of sexual conflict (Iglesias-Carrasco et al. 2018a; Vincent et al. 2020). Previous studies have manipulated the availability of water for adult seed beetles, and concluded that it most likely influences mating behavior by altering male and female condition via effects of hydration (Edvardsson 2007; Vincent et al. 2020). Here we manipulated the water content of beans (distinguished as wet or dry beans), to test whether water availability during development influences mating interactions and/or sexual conflict. We found that even though beetles emerged from the two types of bean of the same size, males reared in wet beans (wet males) mating with females reared in dry beans (dry females) transferred significantly larger ejaculates than any of the other three mating pair combinations. Mating behavior on the other hand was not affected by the rearing environment of either males or females. We also found in the day following the first mating, that females mated to wet males laid fewer eggs than females mated to dry males, and that eggs laid by dry females mated to wet males had lower survival to adulthood than eggs produced by dry-dry pairs. This meant that overall, all pairs produced a similar number of adult offspring. When providing pairs with the opportunity to mate a second time, we found no effect of either male or female rearing treatment on the likelihood of remating, behavior during remating, or female fecundity following the remating. In contrast, the likelihood that eggs survived to adulthood was affected by an interaction between male water treatment and remating status. Specifically, after the first mating, dry females mated to dry males, produced eggs that were more likely to survive to adulthood than wet females. But after the second mating assay, regardless of female treatment, eggs produced by females that remated with dry males, were less likely to survive than eggs produced by those who failed to remate. Despite these differences in egg-to adult-survival, however, the actual number of adult offspring produced by pairs after the second mating was not affected by either the male water treatment, the female water treatment, or remating status. Further, neither male nor female lifespan was affected by either their water treatment or that of their partner. Although males that successfully mated twice did live shorter than those that were not provided the opportunity to remate. Finally, as expected, the body weight of both males and females had important effects on almost all aspects of fitness and reproduction.

Effect of developmental environment on mating traits

Like for many animals that exhibit multiple mating, antagonistic interactions in seed beetles are driven by competing interests of males and females (Arnqvist and Nilsson 2000). In seed beetles, larger ejaculates and longer copulations have been shown to increase male reproductive success (Savalli and Fox 1999; Edvardsson and Canal 2006), but decrease female fitness (Edvardsson and Canal 2006) because these traits are associated with the transfer of seminal toxins (Bayram et al. 2019) and damaging the female reproductive tract (Crudginton and Siva-Jothy 2000). However, male and female mating traits, as well as the benefits and costs of mating, may differ depending on the environment (Fricke et al. 2009), and more specifically, limited resources (Perry and Rowe 2010). In seed beetles, access to water has been shown to benefit female fitness and reproduction (Edvardsson 2007; Ursprung et al. 2009; Vincent et al. 2020) and this may alter the fitness consequences of mating for females and in turn the balance of sexual conflict. In our experiment, we predicted that providing water during development could allow males to transfer larger (i.e. water-rich) ejaculates and reduce female need for water. Our results are consistent with this prediction—wet males transferred larger ejaculates, but only when mated with dry females. This suggests that indeed wet males had the potential to provide larger ejaculates compared with dry males, and dry females were willing to receive larger ejaculates. The fact that wet males didn't transfer larger ejaculates to wet females, even though they were presumably capable of doing so (since they did to dry females), could suggest that wet females acquire more water during development. If females do acquire water from their environment this is likely to reduce the value of water obtained from male ejaculates so that benefits associated with obtaining water no longer offset the costs of receiving a larger ejaculate (e.g. arising from toxin transfer: Crudginton and Siva-Jothy 2000). Alternatively, this pattern could result from strategic ejaculation by males based on some aspect of female quality (i.e. males could be choosing not to transfer large ejaculates to wet females) (Kelly and Jennions 2011; Lupold et al. 2011). Therefore, although ejaculate weight shows the predicted pattern, it is still unclear whether males or females drive the decreased ejaculate size when wet males mate with wet females. Further, understanding the balance between the costs imposed by toxins and the benefits of acquiring water from ejaculates requires more attention.

We also predicted that access to water during development would alter male and female mating behavior. However, despite observing environmentally dependent differences in ejaculate mass, there were no effects of rearing environment on mating behavior (during either the first or second mating trial) nor the propensity of pairs to remate. This differs from previous research which has manipulated male and female access to water during adulthood and found that females who have access to water are less willing to remate (Edvardsson 2007), males who have access to water mate for longer than males that did not (Vincent et al. 2020), and that females who have access to water have shorter kicking durations, but only when they mated with males that had no access to water (Vincent et al. 2020). Differences between the results we present here and this previous research suggest that the effects of water intake on mating interactions differ depending on the life stage during which access to water is experienced (Boggs 2009). To be specific, access to water during development affects ejaculate size, while access to water after emergence appears to mainly influence body condition (as evidenced by differences in body weight and longevity, Vincent et al.

2020), and this, in turn, alters the ability of males and females to persevere/resist during mating (Vincent et al. 2020).

It is worth noting that wet males transferred a larger ejaculate when mating with dry females even though they had the same mating duration as other pair combinations. This could indicate that wet males mated to dry females had a higher average ejaculate transfer speed than other pair combinations, however, this remains to be tested. Previous research shows that ejaculate transfer begins at around 2 min after the beginning of copulation and is usually completed within 6 to 7 minutes (Wilson et al. 2014). Copulations in our experiment lasted from 4 to 20 minutes, so it is possible that mating lasts longer than is necessary for ejaculate transfer, and instead, like in some other insects, prolonged mating may function in mate guarding (Alcock 1994). If this is the case mating duration may not be a good proxy for seminal fluid transfer.

Consequences of developmental environments for fitness

To understand how environmentally mediated differences in mating behavior influence sexual conflict, it is necessary to look at the fitness consequences of mating interactions between individuals from different environments (Fricke et al. 2009). When considering the fitness outcomes of mating between beetles reared in wet and dry beans, if wet beans provide larvae with precious water and the effect lasts into adulthood, then all else being equal, wet-wet pairs might be expected to have greater reproductive output than other pairs. However, if female seed beetles which are adapted to living in dry environments usually obtain water from male ejaculates, as has been previously suggested (Edvardsson 2007), then an increase in available free water could disrupt the relationship between the optimal level of mating trait expression of males and females. If this is the case then we would expect wet-wet pairs to have reduced fitness in comparison to dry-dry pairs of beetles. In contrast to either of these predictions, our results show that females mated to dry males laid more eggs than females mated to wet males in the 24hrs after the first mating and there were no effects of male rearing treatment on female lifespan. Previous research on a range of insects (e.g. seed beetles, fruit flies, Chapman et al. 1995; Herndon and Wolfner 1995; Gillott 2003; and moths, Xu and Wang 2011; Takashi Yamane 2015) has shown that accessory gland proteins increase female egg-laying rate after mating. Thus, one explanation for our result could be that wet males have more dilute ejaculates and thus transfer fewer accessory gland proteins and are less able to stimulate female egg-laying than dry males. Alternatively, dry males could strategically invest more in seminal proteins that promote egg laying (Isaac et al. 2010). Although, how such an effect would be triggered by differential water intake is not clear. Future studies looking at the composition of male ejaculates are needed to test these ideas. To our knowledge this is the first study to show that water availability during development might influence ejaculate composition, however, effects of developmental environment on ejaculate traits are common in studies that manipulate the diet or social environment of developing individuals (e.g. fruit flies, *Drosophila melanogaster*, Morimoto et al. 2016; Wigby et al. 2016; cockroach, *Nauphoeta cinerea*, Harris and Moore 2004 and neriid fly, *Telostylinus angusticollis*, Macartney et al. 2018). The fact that the rearing treatment of males and females interacted to affect the proportion of eggs surviving to adulthood, but that the actual number of eggs surviving to adulthood did not differ between the four pair types, means it is unclear what the long-term fitness consequences of these effects might be.

Access to water is not only expected to affect the first mating, it may also affect the economics of multiple mating (Edvardsson 2007). To explore this, we gave a subset of beetle pairs the opportunity to remate. Our results showed that after the second mating trial, offspring of dry males that successfully remated were more likely to survive until adulthood than those from dry males that failed to remate. But, like for reproductive success after the first mating, the number of adult offspring produced did not differ between the four mating pairs, so the evolutionary consequences of these differences are difficult to determine.

The fact that our developmental water treatment did not affect the lifespan of either males or females is somewhat surprising given that water is known to be a limiting resource and that access to water during adulthood does increase longevity (Iglesias-Carrasco et al. 2018b). That neither emergence weight nor lifespan differ depending on the water treatment suggests that increased availability of water during development does not affect beetle condition, thus the way in which water availability mediates sexual conflict appears to depend on the life stage at which it is acquired.

CONCLUSION

In seed beetles, the effect of water availability during development lasts into adulthood and affects the economics of mating. These effects seem to be primarily driven by differences in ejaculate size. These results are different from those that have been found when manipulating access to water during adulthood (Iglesias-Carrasco et al. 2018a; Vincent et al. 2020), implying that how resources are allocated to different traits depends on the life stage during which they are acquired (Boggs 2009). Our study provides evidence for the importance of the developmental environment in mediating mating interactions, and the fitness consequences of these interactions. However, further studies are needed to tease apart whether and how access to water during development mediates sexual conflict, and the potential interaction between developmental and adult environment. It would be interesting in future studies to look at how access to resources during development affects ejaculate composition and how the adult environment interacts with the juvenile environment to affect multiple mating, levels of sexual conflict, and fitness.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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