The fitness cost to females of exposure to males does not depend on water availability in seed beetles

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Access to multiple males can benefit a female in terms of increased fecundity and/or offspring performance. However, the presence of more males can also impose costs on females that arise from an elevated mating rate (e.g. due to increased genital damage, loss of feeding opportunities) and/or increased harassment. Different environments might influence the relative magnitude of these costs and benefits, because they can influence how often males and females encounter each other as well as the nature of these encounters. In the seed beetle, *Callosobruchus maculatus*, water is a limiting resource for females that can be obtained from male ejaculates. Here we explored whether the net fitness of female seed beetles is affected by breeding in either a dry or a wet environment when housed with differing numbers of males (none, one or four). Consistent with costly male harassment, females housed with four males laid significantly fewer eggs than those housed alone or with a single male, but there was no effect of the number of males on female egg-laying rate, life span, larval development rate or egg–adult survival of offspring. Although females in the wet environment lived significantly longer, there was only tentative evidence that water availability affected the net fitness cost to females of being exposed to more males. We conclude that to understand the evolution of mating systems it is important to explore how the environment affects female fitness by balancing the costs and benefits of being exposed to males.

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Reproductive interactions with males benefit females by providing access to sperm that is needed for offspring production. However, at a certain point, high male density can also be costly for females. In most species the optimal number of matings is lower for females than males (Arnqvist & Nilsson, 2000). This generates sexual conflict over mating and can lead to male harassment (i.e. repeated unsuccessful mating attempts by coercive males), which, in turn, can generate a wide range of costs for females, including reduced feeding opportunities or increased physical injury and energy expenditure when resisting males (Bateman, Ferguson, & Yetman, 2006; Rönn, Katvala, & Arnqvist, 2006; Takahashi & Watanabe, 2010), often with detrimental effects on female fitness (Crudgington & Siva-Jothy, 2000; Eady, Hamilton, & Lyons, 2007). In consequence, females might sometimes opt to mate simply to decrease harassment (i.e. convenience polyandry; Blyth & Gilburn, 2006; Thornhill & Alcock, 1983). Although there are potential

benefits to mating multiply for females, both direct (e.g. greater male services and resources, Hasson & Stone, 2009; Townsend, Clark, & McGowan, 2010) and indirect (e.g. higher genetic quality offspring or the use of compatible sperm to ensure offspring viability, Jennions & Petrie, 2000; Tregenza & Wedell, 2002), mating can impose direct costs that accumulate with each successive mating (Crudgington & Siva-Jothy, 2000; Fedorka, Zuk, & Mousseau, 2004; Wigby & Chapman, 2005). Consequently, the degree to which females increase their mating rate when more males are present is likely to require that they balance the costs of resisting male mating attempts with the change in net fitness from each successive mating. How the various costs and benefits balance out to influence female fitness is important in driving the evolution of mating systems and reproductive behaviour.

Few studies have tested whether the fitness consequences for females of increased interactions with males vary according to the local environment (Sih, Montiglio, Wey, & Fogarty, 2017; Smith, 2007). This is expected because the environment can influence the relative strength of sexual selection or sexual conflict arising from additional matings and/or resisting more mating attempts. For example, population density might influence the optimal mating strategy of females to acquire direct and indirect benefits

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(Kokko & Rankin, 2006), such that female mating preferences are density dependent (Rosenthal, 2017; Welch, 2003). At low densities of males, females are predicted to be less selective due, in part, to reduced mate availability and the increased time and energy costs of locating mates (Hutchinson & Halupka, 2004; Kokko & Mappes, 2005). Higher male densities will, however, tend to increase the number of male mating attempts. This could lead to either a reduction in female willingness to remate (e.g. Martin & Hosken, 2003) because of the increased costs of excess mating or an increase in female mating rate to lower the short-term rate of harassment (e.g. Rowe, Arnqvist, Sih, & Krupa, 1994).

The environment could also affect the magnitude of the direct benefits that females receive from each mating (e.g. the quantity of beneficial substances transferred in ejaculates, such as nutrients and hormonal triggers that increase egg production; Arnqvist & Nilsson, 2000; Yamane, Goenaga, Rönn, & Arnqvist, 2015). For example, low food availability might decrease male body condition and reduce ejaculate size and composition (Iglesias-Carrasco, Jennions, Zajitschek, & Head, 2018; Perry & Rowe, 2010; Polak et al., 2017) and thereby lower the net benefit of mating for females. Similarly, females in an environment with greater access to food have been shown to remate less often as mating gifts from males are then less valuable (Toft & Albo, 2015). Despite the likely role of the environment in determining the net outcome of female mating decisions, relatively few studies have asked how different environments affect male–female interactions due to the potential for correlated changes in both male harassment and the benefits of additional matings (but see Edvardsson, 2007).

The seed beetle, Callosobruchus maculatus, is an ideal model species to investigate the fitness consequences for females of interacting with different numbers of males in different environments. Although females are often polyandrous, the direct benefits of mating multiply are subtle (e.g. males do not provide packaged nuptial gifts such as spermatophores). Previous studies in C. maculatus have yielded mixed results that make it difficult to predict the net fitness effect on females of more males being present, owing to the combination of both direct and indirect benefits of additional matings, alongside the cumulative costs of remating. For example, there is evidence that polyandrous females lay more eggs than those mated to a single male (even after controlling for the number of matings; Eady, Wilson, & Jackson, 2000), possibly because of cryptic female choice. Intriguingly, there are also potential direct benefits of mating. Several studies have suggested that fecundity benefits could be attributed to nutrients and water in ejaculates (Eady et al., 2007; Savalli & Fox, 1999; Ursprung, den Hollander, & Gwynne, 2009). As C. maculatus is a pest of stored legumes, living in an environment with little or no access to water or food for adult beetles, water is expected to be a limiting resource for females that constrains their reproductive output and life span. Male C. maculatus transfer ejaculates that are rich in water (Edvardsson, 2007) so, if male ejaculates are the only water resource available to adult females, we might expect the net benefits of remating to outweigh the costs in dry environments, thereby increasing the mating propensity of females. However, there are other potential costs of mating that could reduce or eliminate the net benefits of polyandry in dry conditions. The costs of copulation in the seed beetle include traumatic wounding of the female's reproductive tract caused by the male's aedeagal spines and the transfer of toxic ejaculatory substances (Crudgington & Siva-Jothy, 2000; Eady et al., 2007; Gay, Eady, Vasudev, Hosken, & Tregenza, 2009). In addition, the presence of more males leads to greater male harassment which can increase the risk of predation and physical damage and reduce the time available for feeding and oviposition, hence shortening female life span (den Hollander & Gwynne, 2009). Surprisingly, despite extensive research in C. maculatus, we still do not know whether the environment alters the balance between these putative costs and benefits of being exposed to males, and the effect that the variation in the number of males has on female fitness (but see Edvardsson, 2007).

Two previous studies in C. maculatus (Edvardsson, 2007; Ursprung et al., 2009) have demonstrated that water availability increases a female's life span and fitness, while reducing her willingness to remate. These findings suggest that water is a limiting resource for females, which might benefit from the water acquired from male ejaculates. Based on these previous findings we predicted that females housed alone in dry conditions would have lower fitness than those in wet conditions. However, we also predicted that females exposed to males would have lower fitness than those housed alone, and that this negative effect would increase with the number of males due to a combination of higher levels of male harassment and the number of mates and/or matings. In the wet environment we expected the costs of being housed with males to be higher than in the dry environment. This is because females can make use of environmental water reducing the advantage of additional matings through the acquisition of water in ejaculates, reducing female willingness to remate and, hence, potentially increasing the level of male harassment and the costs of rejecting males. Therefore, if the costs of increased harassment outweigh the benefits of having access to environmental water and larger ejaculates, we predicted that females housed with males in wet conditions would have lower fitness than those in dry conditions.

METHODS

Study Species

In C. maculatus, resources required for adult survival and reproduction are acquired from the host bean during the larval stage (Messina & Slade, 1997). The life cycle begins with females laying an egg on the surface of a host bean. The hatching larva burrows into the bean and remains inside feeding on it for 3–4 weeks until it emerges as an adult.

We used beetles originating from a stock kept at the University of Western Australia since 2005 in cultures of >500 adults breeding on black-eyed beans, Vigna unguiculata (Dougherty et al., 2017). This stock was maintained in our laboratory in cultures of >500 beetles at 27 ± 1 °C with a 14:10 h light:dark cycle for three generations prior to our experiment. Stock larvae were raised on black-eyed beans and adults were not provided with food or water.

Ethical Note

This work followed the ASAB/ABS guidelines for the treatment of animals in behavioural research. Information about individuals' housing conditions are described below. 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.

Experimental Design

To investigate how the environment (here water availability) influences the costs and benefits for females of being exposed to different numbers of males, we used a 2×3 experimental design where we manipulated water availability (wet or dry) and the number of males (none, one or four) housed with a female after an initial single mating to ensure she had sperm. We had six experimental groups: (1) dry, no male (D0, $N = 49$); (2) wet, no male (W0, $N = 47$); (3) dry, one male (D1, $N = 47$); (4) wet, one male (W1,

$N = 44$); (5) dry, four males (D4, $N = 45$); (6) wet, four males (W4, $N = 45$). Our dry treatment mirrors the natural or stock environment where adult individuals lack access to water.

Experimental Procedure and Measurement of Fitness Traits

To obtain virgins, 70 mated stock females were each placed individually in a petri dish with 20 mung beans, *Vigna radiata*. Our stock performs equally well on mung or black-eyed beans (D. McCorquodale, personal communication, October 2016). We monitored the females constantly: each time they laid an egg, the bean was removed and placed individually in an Eppendorf tube with a pin-hole in the cap for airflow. We repeated this procedure until we had 800 beans, each with a single egg. The adults that started to emerge around 21 days later became the focal individuals for our experiment. The eclosion date was recorded and individuals were given 24 h to attain sexual maturity (Fox, Hickman, Raleigh, & Mousseau, 1995). We then placed a randomly chosen virgin female with a virgin male in an Eppendorf tube and allowed them to mate. After a single copulation, females were randomly assigned to one of our six treatments.

Females were individually placed in a 60 ml plastic container with approximately 40 mung beans. Water was supplied ad libitum to females in the wet treatment by placing soaked cotton wool in a plastic vial lid, which was refilled every 24 h. Females have previously been shown to drink water when it is made available in this way (Edvardsson, 2007). Too few males emerged at the same time as females, so for the first 24 h after copulation (day 1), all females were alone in their designated water treatment. For the next 3 days (days 2, 3, 4), in addition to their corresponding water treatment, females experienced one of the three levels of exposures to males. Since males were housed in the same containers as females, they experienced the same water treatment (dry or wet). Before being placed with females, males corresponding to the wet treatment had no access to water. Females were transferred daily to a new container with approximately 40 mung beans that again, depending on her treatment group, housed either zero, one or four males (drawn from a large stock of previously mated males). On day 5 all males were removed, and females remained in their day 4 container in their designated water treatment until death. Female survival was monitored every 24 h and life span was recorded as the number of days a female survived after her first copulation. We counted the eggs laid in each container to measure both egg-laying rate (i.e. number of eggs laid/day for days 2 and 3) and 'lifetime' egg production (excluding day 1 eggs which were laid prior to exposure to males). For both variables we excluded eggs laid during the first 24 h after the copulation because females were not housed with males during this time. Therefore, any change in the number of eggs would be related exclusively to the water treatment, and not to the interaction between water and male exposure. For the egg-laying rate, we were interested in the number of eggs laid in 24 h, so we also excluded the fourth container, since females were housed in that container for several days until their death. Once eggs on beans were counted, we returned the beans to the controlled temperature room and 21 days later we began to check for emerging adults. We recorded the eclosion date of the first emerging offspring per container to estimate development time. Once offspring started to emerge they were counted and removed each day for 10 days. We used these data to calculate the percentage of eggs that produced emergent offspring (hereafter 'egg–adult survival').

Statistical Analyses

Since too few males emerged at the same time as females, the latter spent the first 24 h after their initial copulation in their designated water treatment but without any males. Our main aim was to test whether and how access to water and exposure to males interact, and we therefore excluded eggs collected on the first day from our analyses. We tested how water availability and the exposure to males influenced egg laying in two ways: 'lifetime' egg production (i.e. from day 2 onwards), and egg-laying rate (i.e. eggs/day for days 2 and 3 in the second and third containers, respectively). 'Lifetime' egg production is indicative of the total resources invested by females in egg production over their whole life and is therefore a good estimate of fitness. Egg-laying rate, on the other hand, provides information about changes in fecundity with age and is not confounded with survival. Male presence and water availability might influence these two variables in different ways. For 'lifetime' egg production we specified a generalized linear model (GLM) with a quasi-Poisson error distribution to account for overdispersion. Water availability, level of exposure to males and their interaction were specified as fixed factors. For egg-laying rate we specified a generalized linear mixed model (GLMM) with a Poisson error distribution. Water availability, level of exposure to males, day (second or third) and all two-way and three-way interactions were specified as fixed factors. We included female identity as a random effect to control for individual variation, and an observation level random effect to correct for overdispersion (Harrison, 2014). Following this correction, our model was underdispersed (dispersion parameter = 0.517). The figure for the 'lifetime' egg production suggested that there could be an interaction between water availability and number of males when excluding the treatment when females were exposed to four males. To explore this possibility further we ran an independent model with water availability, level of exposure to males (in this case zero or one) and the two-way interaction as fixed factors.

To determine whether water availability and level of exposure to males influenced egg–adult survival of offspring (egg–adult survival) we ran a GLMM with a binomial error distribution using the cbind function (number of adults eclosing; number of unhatched eggs). We treated water availability, level of exposure to males, the day the eggs were collected and all two- and three-way interactions as fixed factors. Female identity was treated as a random effect. We constructed a similar model to look at the effects of water availability, level of exposure to males and time since mating on offspring development time, but in this case the best fit model was a GLMM with a Poisson error structure. We corrected for overdispersion using an observation level random effect (Harrison, 2014).

We used Cox proportional hazard models (function coxph, R package survival, Therneau & Grambsch, 2000) to test whether water availability and male exposure affected female survival.

Our general protocol to test whether water availability, level of exposure to males and day interacted to affect female fitness was to initially include two- and three-way interactions in models. We then re-ran models without these interactions. If their removal did not reduce the model fit (LLR test), we interpreted the main effects from the reduced model. If it reduced the fit, we interpreted the interactions by looking at the model parameters. All analyses were conducted in R 3.4 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org).

RESULTS

Model parameter estimates and test statistics are provided in Tables A1–A5 in the Appendix. Tests for main and interaction effects are presented in the text below.

The number of males to which a female was exposed had a significant effect on her lifetime egg production ($X^2_2 = 6.730$, $P = 0.035$; Fig. 1a, Table A1). Females exposed to four males laid

significantly fewer eggs than those that were alone ($P = 0.006$) or with only one male ($P = 0.048$). There was, however, no significant difference in the total number of eggs laid by females that were alone or with only one male ($P = 0.444$). Water availability did not affect the total number of eggs laid ($X^2_1 = 0.141$, $P = 0.707$), nor did it ameliorate the cost of greater exposure to males (water∗number of males: $X^2_2 = 4.755$, $P = 0.093$). Interestingly, in our exploratory analysis, when we compared lifetime egg production between females that were alone and females accompanied by one male, we found a two-way interaction between male exposure and water treatment ($F_1 = 4.484$, $P = 0.036$). Females in dry environments laid more eggs when housed with one male than with no male, while females in the wet environment showed the opposite pattern.

The daily egg-laying rate, the number of eggs laid in a period of 24 h during the second and third days after copulations, decreased between days (Fig. 1b, Table A2), and this decline was significantly greater for females with access to water than those without (day∗water: $X^2_1 = 8.461$, $P = 0.004$). No other interactions had a significant effect on the egg-laying rate (water∗mating: $X^2_2 = 2.899$, $P = 0.235$; day∗number of males: $X^2_4 = 0.027$, $P = 0.987$; water∗number of males∗day: $X^2_4 = 2.256$, $P = 0.324$).

Egg–adult survival was affected by a three-way interaction between the day of laying, the water treatment and the level of exposure to males ($X^2_4 = 14.535$, $P = 0.006$; Fig. 2, Table A3). To investigate this interaction further we analysed each water treatment separately. In the dry environment, the egg–adult survival was lower for eggs laid on day 4 than for those laid on day 2 or 3 ($X^2_2 = 32.525$, $P < 0.001$; Table A3). There was no effect of exposure to males or any interaction between the day and male exposure (both P values > 0.358). For the wet treatment, how the level of exposure to males affected egg–adult survival depended on the day when eggs were laid (i.e. a two-way interaction between male exposure treatment and day: $X^2_4 = 31.398$, $P < 0.001$). On day 2, egg–adult survival decreased when females were exposed to more males, on day 3 egg–adult survival was similar across all male exposure treatments, and on day 4 egg–adult survival was greater when females were exposed to more males.

Larval development time was not affected by the day of egg laying, water availability, the exposure to males or any of their interactions (all $P > 0.931$; Fig. 3, Table A4).

Finally, females with access to water lived for significantly longer ($X^2_1 = 46.71$, $P < 0.001$), but there was no effect of the level of exposure to males on survival ($X^2_2 = 4.59$, $P = 0.100$) nor did it mediate the effect of access to water (interaction: $X^2_2 = 0.51$, $P = 0.775$; Fig. 4, Table A5).

DISCUSSION

Costs and benefits for females of interacting with males are expected to depend on the environment. We found that both the number of males with which a female was housed and the availability of water in the environment had significant fitness consequences for females. Females housed with more males produced fewer eggs over their lifetime, while females with access to water in their environment lived for longer than those in dry environments. However, we found only tentative evidence that water availability modified the fitness cost of interacting with males.

We predicted that encountering a greater number of males would reduce female fitness, owing to costs of increased harassment and/or engaging in superfluous matings. Females housed with four males did indeed have lower lifetime egg production than those housed alone or with a single male. One explanation for the reduced lifetime fecundity of females exposed to several males is that these females engaged in superfluous matings to avoid the costs associated with rejecting males (i.e. convenience polyandry) and that any potential direct benefits of remating were outweighed by mating-associated costs that lowered net fecundity. Mating has been shown to impose direct costs on females in several species due to physical damage during copulation and/or the transfer of toxic ejaculate substances (Chapman, Liddle, Kalb, Wolfner, & Partridge, 1995; Crudgington & Siva-Jothy, 2000; Johnstone & Keller, 2000; Yamane, Miyatake, & Kimura, 2008). In *C. maculatus*, male genital spines can wound females during copulation which facilitates the circulation of seminal fluids into the body cavity (Dougherty & Simmons, 2017; Hotzy, Polak, Rönn, & Arnqvist, 2012), and increased mating rates have previously been shown to increase fecundity but reduce female life span (Eady et al., 2007). A second explanation is that increased exposure to males increases the amount of sexual harassment experienced by females. Male harassment has been suggested to lower female fitness in both vertebrates and invertebrates (e.g. fish: Ojanguren & Magurran, 2007; damselflies: Takahashi & Watanabe, 2010; seed beetles: Gay et al., 2009), owing to a range of costs for females including loss of feeding time (Dadda, Pilastro, & Bisazza, 2005) and higher energy expenditure when resisting male mating attempts (Watson, Arnqvist, & Stallmann, 1998) that often reduce fecundity (Crudgington & Siva-Jothy, 2000; Eady et al., 2007). In *C. maculatus*,
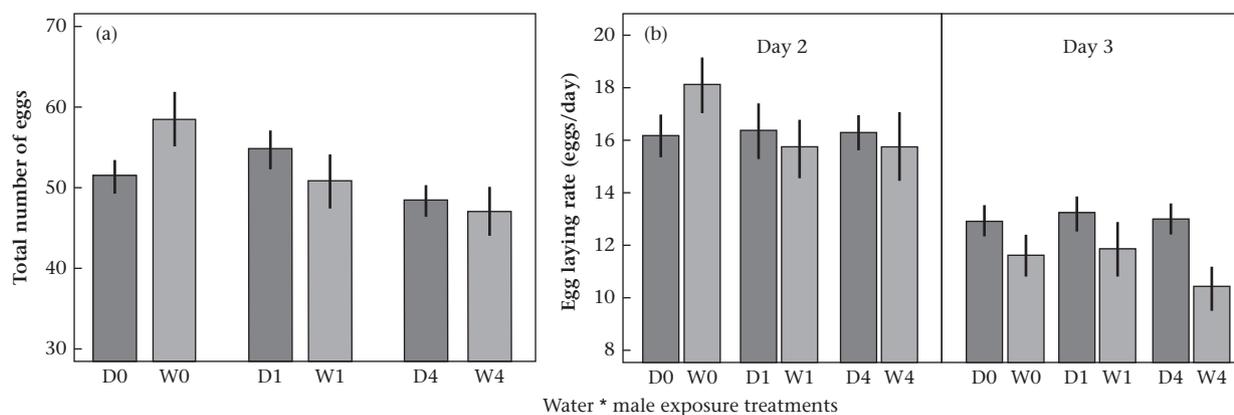
Figure 1. (a) Mean ± SE number of eggs laid over a female's lifetime and (b) mean ± SE number of eggs laid by females on each of the 2 days after copulation (number of eggs in 24 h) in relation to exposure to males and the water treatment (D0: dry, no males; W0: wet, no males; D1: dry, one male; W1: wet, one male; D4: dry, four males; W4: wet, four males).
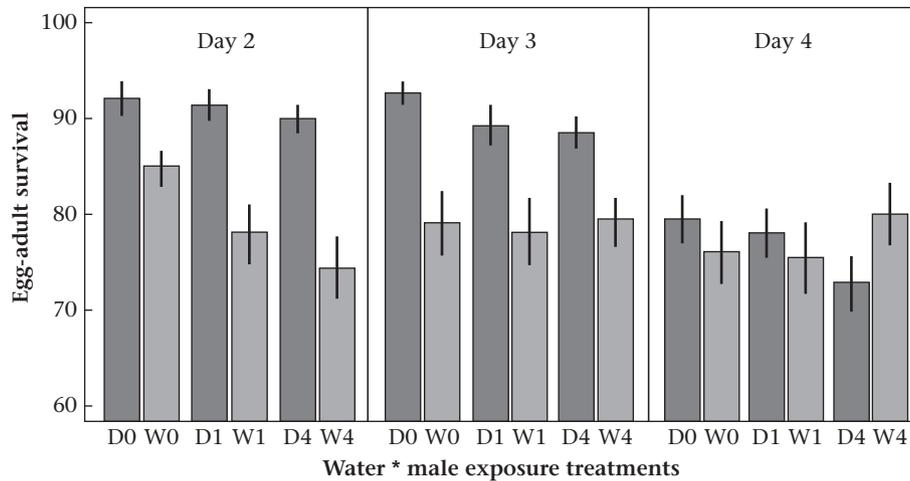
Figure 2. Mean ± SE offspring survival from egg to adult (egg–adult survival, in %) on each of the 3 days after female copulation in relation to exposure to males and the water treatment (D0: dry, no males; W0: wet, no males; D1: dry, one male; W1: wet, one male; D4: dry, four males; W4: wet, four males).

Figure 3. Mean ± SE duration of offspring development for each of the 4 days after female copulation in relation to exposure to males and the water treatment (D0: dry, no males; W0: wet, no males; D1: dry, one male; W1: wet, one male; D4: dry, four males; W4: wet, four males).
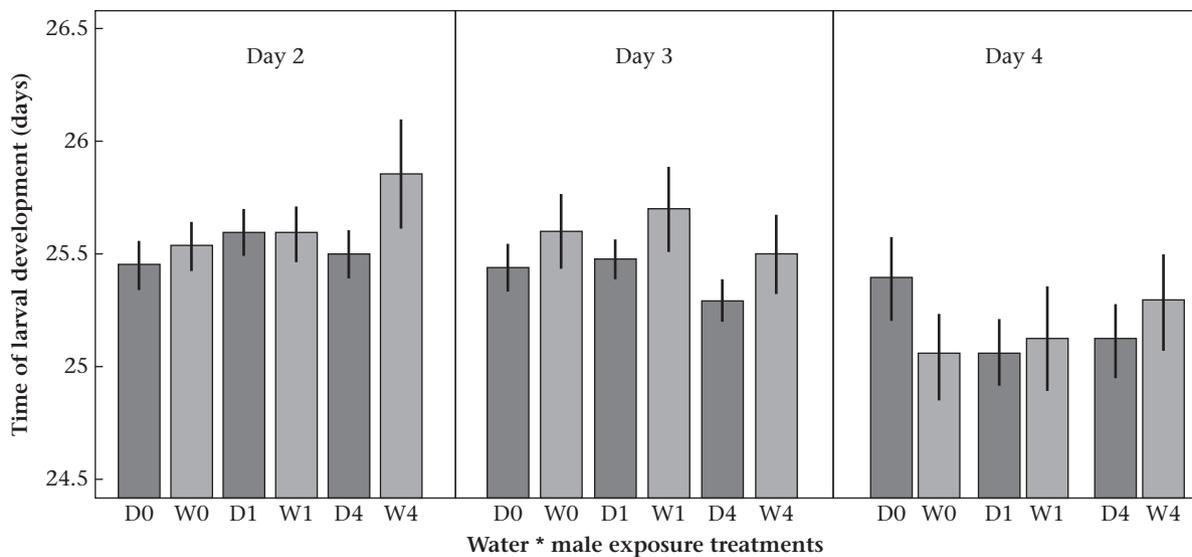
harassment of females by emasculated males (to remove potential effects associated with mating itself) has sometimes been shown to lower reproductive output and survival (den Hollander & Gwynne, 2009), but in other experiments it has not (Zajitschek, Dowling, Head, Rodriguez-Exposito & Garcia-Gonzalez, 2018). In the current experiment we were specifically interested in the net effects on female fitness of exposure to males under different environmental conditions and so we cannot tease apart these potential causes of reduced female fitness.

As well as costs of being housed with more males we also predicted that these costs would be mediated by water availability. If the ability of females to gain water from environmental sources reduces their need to remate to acquire water (Edvardsson, 2007), then we expected the resulting shift in the balance between the costs and benefits of mating to reduce the fitness of females housed with more males in wet environments. Despite clear evidence that being housed with multiple males was costly for females, we found only tentative evidence that water availability moderated these costs. In our original analysis, including all three male exposure

treatments, we found no evidence of an interaction between male exposure treatment and male water availability treatment on female fitness. However, in an unplanned exploratory analysis that excluded the four-male exposure treatment, we found an interaction in the predicted direction. When mated with a male and then housed alone, females that had access to water had greater lifetime egg production than those without water. When housed with a male after mating, however, females from wet environments produced fewer eggs during their lifetime than those from dry environments. These results could suggest that the effects of the environment on the relationship between level of exposure to males and female fitness are swamped by high costs of exposure when there are many males present, and that an effect of the environment is only apparent when the costs of exposure to males are low to moderate. Similar results have been found in studies of inbreeding depression and stressful environmental conditions (Fox, Stillwell, Wallin, Curtis, & Reed, 2011; Marr, Arcese, Hochachka, Reid, & Keller, 2006), where the effect of a moderating variable depends on the absolute level of costs experienced. This result
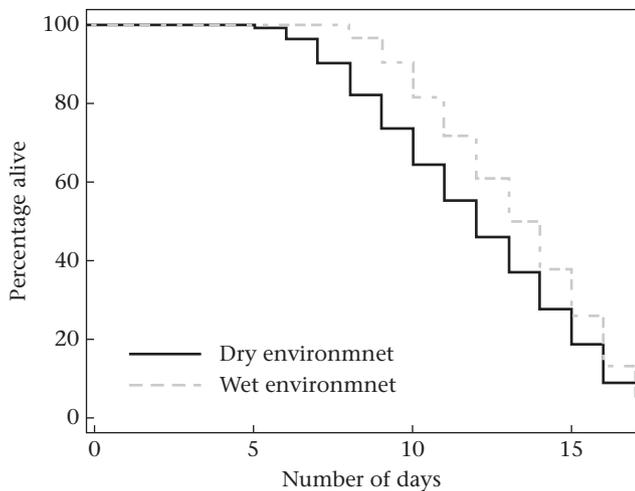
Figure 4. Kaplan–Meier survival plots for females maintained in dry or wet conditions.

highlights a need to consider not only the relative costs but also the absolute costs of a treatment.

In addition to the effects of male number and water availability, we found that female egg-laying rate decreased over time (i.e. fewer eggs laid on day 3 than day 2), and that, in the dry treatment, offspring from eggs laid on later days (i.e. from day 4 onwards) were less likely to survive to adulthood. We believe these findings are likely to be related to maternal age, as previous studies have shown that the number of eggs female seed beetles lay declines with age (Fox, Bush, & Wallin, 2003), and that females more than 5 days old lay smaller eggs with reduced egg–adult survival (e.g. Fox, 1993) presumably due to the depletion of breeding resources (Richards & Myers, 1980). Surprisingly, the age-related decline in the number of eggs laid was significantly greater when females had access to water. One possible explanation for our finding is that, while water is beneficial for adult females (increasing their life span), increased humidity reduces the quality of beans for developing larvae and thus lowers female egg-laying rate (Cope & Fox, 2003; Mainali et al., 2015). Alternatively, since females with water live longer they may partition resources to eggs differently to those without access to water, laying smaller eggs over a longer life span. These explanations could be tested by looking at female preferences for beans that have been kept in different humidities and by measuring the size of eggs laid by females housed with and without water throughout their life (e.g. Fox, 1993).

Conclusions

Here we have shown that both exposure to multiple males and water availability have important fitness consequences for females, but that water availability only appears to moderate these effects when the costs of male exposure are not too high. Our findings provide an insight into the importance of the environment for female fitness and the complexity of understanding the balance between the costs and benefits of exposure to males in different conditions. To fully understand the evolution of mating systems and sexual conflict, future studies should explore how any costs associated with exposure to males and copulations change across a range of ecologically relevant environments. In particular, in laboratory studies these should be biologically realistic environments, where sexual encounters are not excessively frequent, so that the absolute level of male harm is potentially reduced (e.g. Yun, Chen, Singh, Agrawal, & Rundle, 2017).

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.anbehav.2018.06.006.

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Appendix

Table A1
Effect of water availability and exposure to males on the lifetime number of eggs

	Estimate	SE	t	P
Full model				
(Intercept)	3.939	0.048	81.16	**<0.001**
Water (yes)	0.129	0.067	1.93	
Male number (1 male)	0.062	0.068	0.91	
Male number (4 males)	−0.06	0.071	−0.848	
Water∗Male number (1 male)	−0.201	0.097	−2.07	**0.039**
Water∗Male number (4 males)	−0.156	0.100	−1.56	0.119
Reduced model				
(Intercept)	3.997	0.039	101.73	**<0.001**
Water (Yes)	0.015	0.041	0.38	0.707
Male number (1 male)	−0.004	0.049	−0.76	0.444
Male number (4 male)	−0.139	0.050	−2.77	**0.006**
Comparison of fit	Residual (*df*)	Residual deviance	Deviance *F*	*P*
Full model	271	1830.9		
Reduced model		1859.1	−28.194 2.378	0.095

GLM model output from full (including interaction terms) and reduced (main effects only) models (see text), and statistical comparisons of model fits. Significant values are in bold.

Table A2
Effect of water availability and exposure to males, as well as days since mating on rate of egg laying

	Estimate	SE	z	P
Full model				
(Intercept)	2.741	0.06	45.62	**<0.001**
Water (yes)	0.103	0.085	1.21	
Male number (1 male)	−0.005	0.086	−0.05	
Male number (4 males)	0.017	0.087	0.19	
Day 3	−0.219	0.075	−2.92	
Water∗Male number (1 male)	−0.152	0.123	−1.24	0.216
Water∗Male number (4 males)	−0.179	0.123	−1.46	0.144
Water∗Day 3	−0.232	0.108	−2.15	**0.032**
Male number (1 male)∗Day 3	0.019	0.107	0.18	0.854
Male number (4 males)∗Day 3	−0.008	0.108	−0.07	0.941
Water∗Male number (1 male)∗ Day 3	0.139	0.155	0.90	0.368
Water∗Male number (4 males)∗Day 3	0.048	0.156	0.31	0.759
Reduced model				
(Intercept)	2.816	0.043	65.62	**<0.001**
Water (yes)	−0.084	0.041	−2.04	**0.042**
Male number (1 male)	−0.039	0.049	−0.78	0.433
Male number (4 males)	−0.064	0.052	−1.28	0.199
Day 3	−0.298	0.032	−9.17	**<0.001**
Comparison of fit	*df*	Log-likelihood	Deviance χ2	*P*
Full model	7	3649.2	−1802.5	
Reduced model		3681.7	−1796.6 11.73	0.109

GLMM model outputs from full (including interaction terms) and reduced (main effects only) models and statistical comparison of model fits (see text). Significant values are in bold.

Table A3
Effect of water availability, exposure to males and days since mating on the percentage of eggs emerging as adults

	Estimate	SE	z	P
Full model				
(Intercept)	2.752	0.173	15.87	**<0.001**
Water (yes)	−0.892	0.225	−3.97	
Male number (1 male)	−0.331	0.239	−1.39	
Male number (4 males)	−0.407	0.238	−1.71	
Day 3	−0.054	0.206	−0.26	
Day 4	−1.270	0.159	−8.04	
Water∗Male number (1 male)	−0.157	0.314	−0.50	0.617
Water∗Male number (4 males)	−0.361	0.311	−1.16	0.246
Water∗Day 3	−0.336	0.256	−1.31	0.191
Water∗Day 4	**0.738**	**0.200**	**3.68**	**<0.001**
Male number (1 male)∗Day 3	−0.101	0.276	−0.37	0.714
Male number (4 males)∗Day 3	−0.089	0.275	−0.32	0.747
Male number (1 male)∗Day 4	0.236	0.217	1.09	0.278
Male number (4 males)∗Day 4	−0.053	0.102	−0.52	0.605
Water∗Male number (1 male)∗Day 3	0.557	0.349	1.59	0.111
Water∗Male number (4 males)∗Day 3	**0.939**	**0.347**	**2.42**	**0.016**
Water∗Male number (1 male)∗Day 4	0.247	0.281	0.88	0.379
Water∗Male number (4 males)∗Day 4	**0.959**	**0.279**	**3.44**	**0.001**

GLMM model outputs from full models (including interaction terms). Significant values are in bold.

Table A4
Effect of water availability, exposure to males and days since mating on offspring development time

	Estimate	SE	z	P
Full model				
(Intercept)	3.237	0.028	114.3	**<0.001**
Water (yes)	0.003	0.04	0.08	
Mating (1 male)	0.006	0.04	0.14	
Mating (4 males)	0.002	0.041	0.05	
Day 3	−0.001	0.040	−0.02	
Day 4	−0.002	0.040	−0.06	
Water∗Male number (1 male)	−0.004	0.058	−0.07	0.948
Water∗Male number (4 males)	0.011	0.058	0.19	0.85
Water∗Day 3	0.003	0.057	0.06	0.952
Water∗Day 4	−0.017	0.057	−0.29	0.769
Male number (1 male)∗Day 3	−0.004	0.057	−0.07	0.947
Male number (4 males)∗Day 3	−0.007	0.058	−0.12	0.901
Male number (1 male)∗Day 4	−0.018	0.057	−0.32	0.747
Male number (4 males)∗Day 4	−0.013	0.058	−0.22	0.826
Water∗Male number (1 male)∗ Day 3	0.006	0.083	0.07	0.945
Water∗Male number (4 males)∗ Day 3	−0.009	0.083	−0.12	0.907
Water∗ Male number (1 male)∗ Day 4	0.019	0.083	0.24	0.813
Water∗Male number (4 males)∗ Day 4	0.009	0.083	0.11	0.909
Reduced model				
(Intercept)	3.239	0.017	193.94	**<0.001**
Water (yes)	0.004	0.014	0.27	0.784
Mating (1 male)	0.001	0.017	0.03	0.974
Mating (4 males)	0.001	0.017	0.04	0.965
Day 3	−0.003	0.017	−0.2	0.839
Day 4	−0.162	0.017	−0.96	0.338

Comparison of fit	df	Log-likelihood	Deviance	χ2	P
Full model	7	−2079.1	4158.1		
Reduced model	19	−2078.8	4157.7	0.4516	1

GLMM model outputs from full (including interaction terms) and reduced (main effects only) models, and statistical comparison of model fits (see text). Significant values are in bold.

Table A5
Effect of water availability and exposure to males on female survival

	Estimate	SE	z	P
Full model				
Water (yes)	−0.296	0.069	−4.31	**<0.001**
Male number (1 male)	0.007	0.063	0.11	0.91
Male number (4 males)	0.094	0.062	1.51	0.13
Water∗Male number (1 male)	0.067	0.097	0.68	0.49
Water∗Male number (4 males)	0.016	0.096	0.17	0.87
Reduced model				
Water (yes)	−0.269	0.039	−6.82	**<0.001**
Male number (2 males)	0.035	0.048	0.72	0.469
Male number (4 males)	0.1	0.047	2.11	**0.035**

Comparison of fit	Loglik	χ²	df	P
Full model	−18201			
Reduced model	−18201	0.51	2	0.775

Cox proportional hazard model outputs from full (including interaction terms) and reduced (main effects only) models and statistical comparison of model fits (see text). Significant values are in bold.