



Sexual conflict and the environment: teasing apart effects arising via males and females

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When males and females differ in their reproductive interests, each sex attempts to increase its own reproductive success, sometimes to the detriment of its mate. The environment that individuals experience has been shown to be fundamental in mediating this sexual conflict, since it can alter the balance between the costs and benefits that males and females obtain from mating. In the seed beetle *Callosobruchus maculatus* water availability is an important environmental factor that can alter this balance, with consequences for female mating strategies and the strength of sexual conflict. Whether the effect of water availability arises via effects on females or males, however, has not been explored. Here we manipulated access to water independently for adult males and females and explored how this environmental variation affected male and female mating behaviour, female fitness and offspring development. We found that access to water did not alter the size of ejaculates that males transferred, but that males with water available had longer copulation durations. We also found that females with access to water, despite living longer and laying a similar number of eggs during their lifetime, had offspring with longer development times and lower eclosion success than those of females with no access to water. Our results suggest that water availability has detrimental effects on female fitness, but despite changes in male mating behaviour these effects are not related to changes in the costliness of males as mates.

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Sexual conflict, that is, differences in the evolutionary interests of males and females (Parker, 1979), is widespread among sexual species, and is a powerful force driving the evolution of reproductive traits (Arnqvist & Rowe, 2005; Chapman, Arnqvist, Bangham, & Rowe, 2003). Since the lifetime reproductive interests of males and females rarely coincide (Bateman, 1948), sexual conflict can drive the evolution of traits that maximize the fitness of one sex at the expense of the other (Arnqvist & Tuda, 2010). Conflict between the sexes arises for control over several aspects of reproduction, including mating rate, parental effort, female propensity to remate and copulation duration (reviewed in Chapman et al., 2003). For example, long copulations might benefit males if they increase the amount of sperm transferred (Edvardsson & Canal, 2006) and increase fertilization success (Simmons, 2001). However, long copulations might be costly for females, for example if they increase the amount of toxic substances transferred, the risk of disease transmission or the severity of genital injury (Simmons, 2001; Stockley, 1997; Watson, Arnqvist, & Stallmann, 1998). In such circumstances, females may prefer to terminate copulation as

soon as a minimum threshold of sperm has been transferred. The process by which females evolve resistance traits to reduce the costs associated with male persistence traits can lead to an evolutionary arms race where each sex attempts to maximize its own reproductive success (Perry & Rowe, 2015).

The nature and intensity of sexual conflict over reproduction and the optimal outcome for each sex may vary depending on their environment (Claudia Fricke, Perry, Chapman, & Rowe, 2009; Perry, Garroway, & Rowe, 2017; Yun, Chen, Singh, Agrawal, & Rundle, 2017). This is because the costs and benefits and hence the net value of copulating may depend on environmental conditions. Although in nature many environments are likely to change simultaneously for both sexes (e.g. temperature), in order to understand the economics of reproduction and the evolution of mating strategies in response to sexual conflict, it is important that experimental studies manipulate the environment for males and females independently. This is because the environment can affect male and female interests differently. For example, in environments where females have access to resources, there may be less need to obtain nutrition from nuptial gifts and so the benefits of multiple and/or long copulations may be reduced (Edvardsson, 2007; Toft & Albo, 2015). However, males in the same environments might benefit from increasing their copulation rates if their better body condition allows them to increase their performance and

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fertilization success (Aluja, Jácome, & Macías-Ordóñez, 2001; Kaspi, Taylor, & Yuval, 2000). This situation might lead to increased rates of male harassment and hence to increased costliness of males as mates. In consequence, to balance these conflicting concerns, males and females may adjust their mating behaviour to ensure the best potential fitness outcome in each environmental condition.

The seed beetle *Callosobruchus maculatus* is a good model species to explore how the environment affects the strength of sexual conflict. Mating has several costs for females including a spiny aedeagus (reproductive organ) that damages the female reproductive tract during copulation and consequently reduces female life span (Crudgington & Siva-Jothy, 2000). This damage increases with the duration of copulation (Crudgington & Siva-Jothy, 2000), which might, in turn, create conflict over the optimal copulation duration in this species (but see Edvardsson & Canal, 2006). As a counter-adaptation to harm from males, females perform a conspicuous kicking behaviour during mating that has been suggested to assist in terminating matings (Crudgington & Siva-Jothy, 2000; Edvardsson & Tregenza, 2005; but see ; Wilson & Tomkins, 2014; Dougherty et al., 2017). Despite the costs associated with long-lasting matings for females, there are also potential benefits. For example, longer copulations are associated with the transfer of larger ejaculates (Van Lieshout, McNamara, & Simmons, 2014) that confer direct benefits to females, such as water, which increases female fecundity (Edvardsson, 2007). Therefore, if the environment affects the intensity of sexual conflict and, hence, the complex balance between the costs and benefits of prolonging the copulation for each sex, we might expect mating behaviour to vary plastically in relation to the environment (see e.g. Wilson, Buzatto, Robinson, & Tomkins, 2014).

An important environmental factor that might affect the intensity of sexual conflict over mating in seed beetles is the presence of water. *Callosobruchus maculatus* live in dry environments in stored legumes, where male ejaculates are expected to be an important source of water. In such conditions, if the benefits of acquiring water from ejaculates outweigh the costs of copulation, females will be expected to be more willing to prolong copulations and start kicking later. However, in environments where water is present, females might opt for a less costly source of water than male ejaculate (i.e. females drink water when available in the environment, Edvardsson, 2007), and hence alter their mating behaviour accordingly. This might conflict with male interests if males with access to water can improve their fitness by increasing the copulation duration to transfer larger ejaculates. Previous studies have shown strong effects of environmental water on female life span and mating behaviour in *C. maculatus* (Edvardsson, 2007; Iglesias-Carrasco, Bilgin, Jennions, & Head, 2018). However, these studies manipulated the availability of water for both sexes simultaneously, thus limiting the conclusions that can be made, since it is impossible to disentangle whether changes in behaviour are driven by male and/or female responses to water availability.

To explore whether differences in mating behaviour between wet and dry environments are driven by the effects of water availability on males, females or both, we manipulated male and female access to water independently and paired individuals to mate within and across treatments. To see the effect that the manipulation of water availability has on male and female mating traits, we measured male ejaculate size, copulation duration and female kicking behaviour during copula. To determine whether water availability mediates sexual conflict, we also looked at the subsequent fitness consequences for females from wet or dry environments of mating with males from wet or dry environments, by measuring female fitness traits including life span and fecundity, as well as early life fitness traits of offspring including development time and survival to adulthood. We made the following predictions. (1) Males from the wet treatment would transfer larger ejaculates

(i.e. because they contain more water) than those from the dry treatment. (2) Mating duration would depend on the sex that is driving these behaviours. So, if females are driving copulation duration, matings involving females from the dry treatment would be longer to allow enough ejaculate/water transfer. Further, females would have longer copulations when mating with males from the dry treatment. We predicted this because males in dry conditions are expected to transfer less water to females in their ejaculates, and if females need water from male ejaculates then they will mate for longer with males from dry conditions to obtain a similar quantity of water. However, if males are driving copulation duration, copulations involving males from the wet treatment would be longer so males can transfer their larger ejaculates, to reduce female remating and, hence, sperm competition. (3) Females from the wet treatment, or those mating with males from the wet treatment (if these males transfer larger water-rich ejaculates), would have elevated fitness compared to females from the dry treatment that are mated to males from the dry treatment.

METHODS

Study Species

Callosobruchus maculatus is a pest of stored legumes. They lay eggs on the surface of host beans, into which the larvae burrow, and emerge 21–29 days later, depending on the ambient temperature and humidity (Fox, 1993b). We used beetles from a large stock population maintained, at The Australian National University, on mung beans, *Vigna radiata*, for nine generations and incubated at 25 ± 1 °C.

Experimental Design

To investigate how water availability influences mating behaviour, sexual conflict and female fitness, we used a 2×2 factorial experimental design where male and female beetles were divided into two groups, and water availability was manipulated to produce either a wet or dry condition for each sex. The dry treatment is consistent with the conditions in which the stock population is kept (a seed store environment). Males and females were then paired to produce four treatment combinations: a dry-treatment female with a dry-treatment male ($N = 125$); a dry-treatment female with a wet-treatment male ($N = 126$); a wet-treatment female with a dry-treatment male ($N = 128$); and a wet-treatment female with a wet-treatment male ($N = 126$). Approximately half of these pairs were allowed to mate once, while the other half were mated twice.

Experimental Procedure

To obtain virgin beetles, mated females from our stock population were individually placed in petri dishes with five mung beans. Each female was allowed to lay eggs for 1 h, after which each bean was inspected and those with one egg laid on them were placed in individual Eppendorf tubes (if beans had more than one egg, excess eggs were scraped off). This process was repeated multiple times until we had ca. 1500 mung beans each with a single egg on them. Tubes were labelled with their maternal identity to prevent siblings later being paired together. The eggs were incubated at 25 °C and beetles emerged approximately 29 days after being laid. After emergence, each beetle was placed into a 70 ml plastic container and assigned to either the wet or dry treatment. In the wet treatment a vial lid containing water-soaked cotton wool was placed into the container (Edvardsson, 2007; Iglesias-Carrasco, Bilgin, et al., 2018). Males and females remained in these conditions alone for 24 h, to allow them to mature (Fox, Hickman, Raleigh, &

Mousseau, 1995). Owing to time constraints, data were collected over two blocks conducted using consecutive generations of our stock population. Block was included in all analyses to control for potential temporal variation in rearing conditions.

After reaching maturity, each beetle was weighed, immediately before the mating trial, so that natural loss of weight (e.g. due to desiccation) did not confound measures of ejaculate mass. Neither female ($P = 0.074$) nor male body weight ($P = 0.088$) differed between water treatments at this point of the experiment. We then paired males and females according to the treatment combinations outlined above and placed them in an Eppendorf tube (1.5 ml) to record their mating behaviour. During behavioural trials we recorded (1) the time from when copulation began to when a female started kicking (hereafter kicking latency), (2) how long females spent kicking before copulation ended (kicking duration) and (3) the time from when copulation began to when it ended (copulation duration). Each pair combination was given 30 min to initiate copulation; over 98% began mating within 1 min, while the remaining 10 (of $N = 524$) did not copulate. After copulation the male was weighed again to estimate the mass of ejaculate transferred (i.e. weight before mating – weight after mating) and the female was returned to her treatment (wet or dry) along with 40 mung beans. To weigh beetles, we used a Sartorius Cubis microbalance with 0.001 mg precision. Forty-eight hours after the initial mating, approximately half of the females were mated a second time to a new (but previously mated) male of the appropriate water treatment. Again, matings were conducted in an Eppendorf tube. Here, our aim was to explore whether the effects of male water treatment on female fitness resulted from cumulative effects of reduced ejaculate water, so we did not record behavioural measures. However, we did observe the pairs to ensure that they copulated successfully. Copulations were considered successful when males mounted females, inserted their aedeagus into the female and remained stationary in this posture for at least 2 min before females commenced kicking.

Once females had completed their mating trials, they were returned to new individual treatment containers and given 40 fresh beans and water every 24 h, before being left in the final container on day 5. We ensured that the water was kept separate from the beans, to prevent them from going mouldy and we removed the water from the final egg container after 24 h to ensure that larvae hatching from eggs laid on different days all developed in beans that had been exposed to the wet treatment for the same amount of time. Females were kept in the final egg container until death. The date of death was recorded to allow calculation of life span, and the female was removed from the container.

Once a female perished, we counted the eggs laid in each of the sample containers (i.e. from day 1–4). This allowed us to look at female egg laying rate (i.e. number of eggs laid in a 24 h period) over 3 days, as well as the total number of eggs a female laid over her lifetime (i.e. the sum of all four sample containers, hereafter 'female lifetime fecundity'). After an approximate 29-day incubation period offspring began to emerge. We recorded the date of first emergence for each sample container to estimate larval development time. Ten days after the first emergence was recorded, the container was frozen, and the emerged adult offspring were counted to determine egg to adult survival. All counts were conducted blind and by the same observer for consistency. Throughout the experiment all beetles and beans were kept in a controlled temperature room, at 25 °C.

Ethical Note

This work followed the ASAB/ABS guidelines for the treatment of animals in behavioural research. Housing conditions, handling

and experimental monitoring were all conducted in a way to maximize the animals' welfare. We complied with the Australian regulations for experiments on invertebrates.

Statistical Analysis

To look at the independent effects of male and female water environment as well as how they interact to affect mating behaviour, female fitness and offspring traits we analysed our data using the lme4 package (Bates, Maechler, Bolker, & Walker, 2015) in R (version 3.5.0). In all models we included female and male water treatments and their two-way interaction as fixed effects. In all models we also included female weight as a covariate and experimental block as a fixed factor to control for potential temporal variation in rearing conditions. We included block number as a fixed effect rather than a random factor because we had only two levels (Bolker et al., 2009). In models looking at female fitness and offspring traits we additionally included the number of times a female copulated (one or two) as a fixed factor.

Parameter and significance estimates were obtained using the 'summary' function. All models were first run with the two-way interaction between male and female water treatment (full models) and then without the interaction (main effects models) so that we could interpret the main effect of each treatment when the interaction was nonsignificant. When two-way interactions were significant, we conducted post hoc pairwise comparisons using Tukey tests. Further model details are provided below.

Mating trials

Male ejaculate size (i.e. weight before mating – weight after mating) and measures of mating behaviour (i.e. copulation duration, kicking latency and kicking duration) were analysed using linear models (LM). After fitting models, we checked that model residuals met the assumption of normality and homoscedasticity. When they did not (as was the case for all measures of mating behaviour) we transformed the data using the power Transform function in the car package and confirmed assumptions were met following transformation.

Female fitness traits

The effects of male and female water treatment on female egg laying were analysed in two ways. First, we used a generalized linear model (GLM) with a quasi-Poisson error distribution to determine how our treatments influenced female lifetime fecundity (i.e. number of eggs laid in all four containers). Second, to test whether male and female water treatments affected female egg laying rate (i.e. number of eggs laid over 24 h) we used an LM. For this analysis, we only used the third day after the copulation (i.e. the third container). We used this day because it occurred after all matings had taken place. We tested the effect of male and female water treatments on female survival using a Cox proportional hazard model (function coxph, R package 'survival,' Therneau & Grambsch, 2000).

Female and male body weight

To look at differences in water loss between individuals from the wet and dry treatments, we ran an LM that included body weight (the second measurement just before the second copulation and after 2 days of treatment) as the response variable, water treatment as a fixed factor and the number of eggs laid up to that day (for females) or the size of the ejaculate transferred in the first copulation (for males) as covariate.

Offspring traits

Larval development time was analysed using an LM after transforming the data (as above). The number of offspring emerging from beans as adults was analysed using a GLM with a binomial error structure using the cbind function (number of eclosed adults; number of unhatched eggs). For this analysis we excluded 35 females (final $N = 444$) for which our records of survival were greater than 100% (presumably due to errors in egg counting). Again we used day 3 containers only for this, since this day occurred after all copulations had been conducted but before maternal age-related declines in offspring survival (Fox, Bush, & Wallin, 2003).

RESULTS

Full model outputs with parameter estimates are provided in Appendix Tables A1–A5 and full figures with raw data in Appendix Fig. A1.

Ejaculate Size

There was no significant effect of male water treatment, female water treatment or the two-way interaction between them on male ejaculate size transferred during the first copulation (Table A1).

Mating Behaviour

The two-way interaction between male and female water treatments had a significant effect on kicking duration (Fig. 1, Table A2). Females from the wet treatment had shorter kicking durations when mated with males from the dry than from the wet treatment (Tukey pairwise comparison: $P = 0.002$). However, females from the dry treatment showed no difference in kicking duration when mated to males from dry or wet treatments (Tukey pairwise comparison: $P = 0.895$). There were no effects of the two-way interaction between male and female treatments on kicking latency or the copulation duration (Table A2). When looking at main effects after removing interactions from our models, female water treatment had no overall effect on any of the behavioural variables

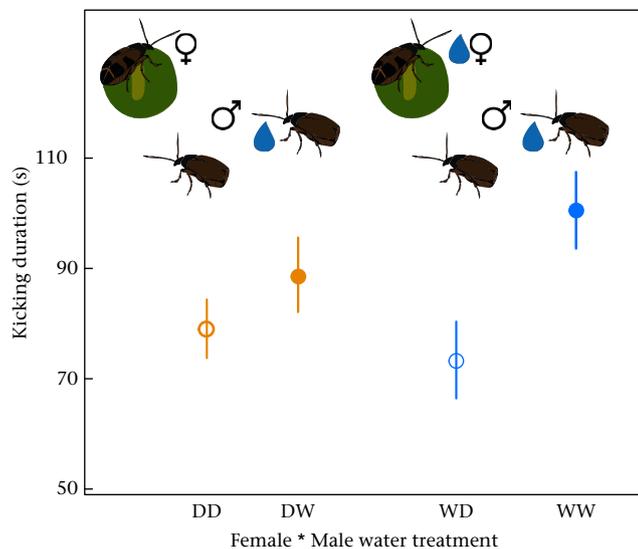


Figure 1. Mean \pm SE kicking duration (time (s) elapsed since females started kicking until the end of the copulation) in relation to female and male water treatments (DD: both female and male in dry treatment; DW: female in dry treatment and male in wet treatment; WD: female in wet treatment and male in dry treatment; WW: both female and male in wet treatment).

measured (Table A2). Male water treatment, on the other hand, had no effect on kicking latency, but did affect copulation duration (Table A2), with males from the wet treatment having longer copulations than males from the dry treatment. Wet-treatment males showed no difference in copulation duration when mated to females from dry or wet treatments (Tukey pairwise comparison: $P = 0.970$).

All the behavioural traits were intercorrelated (Pearson correlations: Kicking latency–Kicking duration: $r = -0.114$, $P = 0.011$; Kicking latency–Total copulation duration: $r = 0.853$, $P < 0.001$; Kicking duration–Total copulation duration: $r = 0.422$, $P < 0.001$).

Female fitness traits

There was no effect of male treatment on female life span; however, females from the wet treatment lived longer than females from the dry treatment (Table A3). Neither the number of copulations nor the two-way interaction between male and female treatment significantly affected female life span (Table A3).

Neither male treatment, female treatment nor the two-way interaction between them affected female lifetime fecundity (Table A4). However, females that copulated twice laid more eggs than those mated just once (Table A4).

We found an effect of the two-way interaction between male and female treatments on egg laying rate (Table A4, Fig. 2): females from the dry treatment that were mated with males from the dry treatment laid significantly more eggs over 24 h than females from the wet treatment mated with males from either wet (Tukey's test: $P = 0.042$) or dry treatments (Tukey's test: $P < 0.001$). When looking at main effects after removing interactions from the model we found no overall effect of male water treatment on female egg laying rate (Table A4). Females from the dry treatment, however, laid more eggs in 24 h than those from the wet treatment. Finally, females mated twice had higher egg laying rates than those mated just once (Table A4).

Female and Male Body Weight

Females from the wet treatment were heavier after 2 days in the treatment than those from the dry treatment (LM: $t = 6.483$, $P < 0.001$). However, water treatment did not affect male weight (LM: $t = -0.165$, $P = 0.869$).

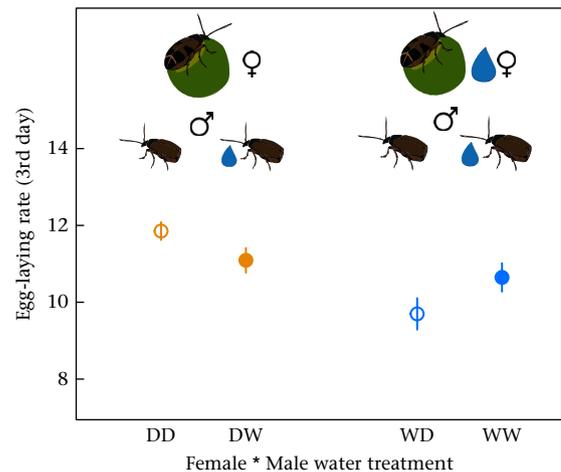


Figure 2. Mean \pm SE number of eggs laid by females over 24 h (corresponding to the third day after the first copulation) in relation to female and male water treatments (DD: both female and male in dry treatment; DW: female in dry treatment and male in wet treatment; WD: female in wet treatment and male in dry treatment; WW: both female and male in wet treatment).

Offspring Traits

We found no effect of male treatment on larval development time or number of adult offspring eclosing (Table A5). However, there was a significant effect of female water treatment on both offspring traits: larvae of females from the wet treatment took longer to eclose (Fig. 3a), and fewer offspring emerged as adults from beans (Table A5, Fig. 3b) than those from dry-treatment females. Neither the two-way interaction between male and female treatment nor the number of copulations had an effect on offspring traits (Table A5).

DISCUSSION

We predicted that male and female mating behaviours and the costs of mating for females would depend on the environmental conditions experienced by males and/or females. To test this prediction, we independently manipulated the environment (dry versus wet) of male and female *C. maculatus* by altering water availability. We found that males from the wet treatment had longer copulation durations than males from the dry treatment, but this effect did not depend on female water treatment. However, despite longer copulations, males from the wet treatment did not transfer larger ejaculates than those from the dry treatment. We also found that females from the wet treatment lived longer than females from the dry treatment. However, females from the wet treatment had similar total lifetime fecundity to those from the dry treatment and their offspring quality was lower. We found no

evidence that water availability modified the fitness costs of mating with males originating from different treatments.

Mating Behaviour

Mating behaviour should reflect a balance between the optimal copulation duration for males and that for females, and this optimum might depend on the environment if it alters the costs and benefits of mating for either sex. For *C. maculatus*, we expect females to start kicking males sooner, to terminate copulation, when the costs of long copulations are high (Edvardsson & Canal, 2006) or at least when these costs outweigh the potential benefits. In the current experiment we predicted that the balance between the costs and benefits of mating would depend on whether females had access to environmental water or not. For example, if females in the wet treatment gained little benefit from the water in large ejaculates, we predicted they would have shorter kicking latencies and copulation durations than females in the dry treatment. However, we found no effect of female water treatment on copulation duration or the latency to kick. Access to water may not alter the preferred mating duration of females for several reasons: (1) the benefits of at least the first copulation are similar for females in both treatments; (2) even for females from the wet treatment, the potential benefits of ending copulations early do not outweigh the energetic costs of resisting male attempts to prolong copulation; and (3) females may simply not obtain water from male ejaculates.

We also predicted that female mating behaviour would depend not only on their own but also their mate's environmental

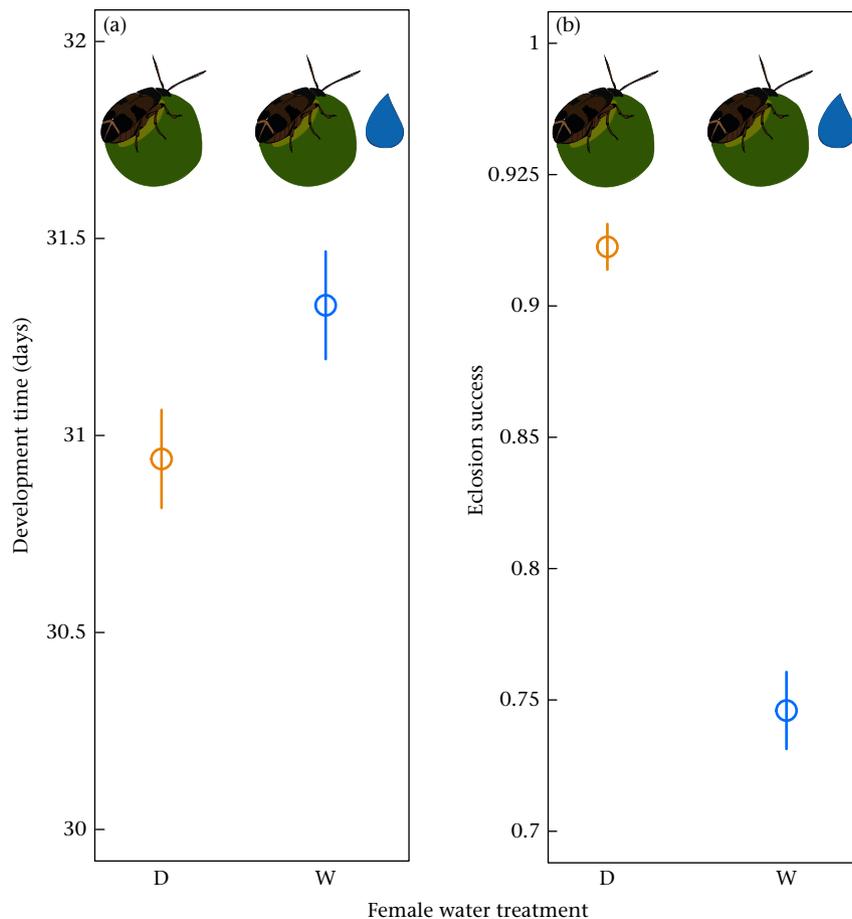


Figure 3. Mean \pm SE (a) larval development time (days) and (b) eclosion success (unlike the statistical model the figure shows the eclosion success as the proportion of eggs eclosing as adults, i.e. number of eclosed individuals/number of eggs) in relation to female water treatments (D: dry treatment; W: wet treatment).

conditions. We predicted that females mating with wet-treatment males would kick sooner if those males transferred larger ejaculates (Wilson et al., 2014) in a shorter time or meet female demands (e.g. for hydration) quicker. However, male water treatment did not affect the latency to kick, suggesting that the time to reach a sufficient amount of ejaculate needed for females to start kicking (Van Lieshout et al., 2014) was similar regardless of male treatment. This is consistent with the fact that males in both treatments transferred ejaculates of similar size. Interestingly, we found that matings involving males from the wet treatment had longer kicking and copulation durations than those involving males from the dry treatment, suggesting that males, rather than females, might be driving these behaviours, a suggestion also made by Wilson and Tomkins (2014). One potential explanation is that males prolong copulation to deliver a larger ejaculate to activate ovulation. However, this is unlikely since ejaculates transferred by males in both treatments did not differ in size. Another potential explanation is that access to environmental water increases male condition and hence wet-treatment males were better able to fight the dislodging attempts of females. This latter explanation is also consistent with the two-way interaction found between male and female treatments for kicking duration. Females from the wet treatment mating with males from the wet treatment had longer kicking durations than wet-treatment females mating with dry-treatment males. This pattern could result from an escalation of male persistence and female resistance traits when both males and females were from the wet treatment, if these individuals were in better condition. That is, if water availability increases performance of both males and females, we would expect females from wet treatments to be better able to kick and dislodge males, but we would also expect males from wet treatments to be better at overcoming being dislodged. This could potentially lead to the longer kicking durations and subsequently longer copulations between wet-treatment males and females that we saw here. Although we found no difference in male weight after 2 days in their respective treatment, weight may not be the best proxy for explaining variation in physiological condition caused by water loss. A challenge for future experiments designed to test these explanations is finding a suitable way to measure condition.

Finally, we found that neither the duration of the copulation nor the size of the ejaculate transferred depended on female water treatment. These results contrast with previous studies that found that males of several insect species adjust the quantity and/or composition of ejaculates in response to factors that correlate with female quality and fecundity (strategic ejaculation; Thomas & Simmons, 2007; Kelly & Jennions, 2011; Harley et al., 2013). There are two potential explanations for the lack of effect of female water treatment on male ejaculate size. The first is that female quality as a mate does not change enough with the presence of environmental water for males to alter their investment in ejaculates. The second is that in *C. maculatus* ejaculate allocation might not be beneficial for males (Rönn, Katvala, & Arnqvist, 2008), since the size/composition of the ejaculate directly affects female fitness. Strategic ejaculate allocation might therefore lead to sperm shortage in females and reduce their lifetime fecundity, which in turn can reduce the male's reproductive success.

Female Fitness and Offspring Traits

Females from the wet treatment lived longer, had lower egg laying rates (i.e. over 24 h), but had similar total lifetime fecundity in comparison to females from the dry treatment. Despite the strong effect of female body mass on all the fitness traits measured (see also Iglesias-Carrasco, Jennions, Zajitschek, Head, 2018), our results were significant even when female body mass was included as a covariate,

suggesting that the effect of water is independent of body size. Our results regarding the effect of water on female fitness traits are similar to a previous study which showed that having access to water increased female life span, but did not affect lifetime fecundity (Iglesias-Carrasco, Bilgin, et al., 2018). However, in that study there was no attempt to distinguish between effects arising through a female's own environment and that of her mate. If having access to water improves female condition and confers a life span advantage we might expect females in wet conditions or females that mated with wet-treatment males that transferred more water in their ejaculates to lay more eggs during their lives, in part because they had more time for egg laying. However, since emerging female *C. maculatus* already have matured eggs in their ovaries (Wilson & Hill, 1989), the costs of egg maturation are likely to be incurred primarily during the larval stage (Johanna Rönn, Katvala, & Arnqvist, 2006). Therefore, resource acquisition for investment in egg production may be more important during larval development than during adulthood. Since all the adults used in our experiment experienced the same dry conditions during development, it is possible that all females had a similar amount of resources to allocate towards egg production, explaining why females in both treatments did not differ in their lifetime fecundity in our study. Further experiments would benefit from exploring whether the larval environmental conditions experienced by females affect their subsequent investment in offspring.

Combined, our results for female life span and lifetime fecundity suggest that there are differences in the way that females in the two water treatments partitioned resources to eggs across their lifetime. In general, females from the dry treatment laid more eggs per day than females from the wet treatment. This effect was detectable across both male water treatments. This suggests that females from the wet treatment laid fewer eggs daily but continued to lay eggs later in life, while dry-treatment females tended to lay more eggs early in life possibly because their chances of survival were lower (i.e. live-fast die-young strategy, e.g. Travers, Garcia-Gonzalez, & Simmons, 2015). Changes in the partitioning of resources could also explain why females from the wet treatment had offspring with lower eclosion success and longer development times than females from the dry treatment. Previous studies in *C. maculatus* have shown that eggs laid by females late in life are smaller and have lower eclosion success (Fox, 1993b), probably due to the depletion of breeding resources (Richards & Myers, 1980). This reduction in maternal investment also leads to longer larval development times (Fox, 1993b). In many insects, offspring from larger eggs hatch earlier, have higher hatching success and develop into larger adults (Mousseau & Fox, 1998). Therefore, the longer development time and lower larval survival that we saw for wet-treatment females could indicate a maladaptive response to the wet environment or decreased maternal investment potentially arising from changes in life history strategies or variation in maternal condition. Alternatively, if our populations are adapted to dry conditions, poor performance of offspring from wet-treatment females might arise from beans being exposed to humidity for 24 h, which could have reduced the quality of the environment for larval development.

Given the results of previous studies (Edvardsson, 2007; Ursprung, Den Hollander, & Gwynne, 2009) we expected that females with water available would have higher fitness than those with no water. However, despite the greater weight of females after 2 days in the treatment, which could indicate reduced dehydration, females from the wet treatment generally performed worse than those from the dry treatment. The prediction that wet-treatment females should do better assumes that water is a limiting resource and that dehydration comes with physiological costs. While this may be the case in many systems, our population of seed beetles is likely to be well adapted to dry environments after

spending many generations in dry grain storage environments. Thus, it is possible that our results reflect local adaptation to dry environments and an inability of individuals to cope with wet conditions. However, it is likely that wild ancestral populations would have had access to water. Doing experimental line evolution might provide a way to determine whether local adaptation to dry environments provides an explanation for the poor performance of females in our wet treatment.

Although male water treatment had an effect on mating behaviour, with copulations involving males from the wet treatment being longer with more kicking than matings involving dry-treatment males, we found no effect of male treatment on female fitness. This is not surprising since despite longer copulation durations of wet-treatment males, they did not transfer larger ejaculates than dry-treatment males; hence, females were unlikely to have received greater direct benefits which could have increased their fecundity (Edvardsson & Canal, 2006; Yamane & Miyatake, 2012). Previous studies have suggested that longer copulation and kicking durations would increase physical damage in females and in consequence decrease female fecundity and life span (Crudginton & Siva-Jothy, 2000). Our results, however, suggest that despite longer copulation and kicking durations when mated with wet-treatment males, the costs associated with wounding female genitalia are not enough, at least after a single copulation, to affect female lifetime fecundity.

Finally, we found that females mated twice, and hence received sperm from two different males, laid more eggs than those mated only once. This is consistent with previous studies in seed beetles that found a positive relationship between multiple mating and female fecundity (Eady, Wilson, & Jackson, 2000). This suggests that the benefits of sperm replenishment might outweigh the direct physical costs of a second copulation. It is also possible that in *C. maculatus* one copulation is not enough to fertilize all the eggs (Fox, 1993a). If polyandry is beneficial for females, we might expect a positive effect on offspring traits of females mated twice compared to those mated only once. However, we did not find any effect of the number of copulations on offspring development time or eclosion success, which suggests that females remate to derive direct benefits from males (i.e. sperm), rather than indirect genetic benefits (see also Eady et al., 2000). However, we cannot discard the possibility that indirect benefits of polyandry, if any, might be detectable after more copulations and/or in other unmeasured offspring traits.

Conclusions

Despite changes in mating interactions that were associated with male water treatment, we found no evidence that the male's access to water affected his costliness as a mate. Nor did we find an effect of a male's environment on the size of the ejaculate transferred to females in each copulation. However, the environment that females themselves experienced had strong effects on the way that they partitioned resources to egg laying across their life which had lasting consequences for offspring fitness. This suggests that changes in the balance between the costs and benefits associated with reproducing in different environments are driven largely by variation in female condition. Our study provides evidence for the importance of the environment for female fitness. If we aim to understand the economics of mating and the evolution of mating strategies future studies would benefit from exploring the costs associated with mating in a variety of environments (e.g. Colpitts, Willisicroft, Sekhon, & Rundle, 2017; Yun et al., 2017).

Data availability

Data will be made available on request.

Declaration of Interest

The authors declare no competing interests.

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Appendix

Tables A1–A5 show model outputs from full (including interaction terms between male and female treatments) and reduced (main effects) models. All models include male and female treatments and block ID as fixed factors and female weight as a random effect. In the models exploring female fitness and offspring traits, the number of times (one or two) that females copulated was included as a covariate.

Table A1
Effect of water treatment on male ejaculate size

	Estimate	SE	t	P
Full model				
(Intercept)	0.1894	0.0334	5.676	<0.001
Male treatment (Wet)	0.0019	0.0080	0.242	0.809
Female treatment (Wet)	-0.0091	0.0079	-1.150	0.251
Block ID	0.0004	0.0058	0.073	0.942
Female weight	0.0050	0.0049	1.018	0.309
Interaction	0.0110	0.0112	0.985	0.325
Main effects model				
(Intercept)	0.1872	0.0333	5.623	<0.001
Male treatment (Wet)	0.0076	0.0056	1.345	0.179
Female treatment (Wet)	-0.0036	0.0056	-0.642	0.521
Block ID	0.0005	0.0058	0.087	0.931
Female weight	0.0049	0.0049	0.993	0.321

Interaction: female treatment*male treatment.

Table A2
Effect of female and male water treatment on behavioural traits

	Estimate	SE	t	P
Kicking latency				
Full model				
(Intercept)	0.0560	0.0028	19.933	<0.001
Male treatment (Wet)	0.0000	0.0007	-0.044	0.965
Female treatment (Wet)	0.0004	0.0007	0.583	0.560
Block ID	-0.0018	0.0004	-4.287	<0.001
Female weight	-0.0073	0.0005	-15.008	<0.001
Interaction	0.0001	0.0009	0.053	0.957
Main effects model				
(Intercept)	0.0559	0.0028	19.999	<0.001
Male treatment (Wet)	0.0000	0.0005	-0.009	0.993
Female treatment (Wet)	0.0004	0.0005	0.874	0.382
Block ID	-0.0018	0.0004	-4.293	<0.001
Female weight	-0.0073	0.0005	-15.024	<0.001
Kicking duration				
Full model				
(Intercept)	4.5223	0.4492	10.067	<0.001
Male treatment (Wet)	0.0758	0.1076	0.704	0.482
Female treatment (Wet)	-0.2043	0.1064	-1.920	0.055
Block ID	-0.2138	0.0662	-3.229	0.001
Female weight	-0.0014	0.0778	-0.017	0.986
Interaction	0.3059	0.1507	2.030	0.043
Main effects model				
(Intercept)	4.4587	0.4495	9.918	<0.001
Male treatment (Wet)	0.2314	0.0758	3.053	0.002
Female treatment (Wet)	-0.0522	0.0758	-0.688	0.492
Block ID	-0.2167	0.0664	-3.264	0.001
Female weight	0.0010	0.0781	0.012	0.990
Copulation duration				
Full model				
(Intercept)	0.0371	0.0021	17.703	<0.001
Male treatment (Wet)	-0.0005	0.0005	-0.917	0.360
Female treatment (Wet)	0.0007	0.0005	1.346	0.179
Block ID	-0.0005	0.0003	-1.668	0.096
Female weight	-0.0048	0.0004	-13.138	<0.001
Interaction	-0.0009	0.0007	-1.269	0.205
Main effects model				
(Intercept)	0.0372	0.0021	17.824	<0.001
Male treatment (Wet)	-0.0009	0.0004	-2.592	0.010
Female treatment (Wet)	0.0002	0.0004	0.638	0.524
Block ID	-0.0005	0.0003	-1.640	0.102
Female weight	-0.0048	0.0004	-13.150	<0.001

Interaction: female treatment*male treatment. Significant results are shown in bold.

Table A3
Effect of male and female water treatment on female survival (Cox proportional hazards)

	χ^2	df	P
Full model			
Male treatment	0.2222	1	0.637
Female treatment	19.7579	1	<0.001
Number of copulations	0.7050	1	0.401
Female weight	5.7435	1	0.017
Block ID	2.1649	1	0.141
Interaction	0.0434	1	0.835
Main effects model			
Male treatment	0.2222	1	0.637
Female treatment	19.7479	1	<0.001
Number of copulations	0.7050	1	0.401
Female weight	5.7435	1	0.017
Block ID	2.1649	1	0.141

Table A4
Effect of male and female water treatment on female reproduction

	Estimate	SE	z	P
Female lifetime fecundity				
Full model				
(Intercept)	3.7200	0.0736	50.537	<0.001
Male treatment (Wet)	-0.0070	0.0172	-0.406	0.685
Female treatment (Wet)	-0.0144	0.0173	-0.834	0.405
Number of copulations (2)	0.0751	0.0123	6.086	<0.001
Female weight	0.1445	0.0109	13.318	<0.001
Block ID	-0.0338	0.0126	-2.684	0.008
Interaction	0.0358	0.0245	1.461	0.145
Main effects model				
(Intercept)	3.7115	0.0735	50.526	<0.001
Male treatment (Wet)	0.0107	0.0123	0.867	0.386
Female treatment (Wet)	0.0034	0.0123	0.275	0.783
Number of copulations (2)	0.0760	0.0123	6.160	<0.001
Female weight	0.1443	0.0109	13.285	<0.001
Block ID	-0.0333	0.0126	-2.647	0.008
Egg laying rate				
Full model				
(Intercept)	4.3294	1.9375	2.235	0.026
Male treatment (Wet)	-0.9076	0.4618	-1.965	0.050
Female treatment (Wet)	-1.9868	0.4589	-4.330	<0.001
Number of copulations (2)	1.4395	0.3301	4.360	<0.001
Female weight	1.1898	0.2874	4.140	<0.001
Block ID	-0.2930	0.3373	-0.869	0.386
Interaction	1.6692	0.6547	2.549	0.011
Main effects model				
(Intercept)	3.9677	1.9435	2.042	0.042
Male treatment (Wet)	-0.0788	0.3299	-0.239	0.811
Female treatment (Wet)	-1.1687	0.3299	-3.543	<0.001
Number of copulations (2)	1.4756	0.3317	4.448	<0.001
Female weight	1.1734	0.2890	4.060	<0.001
Block ID	-0.2694	0.3391	-0.794	0.427

Interaction: female treatment*male treatment. Significant results are shown in bold.

Table A5
Effect of male and female water treatment on offspring traits

	Estimate	SE	z	P
Larval development time				
Full model				
(Intercept)	0.0114	0.0004	28.143	<0.001
Male treatment (Wet)	0.0000	0.0001	-0.298	0.766
Female treatment (Wet)	-0.0002	0.0001	-2.198	0.028
Number of copulations (2)	0.0000	0.0001	-0.150	0.881
Female weight	0.0003	0.0001	5.696	<0.001
Block ID	0.0022	0.0001	31.131	<0.001
Interaction	0.0000	0.0001	0.235	0.815
Main effects model				
(Intercept)	0.0114	0.0004	28.245	<0.001
Male treatment (Wet)	0.0000	0.0001	-0.187	0.852
Female treatment (Wet)	-0.0002	0.0001	-2.840	0.005
Number of copulations (2)	0.0000	0.0001	-0.141	0.888
Female weight	0.0003	0.0001	5.699	<0.001
Block ID	0.0022	0.0001	31.191	<0.001
Eclosion success				
Full model				
(Intercept)	2.4791	0.5086	4.875	<0.001
Male treatment (Wet)	-0.0867	0.1543	-0.562	0.574
Female treatment (Wet)	-1.3635	0.130	-10.472	<0.001
Number of copulations (2)	0.0010	0.083	0.012	0.990
Female weight	0.0738	0.0744	0.992	0.321
Eggs on day 3	0.0990	0.0046	21.592	<0.001
Block ID	-0.2285	0.0862	-2.650	0.008
Interaction	-0.0389	0.1818	-0.214	0.831
Main effects model				
(Intercept)	2.4954	0.5030	4.962	<0.001
Male treatment (Wet)	-0.1146	0.0821	-1.396	0.163
Female treatment (Wet)	-1.3834	0.091	-15.150	<0.001
Number of copulations (2)	0.0009	0.0827	0.011	0.991
Female weight	0.0735	0.0744	0.988	0.323
Eggs on day 3	0.0991	0.0031	31.715	<0.001
Block ID	-0.2289	0.0862	-2.655	0.008

Interaction: female treatment*male treatment. Significant results are shown in bold.

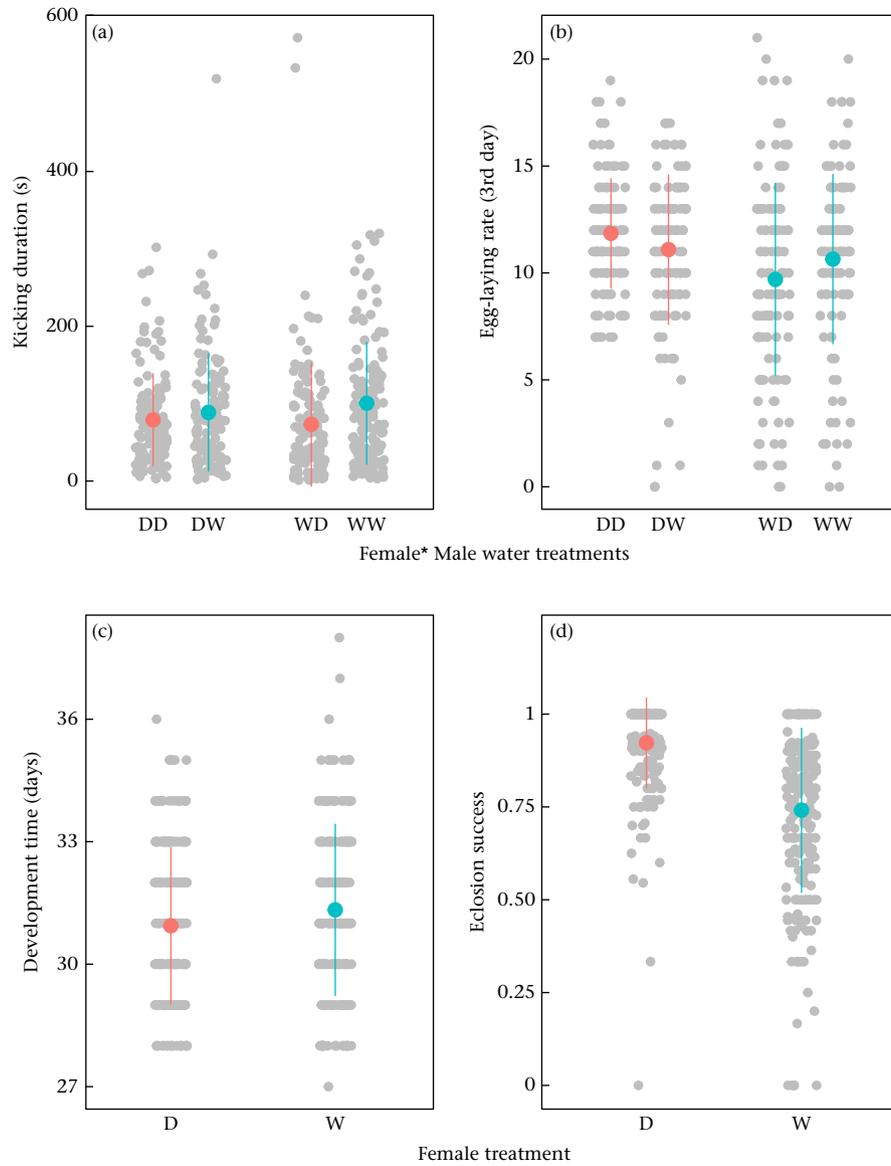


Figure A1. Raw data, mean and SD of (a) kicking duration (time (s) elapsed since females started kicking until the end of the copulation), (b) number of eggs laid by females over 24 h (corresponding to the third day after the first copulation), (c) larval development time (days) and (d) eclosion success (unlike the statistical model the figure shows the eclosion success as the proportion of eggs eclosing as adults, i.e. number of eclosed individuals/number of eggs) in relation to (a, b) female and male water treatments (DD: both female and male in dry treatment; DW: female in dry treatment and male in wet treatment; WD: female in dry treatment and male in dry treatment; WW: both female and male in wet treatment) and (c, d) female water treatments (D: dry treatment; W: wet treatment).