



## Disentangling the costs of mating and harassment across different environments

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Why females mate multiply has been a long-standing question in evolutionary ecology. In attempts to answer this question, many studies on diverse taxa have highlighted various costs and benefits associated with reproduction. However, how the costs of mating differ from the costs of harassment and whether they vary with environmental conditions are unknown. To explore this, we compared various fitness traits of singly mated *Callosobruchus maculatus* females, with or without access to water, subjected to different levels of male exposure: no male (i.e. no mating or harassment), ablated male present (i.e. harassment only), intact male present (i.e. both mating and harassment), exposure to males for a short time (i.e. repeated mating but with minimal harassment). We found that, overall, females lived longer in wet than in dry environments, and that the effects of male exposure on female life span differed between environments: wet environments showed cumulative costs of harassment and mating, while differing levels of male exposure had no effect in dry environments. Further, while females laid more eggs in dry environments, females mating repeatedly were more fecund than females housed with ablated males in both environments, suggesting both benefits of repeated mating and costs of harassment, which are environmentally independent. Finally, offspring survival was dependent on a complex interaction between environment and male exposure, potentially reflecting environmental differences in resource allocation trade-offs.

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The benefits and costs associated with reproduction often differ for males and females. While males tend to increase their fitness by maximizing the number of times they mate, females, in theory, could mate just once to obtain enough viable sperm to fertilize all their eggs (Bateman, 1948). In nature, however, females of many species are polyandrous. Explaining why this is has been a major challenge in the fields of behavioural and evolutionary ecology (reviewed in Arnqvist & Nilsson, 2000). As such, many studies have focused on identifying the benefits females gain from mating multiply. These include direct benefits that females receive during mating, such as nuptial gifts, accessory substances or resources (Fedorka & Mousseau, 2002) that increase female survival (Parker & Simmons, 1989), egg-laying rate and fecundity (Villarreal et al., 2018; Yamane, Goenaga, Rönn, & Arnqvist, 2015), and genetic benefits, which elevate offspring fitness (Jennions & Petrie, 2000;

Rodríguez-Muñoz, Bretman, Slate, Walling, & Tregenza, 2010). If we are to understand the evolution of polyandry, however, we need to understand not only the benefits of multiple mating, but also the costs. Many studies have shown that mating can be costly for females. For example, it can take time and energy away from other activities such as feeding (Magurran & Seghers, 1994), it can cause physical injury (Dougherty & Simmons, 2017; Hosken & Stockley, 2004) or it can increase risk of infection (Rolff & Siva-Jothy, 2002) or predation (Darden & Croft, 2008; Wing, 1991), all of which can negatively impact female reproductive success (Arnqvist & Nilsson, 2000).

In addition to costs and benefits of mating per se, female mating decisions may be influenced by the costs and benefits of rejecting males. Even in polyandrous species, the optimal female mating rate is usually considerably lower than that of males (Arnqvist & Nilsson, 2000). This generates sexual conflict over mating and may lead to the evolution of male mate harassment (e.g. chasing, Clutton-Brock, Price, & MacColl, 1992; Sundaresan, Fischhoff, & Rubenstein, 2007; forced copulation, Emlen & Wrege, 1986; Kwan, Cheng, Rodd, & Rowe, 2013) which has the potential to physically harm females (Blanckenhorn, 2002; Crudgington & Siva-

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Jothy, 2000), reduce their food intake (Takahashi & Watanabe, 2010) and negatively impact various physiological traits (Bretman & Fricke, 2019), survival and reproduction (Bateman, Ferguson, & Yetman, 2006; Eady, Hamilton, & Lyons, 2007; Gosden & Svensson, 2007; Nandy et al., 2013). If the costs of harassment outweigh the costs of mating, females may practise 'convenience polyandry' simply to avoid costs of harassment (Thornhill & Alcock, 1983). To understand why females mate multiply we need to understand how these different costs and benefits are balanced.

The environment in which animals are found can affect the strength of sexual conflict over mating and, consequently influence the evolution of mating traits. For example, environmental factors such as resource availability, environmental temperature, population density and the operational sex ratio may alter the cost of resisting male mating attempts (Rowe, 1992) or the need for nutritious ejaculates (Markow, Gallagher, & Krebs, 1990). In such cases, the fitness consequences of additional matings are likely to be context dependent. For example, the impact of male harm on female fitness has been shown to change with varying temperatures (García-Roa, Chirinos, & Carazo, 2019), as have costs of reproduction (Grazer & Martin, 2012). Given this variation in responses across environmental conditions, females may be expected to alter their mating behaviour depending on their environment (Arnqvist & Nilsson, 2000; Droge-Young, Belote, Eeswara, & Pitnick, 2016). For instance, starved (Prokop & Maxwell, 2009; Toft & Albo, 2015) and dehydrated (Edvardsson, 2007) females mate more frequently than those with adequate resources due to the increased value of obtaining mating gifts from males. The environment could also alter the ability of males to provide direct benefits to females. For example, studies have shown that food-deprived males have smaller ejaculates and altered ejaculate composition compared to well-fed males (Iglesias-Carrasco, Jennions, Zajitschek, & Head, 2018; Perry & Rowe, 2010), potentially reducing the effective benefit of mating for females. However, whether these effects are a result of environmentally induced changes in the balance between the costs and benefits of additional matings or male harassment remains largely unexplored.

Seed beetles, *Callosobruchus maculatus*, often live in arid environments where they develop and feed within stored seeds. They are also known to infest their host plants in fields where they are exposed to changing weather conditions, including humidity levels (Alzouma, 1981). Such fluctuating humidity conditions in the field have been shown to affect different aspects of female fitness in this species (Ouedraogo et al., 1996). Once seed beetles emerge from seeds as adults, they are able to survive in the absence of additional food or water (Bellows, 1982); however, they will eat and drink if given the opportunity (Edvardsson, 2007; Ursprung, Den Hollander, & Gwynne, 2009). This suggests that water might be a limited resource that constrains female fitness. In dry environmental conditions, females might acquire hydration benefits from male ejaculates that are rich in water (Edvardsson, 2007; Ursprung et al., 2009). In another study, it was shown that females housed in nutrient-deficient environments were more likely to remate than those housed with abundant nutrients (Fox & Moya-Laraño, 2009; Savalli & Fox, 1999) suggesting that the ejaculate may also provide nutritional benefits to females. Evidence to support benefits of mating in this species also comes from studies showing that multiple mating increases female fitness (Fox, 1993; Wilson, Tufton, & Eady, 1999). However, there are also substantial costs of mating in *C. maculatus* that might limit the optimal number of copulations for females. For example, the male genitalia bear sclerotized spines that wound the female's reproductive tract, consequently decreasing female life span (Crudgington & Siva-Jothy, 2000). There is also evidence for the transfer of toxic substances through the ejaculate (Eady et al., 2007) and intensive male harassment (Gay,

Eady, Vasudev, Hosken, & Tregenza, 2009; but see ; den Hollander & Gwynne, 2009) which reduce female fitness.

Water availability is an environmental parameter that is likely to be important for mediating the costs and benefits of mating in seed beetles. Previous studies have shown that when water is available, females reduce their propensity to mate when exposed to males for a short period of time (Edvardsson, 2007; Ursprung et al., 2009). In wet conditions, females might make use of environmental water to avoid superfluous matings. Similarly, males in wet conditions might transfer ejaculates that are richer in water, reducing the need for females to copulate multiply. Interestingly, if females are continuously exposed to males, a reduction in female mating tendency might increase male harassment and hence the costs associated with avoiding males (Gay et al., 2009), and females may resort to engaging in convenience polyandry. However, despite the likely importance of the environment in determining the strength of sexual selection or sexual conflict in *C. maculatus*, little is known about how differing environmental conditions alter the costs and benefits of mating and harassment.

The effect of exposure to different numbers of males on female fitness in seed beetles has been shown to depend on water availability. Iglesias-Carrasco, Bilgin, et al. (2018) and Iglesias-Carrasco, Jennions, et al. (2018) found that females housed alone laid more eggs in wet than in dry conditions, and this result was reversed when females were housed with a male, suggesting that the costs of interacting with males outweigh the benefits when resources can be acquired externally. However, this previous study failed to disentangle the costs of mating from the costs of male harassment. If we aim to understand how different environmental conditions drive the evolution of mating strategies and reproductive behaviour, it is important to disentangle how the various costs and benefits of copulation and male harassment balance to influence female fitness. Here, we explored how the environment (dry versus wet) and different levels of male exposure (male absent, i.e. no mating or harassment; ablated male present, i.e. harassment only; intact male present, i.e. both mating and harassment; exposure to males for a short time, i.e. repeated mating but with minimal harassment) interact to affect female fitness traits including life span, lifetime egg production, offspring development and offspring survival to adulthood. We predicted that: (1) females in wet environments would have longer life spans, higher fecundity and greater offspring fitness than those in dry environments (Edvardsson, 2007); (2) in both wet and dry conditions, females with no exposure to males after an initial mating would have the greatest fitness as they remain shielded from the costs of both mating and harassment; (3) in dry environments, females experiencing repeated matings would be better off than females housed alone because they receive hydration benefits via male ejaculates, while in wet environments females experiencing repeated matings will be no better off than those housed alone because, here, females obtain water from their environment and ejaculates become less valuable; (4) since females housed with intact males will experience costs of both mating and harassment, they would have the lowest fitness.

## METHODS

### *Study Species*

The seed beetle is a common pest of stored legumes. Females lay eggs on the surface of beans, the eggs then hatch and the larvae burrow into the bean where they develop for 3–4 weeks (depending on temperature), following which they emerge as adults.

The beetles used for this study came from a stock population of *C. maculatus* raised on black-eyed beans, *Vigna unguiculata*, at the Australian National University at a constant temperature ( $25 \pm 1^\circ\text{C}$ ).

### Experimental Design

To investigate the role that water availability plays in altering the costs and benefits of harassment and mating, we used a  $2 \times 4$  factorial experimental design. Mating pairs were housed in one of two environmental conditions, wet or dry. Water was made available externally to beetles in the wet treatment while our dry treatment mirrored stock conditions where beetles were not provided with water. Within these environments we also independently manipulated the level of female exposure to males. Following an initial mating (see below), females were housed: (1) on their own (no male); (2) with an emasculated male which was able to harass the female but was incapable of mating (ablated male); (3) with the male they initially mated with until either died (intact male) so that males could mate and harass the females; and (4) housed on their own but presented with their initial mating partner for 30 min every day over a period of 4 days, thus allowing repeated mating, with minimal harassment (repeated mating). In total there were eight experimental treatments, each with a unique environment–mating/harassment combination. The numbers of replicates in each treatment were: dry/ablated:  $N = 63$ ; dry/intact:  $N = 66$ ; dry/repeated mating:  $N = 63$ ; dry/no male:  $N = 62$ ; wet/ablated:  $N = 63$ ; wet/intact:  $N = 64$ ; wet/repeated mating:  $N = 61$ ; wet/no male:  $N = 61$ . The experiment was conducted in two approximately equal sized blocks, separated in time, due to logistical constraints.

### Experimental Procedure

To obtain virgin beetles for our experiment, mated females from our stock population were placed individually in petri dishes, each containing ca. 20 mung beans, *Vigna radiata*. Females were left to lay eggs on these beans for 30 min to 3 h after which beans were inspected to ensure that each had only one egg on it. If there was more than one egg on the bean, the extras were scraped off. Beans were placed in individual Eppendorf tubes with small holes in their lids for ventilation. We kept a maximum of 10 beans per female and the female from which each bean came from was recorded to prevent pairing of siblings during the experiment. Beans with a single egg were then incubated at  $25 \pm 1^\circ\text{C}$  for around 28 days, following which virgin adults started to emerge.

Beans were checked daily for emerging adults. On the day they emerged, adults were sexed, given an ID, weighed (to 0.001 mg, Sartorius microbalance) and had their mother's ID recorded. Equal numbers of males and females were then randomly assigned to the wet or dry treatment, where each adult was placed in a 70 ml sample container either with or without a vial lid containing cotton wool soaked in water (wet or dry, respectively) for 24 h. The next day, one male and one female from the same water treatment were randomly paired (except that brothers and sisters were never paired) in Eppendorf tubes for 30 min where it was ensured that mating occurred. Previous studies show that mating temporarily reduces mating receptivity in this species (Shu, Koepnick, Mbata, Cork, & Ramaswamy, 1996) and that females do not remate for up to 8 h after their first mating (den Hollander & Gwynne, 2009), so it is highly unlikely that beetles mated more than once in this 30 min period.

After their initial mating, females were randomly assigned to one of four male exposure treatments (no male, ablated male, intact male, repeated mating). For the no male treatment, females were transferred into a sample container with approximately 40 mung beans and no males. For the ablated male treatment, females were

transferred into a sample container with approximately 40 mung beans and an ablated male. These randomly chosen males were of the same age as the females and had had their genitalia ablated upon emergence, after weighing, but prior to being placed in the wet or dry treatment. The ablation procedure was performed by anaesthetizing the adults with a mild dose of  $\text{CO}_2$  on a fly pad. Once in 'coma', their posteriors were lightly squeezed using a pair of blunt tweezers so that their aedeagi were extruded. The spiny head of the club-shaped genitalia facilitates successful copulation (Crudgington & Siva-Jothy, 2000; Hotzy, Polak, Rönn, & Arnqvist, 2012). To emasculate the male, the head of the aedeagus was cut off using a pair of microscissors. All males, including those not destined for ablation, were subjected to the same treatment up until the ablation step to control for effects of handling and  $\text{CO}_2$  exposure. All males revived from the coma within in 3–4 min and preliminary tests confirmed that all males behaved normally (i.e. harassed females) post revival. For the intact male treatment, females were transferred into a sample container with approximately 40 mung beans and an intact (sham-ablated) male. For the repeated mating treatment, females were transferred into a sample container with approximately 40 mung beans where they were housed alone but were exposed to their mating pair male for 30 min, every 24 h, for 4 more days after the initial mating. The same male was used on all 4 days to avoid confounding effects of increased egg laying and offspring fitness that may occur when females mate with multiple males (Eady, Wilson, & Jackson, 2000; Zajitschek, Dowling, Head, Rodriguez-Exposito, & Garcia-Gonzalez, 2018).

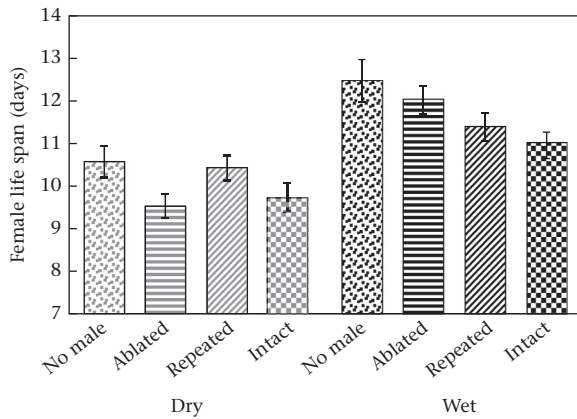
Each female remained in its water availability and male exposure treatment for 5 days during which it was transferred to a new sample container with approximately 40 new mung beans every 24 h. This ensured that females always had plenty of fresh beans on which to lay eggs and allowed us to start counting eggs before larvae started to emerge. During these 5 days, if any ablated male died, it was replaced with another ablated male that had experienced the same water treatment ( $N = 31$  of 126). However, when a male from the intact or repeated mating treatment died before its mate ( $N = 6$  and  $N = 2$ , respectively), it was not replaced as this would have led to progeny being sired by multiple males, which could influence female egg laying (Eady et al., 2000) and offspring fitness (Zajitschek et al., 2018).

We removed males, along with the soaked cotton to prevent fungal growth, 24 h after setting up the day 5 containers. The females remained in these containers, along with the beans, until they died. Female survival was monitored every 24 h and life span was recorded as the number of days the female survived after its initial copulation.

The number of eggs laid by a female in each container was counted before offspring emerged from the beans and the number of eggs across all a female's containers was summed to give the female's lifetime fecundity. Around 28 days after the female had laid eggs, adult offspring started to emerge. The first day of emergence was recorded for all containers and we used the average emergence time across all a female's containers as the larval development time. Following the first emergence, the containers with eggs were maintained for a further 10 days. In previous experiments we have observed that no adults emerge after 10 days from the emergence of the first individual. After this, the containers were put in the freezer, and the number of emerged offspring was counted for each container and then summed to give the number of adult offspring produced by each female.

### Ethical Note

This work followed the ASAB/ABS guidelines for the treatment of animals in behavioural research. Ablations of male genitalia were



**Figure 1.** Effects of water availability and male exposure treatments (no male, ablated male, repeated mating and intact male) on female life span (days). Raw means  $\pm$  SE are presented.

conducted under CO<sub>2</sub> anaesthesia, and a subset of males was monitored shortly after ablation to ensure normal behaviour. Housing conditions, handling and experimental monitoring were all conducted in a way to maximize the animals' welfare. We complied with the Australian National University regulations for experiments on invertebrates.

#### Data Analysis

To determine the effects of water availability and level of male exposure on female fitness traits we ran separate generalized linear models for each fitness trait. All models included water availability, level of male exposure as well as their interaction as fixed effects. Female weight and block were also included as fixed effects in all models to control for potential variation in our data arising from variation in female size or because the two blocks were carried out at different times. Block was included as a fixed rather than random effect following Bolker et al. (2009) because there were only two levels. Female life span, female lifetime fecundity and offspring development time were analysed using linear models. For female life span we also ran a model including lifetime fecundity as a covariate, as life span and reproductive investment may be expected to be traded off. While female life

span and lifetime fecundity did show the expected negative correlation, this did not alter the effects found for other parameters in the model, and so we report results of the model without lifetime fecundity. Residuals of all models were visualized to ensure the assumptions of normality and homoscedasticity were met, and when they were not (in the case of female lifetime fecundity) the data were transformed using the 'powerTransform' function in the 'car' R package (Fox and Weisberg, 2019). Offspring egg to adult survival was analysed using a generalized linear model with a binomial error structure. In this case we used the 'cbind' function to specify our response variable (number of adults eclosing number of unhatched eggs). For all traits, effects of the water availability by male exposure interaction were interpreted from full models, while main effects were interpreted from models with no interaction. When interactions were present, we followed up our analysis with Tukey's tests run within each water availability treatment to determine which male exposure treatments differed. All analyses were conducted in R (version 3.5.0, The R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>).

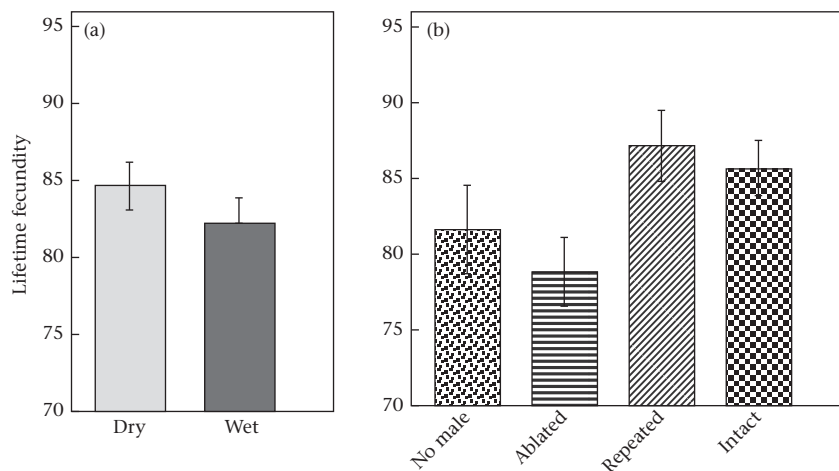
## RESULTS

### Female Life Span

Overall, females that had access to water lived longer than those that did not (Appendix Tables A1, A2, Fig. 1). However, the effect of male exposure treatment on life span was dependent on whether the female experienced wet or dry conditions (Appendix Table A2). In the wet environment the effects of harassment and mating appear to be cumulative with females housed alone having the longest life span, females housed with intact males having the shortest life span and females housed with ablated males or only being exposed to males for short periods having intermediate life spans (Appendix Table A3, Fig. 1). In the dry environment there was no effect of male exposure treatment on female life span (Appendix Table A3, Fig. 1).

### Female Lifetime Fecundity

Overall, females with access to water laid fewer eggs than those with no access to water (Appendix Tables A4, A5, Fig. 2a). Further, water availability did not mediate the effect of male exposure



**Figure 2.** Effects of (a) water availability and (b) male exposure treatments (no male, ablated male, repeated mating and intact male) on female lifetime fecundity. Raw means  $\pm$  SE are presented.

treatment on female lifetime fecundity, but there was a main effect of male exposure treatment (Appendix Tables A4, A5, Fig. 2b), which revealed that females housed with ablated males had lower lifetime fecundity than those that mated daily with males but were not housed with them (Appendix Table A6).

#### Offspring Development Time

Offspring development time was not affected by water availability, male exposure treatment or the interaction between them (Appendix Tables A7, A8).

#### Offspring Survival

Overall, females housed with water had lower offspring survival than those with no water available (Appendix Tables A9, A10, Fig. 3). The effect of male exposure treatment on offspring survival was dependent on water availability (Appendix Tables A9, A10, Fig. 3). When females had no access to water, those exposed to ablated males had offspring with a greater probability of surviving to adulthood than any of the other exposure treatments (Appendix Table A11). However, when females had access to water those that only mated with males (i.e. the repeated mating treatment) had offspring with the greatest probability of survival (Appendix Table A11), although the survival of offspring was not significantly different for females mating repeatedly or housed with no male (Appendix Table A11).

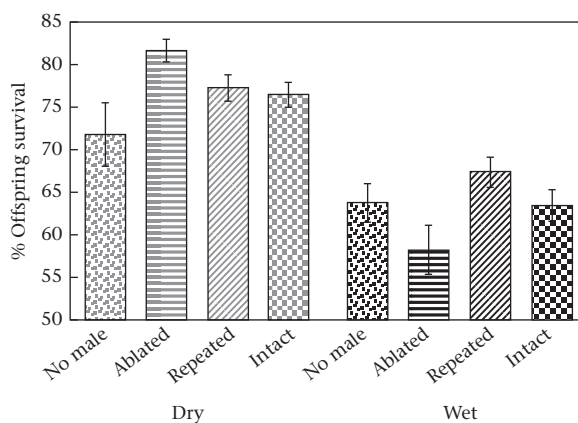
## DISCUSSION

It has been shown that water availability (Edvardsson, 2007; Ursprung et al., 2009) and male exposure (Fox, 1993; Gay et al., 2009; Wilson et al., 1999) independently affect female fitness in *C. maculatus*, but how they interact has received little attention. Exploring such interactions is likely to be important for understanding the evolution and ecology of sexual conflict in this system, because under natural conditions seed beetle populations are likely to experience varying humidity levels. In this study we teased apart the effects of mating and harassment on the fitness of female seed beetles housed either with or without access to water. We found that wet environments were beneficial for female longevity and that survival costs relating to varying levels of male exposure were only apparent in wet environments. Wet environments were also

generally detrimental for female fecundity and offspring survival. We also found that our male exposure treatment influenced female fecundity, with females mating daily having greater fecundity than females housed with ablated males, suggesting costs of harassment and benefits of repeated mating (at least at a rate of once per day) across both wet and dry environments. Finally, the effect of our male exposure treatment on offspring survival was dependent on whether there was water available or not. Females housed with ablated males had the greatest offspring survival when they had no access to water, whereas when water was available, females that were mated repeatedly but not harassed had the greatest offspring survival. Our results highlight that the way in which the environment influences the fitness consequences of mating interactions may be complex, depending on what component of fitness is measured, the way in which the environment differs and the mating system of the species under consideration.

We found that, overall, females with access to water lived longer than females in dry conditions. This finding supports previous studies in *C. maculatus* (Edvardsson, 2007; Iglesias-Carrasco, Bilgin, et al., 2018; Ursprung et al., 2009), and suggests that environmental water might be beneficial for female life span, perhaps because it helps to avoid dehydration. More interestingly, we found that the life span costs associated with varying exposure to males differed between environments: in dry environments differing types of exposure to males did not affect female life span, but in wet environments females housed with males that could both mate and harass had lower survival than those housed alone and females housed with males that could either harass or mate (but not both) had intermediate life spans. This suggests that in wet conditions costs of mating and costs of harassment may be cumulative and not counteracted by potential benefits of mating, while in dry environments potential benefits of obtaining hydration through mating may mask the costs of mating and harassment. Other studies looking at how the environment influences the way in which exposure to males impacts survival have found mixed results. For instance, a recent study on fruit flies, *Drosophila melanogaster*, looking at how the thermal environment and sexual conflict influence female life span also found an interaction effect, with the survival costs of sexual conflict being greater in cooler environments (García-Roa et al., 2019). Another study on fruit flies looked at the effects of simple and complex physical environments on male-induced harm to females (Yun, Chen, Singh, Agrawal, & Rundle, 2017). The authors found that both the environment and the degree of male exposure affected female survival and fecundity, but the interaction between these two factors had significant effects only on fecundity and not female viability. Combined, our results and those of previous studies suggest that whether the relationship between male exposure and survival differs between environments may depend on the specific environments of interest. This means that when making predictions about how the environment might influence the ecology and evolution of species with mating systems that are characterized by sexual conflict it is important to consider how the environmental parameter of interest impacts interactions between males and females in that species.

We found that, overall, females with water available laid fewer eggs than females with no water. This contrasts with previous studies in *C. maculatus* that found either increased fecundity of females that had water available (Edvardsson, 2007; Fox, 1993) or no effect (Iglesias-Carrasco, Bilgin, et al., 2018; Vincent, Head, & Iglesias-Carrasco, 2020). If females with access to water were in better condition, we expected them to have higher lifetime fecundity than those in dry conditions, mirroring the overall effect found for life span. However, our data show a negative relationship between life span and fecundity



**Figure 3.** Effects of water availability and male exposure treatments (no male, ablated male, repeated mating and intact male) on survival of offspring to adulthood. Raw means  $\pm$  SE are presented.

(across all females:  $r = -0.24$ ,  $t = -5.22$ ,  $N = 453$ ,  $P < 0.001$ ). Previous studies have found a trade-off between female life span and investment in egg production in several species (Barnes & Partridge, 2003; Rose, 1984) including seed beetles (Rönn, Katvala, & Arnqvist, 2006). Therefore, one possible explanation for our results is that females in the wet environment lived longer because they laid fewer eggs. However, this seems unlikely since in a post hoc analysis of female life span where we included lifetime fecundity as a covariate, the effect of water availability on life span remained. Alternatively, in many insect species, repeated matings are necessary to fertilize all of a female's eggs (Hunter, Petrie, Otronen, Birkhead, & Møller, 1993; Ridley, 1988) and females of *C. maculatus* in wet conditions have a lower propensity to remate (Edvardsson, 2007; Ursprung et al., 2009). Thus, females in the wet environment may have received fewer sperm resulting in lower lifetime fecundity. This explanation is unlikely, however, because generally only a few matings are required to achieve maximum fertilization success in *C. maculatus* (Eady, 1994; Wilson et al., 1999). Finally, given that *C. maculatus* females are known to lay eggs preferentially on high-quality hosts (e.g. large seeds, Cope & Fox, 2003; seeds with low egg densities, Messina & Renwick, 1985), another possible explanation for reduced fecundity in the wet environment is that females avoided laying eggs because possible increased humidity reduces bean quality. This final possibility could be easily tested in the future using host preference trials.

We also found that, independent of the water environment, the lifetime fecundity of females housed with an ablated male was significantly lower than that of females allowed to mate repeatedly. This could suggest a cost of harassment on female fecundity similar to that found previously in this (Gay et al., 2009) and other related species (*Callosobruchus chinensis*, Sakurai & Kasuya, 2008) that is counteracted by the benefits of mating repeatedly if male ejaculates provide oviposition stimulants or if sperm is limiting (Wilson et al., 1999). Our result could also reflect a cost of interference derived from males continuously chasing females when trying to lay eggs (Bacon & Barbosa, 2020; Córdoba-Aguilar, 2009). Note that ablation of males may overestimate the effects of harassment. Intact males are also expected to chase egg-laying females; however, the level of disturbance is likely to be lower than for ablated males due to male refractory periods after successful copulation. This potentially explains the extreme effect of ablated males on fecundity and only intermediate effects of intact males.

Independent of the type of exposure to males, offspring survival was higher when females were in the dry environment. One possible explanation for this finding is that exposure to environmental water increases the water content of beans in the female's container, hence reducing the quality of the larval rearing environment. Alternatively, the reduction in offspring survival in the wet environment might be related to the age at which females laid the eggs (see also Iglesias-Carrasco, Bilgin, et al., 2018). Since females lived longer in the wet environment, it is possible that a greater proportion of their eggs were laid later in life, when egg size is smaller and survival to eclosion is lower (Fox, 1993). We also found an effect of male exposure treatment on offspring survival that was dependent on water availability: in the dry treatment, the offspring of females housed with ablated males performed the best, while in the wet treatment, the offspring of repeatedly mated females had the highest survival (albeit not significantly greater than when females were housed alone). Our results for females housed in dry conditions, combined with the fact that our results show a negative correlation between female life span and offspring survival (across all females:  $r = -0.56$ ,  $t = -13.51$ ,  $N = 404$ ,  $P < 0.001$ ) could suggest a trade-off between female life span and egg investment. Variation in life span–fecundity trade-offs that depend on environmental conditions (e.g. dietary restriction) have been shown in several other

insect species (Adler, Cassidy, Fricke, & Bonduriansky, 2013; Gruber, Soon, & Halliwell, 2007; Messina & Slade, 1999), and are expected to arise from the reallocation of resources from reproduction to survival. If the trade-off we saw here was a result of limited water, it is not surprising that we did not see the same pattern for females housed in wet conditions. However, the patterns we did see in wet conditions are difficult to explain. Here, females that mated repeatedly had offspring with greater survival than both females housed with ablated and intact males, but not greater than females housed alone. One potential, albeit speculative explanation is that repeated mating produces better quality offspring (Eady et al., 2000). If this is the case, then these offspring may be better at coping with the poor-quality developmental environment that moist beans pose. Another potential explanation is that females in the wet environment obtained all the benefits of mating repeatedly (e.g. sperm replenishment) without the transgenerational costs of continuous harassment that are known to have negative effects on offspring traits (Gasparini, Devigili, & Pilastro, 2012).

### Conclusions

Since an animal's physical environment can change the costs and benefits of mating and reproduction (Yun et al., 2017), it is critical to consider the role of the local environment if we aim to understand the economics of sexual interactions (Fricke, Perry, Chapman, & Rowe, 2009). Our study shows that costs of mating and harassment can vary across different environmental conditions. We found that although access to water increased female life span, it also presented fitness costs in terms of female lifetime fecundity and offspring survival. This may suggest that this population of seed beetles is locally adapted to dry conditions: although they survived longer in wet environments, their lifetime fecundity was greater in their ancestral dry environment. We also suggest that different ecological parameters may influence the costs of mating and harassment differently; altering some environmental parameters might be more crucial than others depending on how the parameter of interest influences various potential costs and/or benefits of mating. Moreover, the female fitness responses we observed may be specific to certain mating systems. Therefore, to understand the ways in which sexual conflict manifests, it is essential that research explores how the costs of sexual interactions differ across a range of biologically relevant environmental conditions and in a wide variety of taxa.

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## Appendix

**Table A1**  
Effects of water availability and male exposure treatment on female life span

	Estimate	SE	t	P
<b>Full model</b>				
(Intercept)	7.595	0.394	19.278	< 0.001
Water availability (W)	2.552	0.440	5.806	< 0.001
Exposure treatment (I)	0.302	0.438	0.690	0.491
Exposure treatment (R)	1.066	0.442	2.413	0.016
Exposure treatment (N)	1.101	0.445	2.473	0.014
Female weight	0.003	0.001	3.830	< 0.001
Block (2)	2.001	0.223	8.977	< 0.001
Water(W)*Exposure(I)	-1.307	0.617	-2.120	0.035
Water(W)*Exposure(R)	-1.829	0.626	-2.922	0.004
Water(W)*Exposure(N)	-0.780	0.626	-1.246	0.213
<b>Main effects model</b>				
(Intercept)	8.137	0.337	24.127	< 0.001
Water availability (W)	1.576	0.222	7.091	< 0.001
Exposure treatment (I)	-0.363	0.310	-1.170	0.242
Exposure treatment (R)	0.151	0.314	0.480	0.632
Exposure treatment (N)	0.699	0.315	2.222	0.027
Female weight	0.003	0.001	3.646	< 0.001
Block (2)	1.986	0.224	8.855	< 0.001

Full model provides parameter estimates and significance values for interpreting interaction effects (main effects in this model are shown for transparency but should not be interpreted). 'Main effects' model does not fit interactions, and provides parameter estimates and significance values for interpreting the main effects. Results are from linear models performed using the lme4 package in R (version 3.5.0). W: wet; I: intact male; R: repeated mating; N: no male.

**Table A2**  
ANOVA outputs showing effects of water availability and male exposure treatment on female life span

	Sum of squares	df	F	P
<b>Full model</b>				
(Intercept)	2205.91	1	371.626	< 0.001
Water availability	200.12	1	33.714	< 0.001
Exposure treatment	55.63	3	3.124	0.026
Female weight	87.08	1	14.670	< 0.001
Block	478.37	1	80.591	< 0.001
Water*Exposure	55.69	3	3.128	0.026
Residuals	2855.13	481		
<b>Main effects model</b>				
Intercept	3500.8	1	582.101	< 0.001
Water availability	302.4	1	50.283	< 0.001
Exposure treatment	71.7	3	3.975	0.008
Female weight	79.9	1	13.293	< 0.001
Block	471.5	1	78.406	< 0.001
Residuals	2910.8	484		

Full model provides significance values for interpreting interaction effects (main effects in this model are shown for transparency but should not be interpreted). 'Main effects' model does not fit interactions, and provides significance values for interpreting the main effects. Rows of specific interest are in italics. Significance values were obtained using the 'Anova' function with type 3 sums of squares in R (version 3.5.0).

**Table A3**  
Tukey's post hoc tests showing how female life span differs between male exposure treatments within each water availability

Contrast	Estimate	SE	df	t	P
<b>Dry treatment</b>					
None–Ablated	1.101	0.445	481	2.473	0.065
None–Repeated	0.035	0.441	481	0.079	0.999
None–Intact	0.799	0.438	481	1.824	0.263
Ablated–Repeated	-1.066	0.442	481	-2.413	0.076
Ablated–Intact	-0.302	0.438	481	-0.69	0.901
Repeated–Intact	0.764	0.434	481	1.759	0.295
<b>Wet treatment</b>					
None–Ablated	0.321	0.440	481	0.730	0.885
None–Repeated	1.084	0.447	481	2.425	0.074
None–Intact	1.326	0.440	481	3.014	0.014
Ablated–Repeated	-0.763	0.442	481	-1.725	0.312
Ablated–Intact	1.005	0.434	481	2.315	0.096
Repeated–Intact	0.242	0.443	481	0.547	0.947

Contrasts were obtained using full models.

**Table A4**  
Effects of water availability and male exposure treatment on female lifetime fecundity

	Estimate	SE	t	P
<b>Full model</b>				
(Intercept)	891.067	54.938	16.219	<0.001
Water availability (W)	-65.778	61.473	-1.070	0.285
Exposure treatment (I)	114.269	61.483	1.859	0.064
Exposure treatment (R)	128.746	61.769	2.084	0.038
Exposure treatment (N)	61.908	63.818	0.970	0.333
Female weight	1.167	0.126	9.296	<0.001
Block (2)	-271.593	31.806	-8.539	<0.001
Water(W)*Exposure(I)	-20.827	86.391	-0.241	0.810
Water(W)*Exposure(R)	-1.565	87.685	-0.018	0.986
Water(W)*Exposure(N)	33.363	89.436	0.373	0.709
<b>Main effects model</b>				
(Intercept)	889.654	47.021	18.920	<0.001
Water availability (W)	-64.039	31.154	-2.056	0.040
Exposure treatment (I)	103.679	43.068	2.407	0.016
Exposure treatment (R)	127.958	43.648	2.932	0.004
Exposure treatment (N)	79.039	44.595	1.772	0.077
Female weight	1.170	0.125	9.380	<0.001
Block (2)	-271.804	31.701	-8.574	<0.001

Full model provides parameter estimates and significance values for interpreting interaction effects (main effects in this model are shown for transparency but should not be interpreted). 'Main effects' model does not fit interactions, and provides parameter estimates and significance values for interpreting the main effects. Results are from linear models on power transformed data, performed using the lme4 package in R (version 3.5.0). W: wet; I: intact male; R: repeated mating; N: no male.



**Table A5**  
ANOVA outputs showing effects of water availability and male exposure treatment on female lifetime fecundity.

	Sum of squares	df	F	P
<b>Full model</b>				
(Intercept)	290 56 605	1	263.071	<0.001
Water availability	126 465	1	1.145	0.285
Exposure treatment	591 312	3	1.785	0.149
Female weight	9 543 865	1	86.408	<0.001
Block	8 053 659	1	72.916	<0.001
<i>Water*Exposure</i>	<i>41 829</i>	<i>3</i>	<i>0.126</i>	<i>0.945</i>
Residuals	49 261 426	446		
<b>Reduced model</b>				
Intercept	39 308 586	1	357.980	<0.001
<i>Water availability</i>	<i>463 981</i>	<i>1</i>	<i>4.225</i>	<i>0.040</i>
<i>Exposure treatment</i>	<i>1 074 221</i>	<i>3</i>	<i>3.261</i>	<i>0.021</i>
Female weight	9 661 381	1	87.985	<0.001
Block	8 072 209	1	73.513	<0.001
Residuals	49 303 255	449		

Full model provides significance values for interpreting interaction effects (main effects in this model are shown for transparency but should not be interpreted). 'Main effects' model does not fit interactions, and provides significance values for interpreting the main effects. Rows of specific interest are highlighted in italics. Significance values are obtained using the 'Anova' function with type 3 sums of squares in R (version 3.5.0).

**Table A6**  
Tukey's post hoc tests showing how female lifetime fecundity differs between water availability treatments and between male exposure treatments

Contrast	Estimate	SE	df	t	P
<b>Water availability</b>					
Dry–Wet	63.2	31.2	445	2.026	0.043
<b>Male exposure</b>					
None–Ablated	77.4	44.6	445	1.737	0.306
None–Repeated	-49.1	44.9	445	-1.094	0.694
None–Intact	-25.3	44.5	445	-0.570	0.941
Ablated–Repeated	-126.6	43.6	445	-2.901	0.020
Ablated–Intact	-102.8	43.1	445	-2.382	0.082
Repeated–Intact	23.8	43.5	445	0.548	0.947

Contrasts were obtained using full models.

**Table A7**  
Effects of water availability and male exposure treatment on offspring development time

	Estimate	SE	t	P
<b>Full model</b>				
(Intercept)	30.960	0.166	186.706	<0.001
Water availability (W)	0.166	0.189	0.878	0.380
Exposure treatment (I)	-0.137	0.184	-0.746	0.456
Exposure treatment (R)	-0.258	0.189	-1.366	0.172
Exposure treatment (N)	-0.081	0.191	-0.426	0.671
Female weight	-0.002	0.000	-5.028	<0.001
Block (2)	6.564	0.095	69.262	<0.001
Water(W)*Exposure(I)	-0.032	0.263	-0.120	0.904
Water(W)*Exposure(R)	-0.065	0.269	-0.241	0.809
Water(W)*Exposure(N)	-0.053	0.271	-0.196	0.845
<b>Reduced model</b>				
(Intercept)	30.980	0.142	218.220	<0.001
Water availability (W)	0.129	0.094	1.371	0.171
Exposure treatment (I)	-0.153	0.131	-1.167	0.244
Exposure treatment (R)	-0.290	0.133	-2.173	0.030
Exposure treatment (N)	-0.108	0.135	-0.795	0.427
Female weight	-0.002	0.000	-5.080	<0.001
Block (2)	6.564	0.094	69.492	<0.001

Full model provides parameter estimates and significance values for interpreting interaction effects (main effects in this model are shown for transparency but should not be interpreted). 'Main effects' model does not fit interactions, and provides parameter estimates and significance values for interpreting the main effects. Results are from linear models performed using the lme4 package in R (version 3.5.0). W: wet; I: intact male; R: repeated mating; N: no male.

**Table A8**  
ANOVA outputs showing effects of water availability and male exposure treatment on offspring development time

	Sum of squares	df	F	P
<b>Full model</b>				
(Intercept)	37 165	1	34859.189	<0.001
Water availability	1	1	0.771	0.381
Exposure treatment	2	3	0.656	0.579
Female weight	27	1	25.277	<0.001
Block	5115	1	4797.185	<0.001
<i>Water*Exposure</i>	0	3	0.022	0.996
Residuals	501	470		
<b>Reduced model</b>				
Intercept	50 455	1	47619.901	<0.001
<i>Water availability</i>	2	1	1.880	0.171
<i>Exposure treatment</i>	5	3	1.620	0.184
Female weight	27	1	25.804	<0.001
Block	5117	1	4829.110	<0.001
Residuals	501	473		

Full model provides significance values for interpreting interaction effects (main effects in this model are shown for transparency but should not be interpreted). 'Main effects' model does not fit interactions, and provides significance values for interpreting the main effects. Rows of specific interest are highlighted in italics. Significance values are obtained using the 'Anova' function with type 3 sums of squares in R (version 3.5.0).

**Table A9**  
Effects of water availability and male exposure treatment on offspring survival to adulthood

	Estimate	SE	z	P
<b>Full model</b>				
(Intercept)	1.788	0.048	37.424	<0.001
Water availability (W)	-0.995	0.050	-19.920	<0.001
Exposure treatment (I)	-0.299	0.052	-5.715	<0.001
Exposure treatment (R)	-0.292	0.052	-5.614	<0.001
Exposure treatment (N)	-0.217	0.056	-3.878	<0.001
Female weight	0.000	0.000	-2.086	0.037
Block (2)	-0.410	0.025	-16.596	<0.001
Water(W)*Exposure(I)	0.330	0.068	4.833	<0.001
Water(W)*Exposure(R)	0.498	0.069	7.207	<0.001
Water(W)*Exposure(N)	0.318	0.072	4.390	<0.001
<b>Main effects model</b>				
(Intercept)	1.603	0.038	41.826	<0.001
Water	-0.706	0.024	-28.819	<0.001
Mating	-0.105	0.033	-3.155	0.002
Mating (R)	-0.009	0.034	-0.273	0.785
Mating (N)	-0.030	0.035	-0.860	0.390
Female weight	0.000	0.000	-1.680	0.093
Block (2)	-0.401	0.025	-16.281	<0.001

Full model provides parameter estimates and significance values for interpreting interaction effects (main effects in this model are shown for transparency but should not be interpreted). 'Main effects' model does not fit interactions, and provides parameter estimates and significance values for interpreting the main effects. Results are from binomial models performed using the lme4 package in R (version 3.5.0). W: wet; I: intact male; R: repeated mating; N: no male.

**Table A10**  
ANOVA outputs showing effects of water availability and male exposure treatment on offspring survival to adulthood

	$\chi^2$	df	P
<b>Full model</b>			
Water availability	415.910	1	<0.001
Exposure treatment	42.390	3	<0.001
Female weight	4.350	1	0.037
Block	279.600	1	<0.001
<i>Water*Exposure</i>	54.130	3	<0.001
<b>Reduced model</b>			
<i>Water availability</i>	850.170	1	<0.001
<i>Exposure treatment</i>	12.490	3	0.006
Female weight	2.820	1	0.093
Block	268.950	1	<0.001

Full model provides significance values for interpreting interaction effects (main effects in this model are shown for transparency but should not be interpreted). 'Main effects' model does not fit interactions, and provides significance values for interpreting the main effects. Rows of specific interest are highlighted in italics. Significance values are obtained using the 'Anova' function with type 3 sums of squares in R (version 3.5.0).

**Table A11**  
Tukey's post hoc tests showing how offspring survival to adulthood differs between male exposure treatments within each water availability

Contrast	Estimate	SE	df	t	P
<b>Dry treatment</b>					
None–Ablated	-0.217	0.056	Infinite	-3.878	0.001
None–Repeated	0.074	0.053	Infinite	1.401	0.499
None–Intact	0.082	0.054	Infinite	1.527	0.421
Ablated–Repeated	0.292	0.052	Infinite	5.614	<0.001
Ablated–Intact	0.299	0.052	Infinite	5.715	<0.001
Repeated–Intact	0.007	0.049	Infinite	0.149	0.999
<b>Wet treatment</b>					
None–Ablated	0.101	0.046	Infinite	2.196	0.124
None–Repeated	-0.105	0.047	Infinite	-2.262	0.107
None–Intact	0.069	0.045	Infinite	1.543	0.412
Ablated–Repeated	-0.206	0.045	Infinite	-4.545	<0.001
Ablated–Intact	-0.031	0.044	Infinite	-0.703	0.896
Repeated–Intact	0.175	0.045	Infinite	3.914	0.001

Contrasts were obtained using full models.