The effect of water on fitness and mating in seed beetles

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Declaration

The work presented in this thesis is the original work by the author unless otherwise stated. All the experiments were performed and analyzed under the supervision of Dr. Megan Head. All figures presented in this thesis have been designed by the author.

This thesis has not been submitted for the purpose of obtaining any other degree at this or other universities and conforms to the Australian National University guidelines and regulations.

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Abstract

How animals adapt to environmental stress has long been of concern. Studies looking at effects of resource limitation often focus on food. However, water is also essential for life and can be limited in the environment. Despite this, little is known about how water availability affects the life history and mating traits of animals. Here, I use the seed beetle, *Callosobruchus maculatus*, to explore how water availability affects oviposition preferences of mothers and the development of their offspring (Chapter 2). I also take an in depth look at how the water availability experienced by males and females during development affects mating interactions and the subsequent fitness outcomes of these interactions (Chapter 3). And finally, I look at the relative and interactive effects of water acquired during development and adulthood on male investment in reproduction throughout their adult life (Chapter 4). In chapter 2 I show that the moisture content of beans (on which females lay their eggs and in which offspring develop) does not affect oviposition preferences or offspring fitness. These results contrast with theoretical expectations, possible reasons for which are discussed. In chapter 3, I found that manipulating the availability of water to males and females during development affected their mating interactions and subsequent fitness as adults. Specifically, males reared in wet environments transferred larger ejaculates to females, but only when the female they mated with was reared in a dry environment. In this

experiment I also found that females mated to males reared in dry environments laid more eggs than those mated to males from wet environments. And that eggs laid by females reared in dry conditions had greater survival when they had mated to males reared in dry than wet environments. These results shed light on how resource limitation mediates the fitness consequences of mating interactions and sexual conflict. Finally, in chapter 4, when manipulating water content during both development and adulthood, I found that greater water content of beans during development had no effect on male body weight at emergence nor their ejaculate weight, but did affect aspects of mating behavior. These effects contrasted with the effects of water during adulthood. Early in adulthood males with access to water lost more weight and had larger ejaculates than males without access to water, but later in life, access to water allowed males to maintain their body weight and prolong their lifespan but did not affect ejaculate size. These results improve our understanding of how water availability experienced at different life stages is allocated to key male fitness traits. When combined, the results from my three data chapters, demonstrate that although water acquired during both development and adulthood can increase the ability of males to invest in mating, the effects of water can differ depending on the trait measured, and the life-stage at which water was acquired. Further, the fitness consequences of such effects may depend on whether or not their mate has had access to water. In the final chapter of my thesis, I bring my results

together and discuss their implications for evolutionary responses to stressful environments.

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1 Introduction

Resource intake is an essential process for animals. Individuals have to absorb nutrition for their metabolism and development (Boggs, 2009; Lindström, 1999). However, in some situations, resources can be insufficient, and this inevitably affects the fitness of individuals (Brommer et al., 1998; Cruz-Rivera and Hay, 2000; Le Gall and Behmer, 2014). For instance, zebra finches, *Taeniopygia guttata*, reared on a seed-only diet have a lower development rate, reduced cell-mediated immune function and survival rate than finches reared on supplemented diets (Birkhead et al., 1999). More generally, a recent meta-analysis showed that ejaculate traits are strongly reduced in the context of nutritional restriction (Macartney et al., 2019).

In addition to simply obtaining enough resources to maintain bodily function and optimize fitness throughout life, the timing of resource acquisition may be important (Boggs, 2009). In insects, resources acquired at each life-stage may be allocated to various traits and functions differently (Boggs, 2009). For instance, in the fruit fly, *Drosophila melanogaster*, individuals with a poor larval diet had lower body mass and smaller appendages (wing area and femur length) as adults than those fed a rich larval diet. Further, those that were fed a poor larval diet and subsequently fed a rich adult diet increased their body weight but not their appendage size (Poças et al., 2020). These results indicate that improved adult diets may compensate for poor larval diets for some traits but not others. Depending on whether they are capital or income breeders, resources acquired during early life-stages can also mediate

reproduction. For example, limited nutrition in male scorpion fly larvae, a kind of income breeder, not only restricts their adult body weight but also their relative salivary gland weight, a sexually selected trait that influences mating duration and male fitness (Engels and Sauer, 2007).

In addition to food, water is also an important resource that is needed for organisms to survive and reproduce (Chown et al., 2011; Danks, 2000), especially for those that inhabit dry conditions with little access to water. Consequently, the availability of water in the environment is expected to affect a range of traits expressed throughout life. For instance, access to water during adulthood can increase lifespan and fecundity of female crickets, *Gryllodes sigillatus* (Ivy et al., 1999), and seed beetles, *Callosobruchus maculatus* (Ursprung et al., 2009; Vincent et al., 2020). Access to water has also been shown to affect female fecundity (Ursprung et al., 2009; Vincent et al., 2020), and mating behavior in species where water can also be obtained from ejaculates (Edvardsson, 2007; Iglesias-Carrasco et al., 2018; Ryne et al., 2004). However, compared with food, exploration of the effect of water on life history is limited. Although it is clear that not enough water is generally detrimental for organisms, the details of this relationship are largely unknown.

To advance our knowledge of how variation in water availability experienced at different life stages affects insects, I used the seed beetle, *Callosobruchus maculatus*, as a model species to carry out my research. Seed beetles are pests of stored grains. Females lay eggs on the surface of beans and newly hatched larvae burrow into the endosperm of the bean on which they were laid. Larvae then develop

within the bean and emerge several weeks later as an adult. As capital breeders, adult seed beetles, acquire all of the resources that they need for their life from the bean in which they developed, but further resources can be acquired during adulthood, and may enhance fitness (Edvardsson, 2007; Messina and Fry, 2003). Since seed beetles inhabit stored dry beans, water can be considered a limited resource in their natural environment. Thus, providing additional water to beetles at different stages of life is expected to influence life-history and fitness traits (Edvardsson, 2007; Iglesias-Carrasco et al., 2018; Ursprung et al., 2009; Vincent et al., 2020). Further, water has been hypothesized to be an important resource that females can obtain from males during mating (Edvardsson, 2007; Ursprung et al., 2009). Males transfer a large, water rich ejaculate during mating, which may benefit females (Edvardsson, 2007; Ursprung et al., 2009). This may also lead to availability of water in the environment being an important mediator of mating interactions, reproduction and possibly even parental effects (Vincent et al., 2020).

In my thesis, I look at how water experienced at different times throughout the life of seed beetles affects life-history, fitness and mating traits. In chapter 2, I manipulate the water content of beans to test whether females prefer to lay their eggs on wet or dry beans. I also measure the developmental performance of larvae reared in these beans. In chapter 3, I mate beetles that have been reared in wet or dry beans to explore the effect of male and female developmental environment on mating interactions. I also measure several fitness traits of males and females after mating to explore how environmental water might mediate sexual conflict. Finally, in chapter

4, I manipulate the availability of water both during development and adulthood, for males only, to take a closer look at the interactive and relative effects that water experienced at different life-stages can have on male investment in reproduction throughout their adult life.

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2 Oviposition preference on wet or dry beans in seed beetles

2.1 Abstract

The environment in which an individual develops can be important for determining their likelihood of surviving to adulthood as well as a range of other fitness traits. In animals with limited juvenile mobility, oviposition preferences of mothers may be important for determining the quality of offspring developmental environments. Here I tested whether female seed beetles prefer to lay their eggs on wet or dry beans and investigated how water availability experienced during development affected offspring fitness. Female seed beetles showed no preference for wet or dry beans and neither development time nor egg to adult survival of offspring was affected by the water content of beans. One explanation for the lack of female preferences for wet or dry beans is that the lack of short term fitness consequences for larvae developing in these beans means there is little or no adaptive advantage of such preferences. Alternatively, water induced changes in bean chemistry, which have been shown in previous studies, may counteract female preferences for wet beans. Explanations and evolutionary implications for my results are discussed.

2.2 Introduction

The environment experienced by organisms early in life can often have a long-lasting impact on individuals (Bateson et al., 2014; Bradshaw, 1965; Eyck et al., 2019).

Various aspects of the environment that animals are exposed to during development can affect a diverse range of traits (Bradshaw, 1965; Jonsson and Jonsson, 2014). For instance, temperature during embryogenesis and egg development, egg size, parental care, predator stress and resource availability can all affect growth rate, adult size, age at maturity, fecundity and lots of other traits throughout life in fish (reviewed by Jonsson and Jonsson, 2014), while low food quality, environmental temperature and their interaction can affect development, survival rate, mating performance and fecundity in insects (e.g. Atkinson, 1994; Holmes et al., 2020; Iglesias-Carrasco et al., 2018b; Kutz et al., 2019). Notably, effects of early life environments can last into adulthood, affecting reproductive performance and fitness of the next generation (Dmitriew and Rowe, 2011).

In many species, the developmental environment is determined by where mothers lay their eggs. As such maternal oviposition preferences can determine food quality, predation risk and the social environment that offspring experience (Agrawal et al., 2002; Ballabeni et al., 2001; Craig et al., 1990). Consequently, maternal oviposition preferences can have a strong effect on offspring performance and viability, especially in species with limited juvenile mobility (Craig et al., 1989; Li and Liu, 2015; Mainali et al., 2015; Rajapakse and Walter, 2007). These effects can also have a lasting impact on adult traits (Mainali et al., 2015). For instance, early larval experience resulting from differences in oviposition site have been shown to be important in the cotton leafworm, *Spodoptera littoralis* (Anderson et al., 2013). In this species males are more attracted to sex pheromones produced by females reared on

the same host plant that they themselves experienced as larvae, and females prefer to lay eggs on the same host they were reared on (Anderson et al., 2013). In addition, maternal oviposition preferences may affect adult performance through differences in body condition that result from variation in resource quality during development (Dmitriew and Rowe, 2011).

Here, I tested whether female seed beetles, *Callosobruchus maculatus*, show an oviposition preference for wet or dry beans. Water is often limiting for seed beetles as they are pests of stored dry beans (Fox, 1993). Further, previous studies have shown that water is an important resource for seed beetles - access to water during adulthood can prolong their lifespan, increase their parental investment and mediate the costs and benefits of mating (Edvardsson, 2007; Iglesias-Carrasco et al., 2018a; Ursprung et al., 2009; Vincent et al., 2020), however the effect of water during development is still not clear. In addition, seed beetle larvae spend their entire development in the bean on which their mother laid their egg and feeding or drinking as adults is facultative, meaning that they gain most of their nutrition from inside their developmental bean (Edvardsson, 2007; Messina and Fry, 2003). This means the ability to distinguish beans with a high or low water content and a preference for wet beans should be favored by natural selection. I predict that female seed beetles will show an oviposition preference for wet beans, and that if water is beneficial for larvae (as it appears to be for adults), then larvae that develop in wet beans will have shorter development times and higher survival.

2.3 Method

Study species

Seed beetles used in my study were from a large stock population maintained at The Australian National University, on mung beans, *Vigna radiata*, for 24 generations and incubated at 25 ± 1 °C.

Preparation of beans

Mung beans used in the experiment were treated to manipulate their water content. I used only undamaged shop bought beans. These were placed in plastic vials (70ml) in groups of 10 (for two-choice) or 20 (for no-choice test). To create wet beans, plastic vials were incubated with the lid open, in an enclosed box with a wet towel on the bottom for one day at 23.5±0.5ºC. To create dry beans, another set of plastic vials were treated in the exact same way except there was no wet towel in the box. All groups of beans were weighed to 0.1 mg using an electronic balance (Mettler Toledo AG135) before and after the treatment to evaluate their moisture content after treatment. Beans in the wet treatment gained 1.57 ± 0.78% (mean ± SE) of their original weight and beans in the dry treatment gained 0.11 ± 0.10% (mean ± SE) of their original weight. The statistical difference in bean weight gain between treatments of both tests was verified (p-value < 0.001) using “t.test” function in R version 3.6.2 (R-Development-Core-Team, 2013).

Oviposition preference tests

To test whether female beetles prefer to lay their eggs on wet or dry beans, I conducted two types of preference test: a two-choice test, and a no-choice test with 100 mated females, which were picked randomly from our lab stock of seed beetles. In the two-choice assay, each female was placed in a petri dish with 10 wet and 10 dry beans. For each female, either the wet or dry beans were marked with a black marker, so that I could later identify which type of bean the female had laid her eggs on (otherwise they looked the same). To control for any potential effect of marking the beans, the treatment that was marked was decided randomly by coin flipping for each female. Females were left in the petri dish for two hours to lay eggs (Cope and Fox, 2003), after which, beans were checked, and the total number of eggs on each type of bean was recorded.

After each female completed the two-choice test they were then used in a no-choice test, each female was placed into a vial with either 20 wet or 20 dry beans for 24 hours to lay more eggs. After 24 hours the female was removed from the vial and placed with the opposite bean treatment for a further 24 hours. The order that the beans were presented was decided by coin flipping to control for the potential effects of aging and order. After that, beans were checked to record the total number of eggs laid on each type of bean and the female was euthanized by freezing.

The reason for running both types of preference test is that they provide different information. The no-choice test can be considered more realistic of a natural situation

(i.e., it is unlikely that females will have a direct choice between wet and dry beans). In this situation however, choice may be costly (and thus preferences may be weaker), because it is expressed by postponing egg laying until a better option comes along. The two-choice test on the other hand may be less realistic but it provides a “no cost” scenario in which preferences should be expressed if they exist. If females are able to distinguish between different types of beans and there is a benefit of doing so, then females presented with two types of beans simultaneously should choose, because there is no potential cost of delaying reproduction.

Measurement of offspring development

To determine the effect of water on offspring, I measured their development time, survival to emergence and body weight at emergence. After the number of eggs on beans were counted, I ensured that all beans had only one egg on them by scraping excess eggs off each bean. Beans with one egg were then given a unique ID and incubated in Eppendorf tubes at 25°C till an adult beetle emerged. From the two-choice tests I kept 576 eggs (294 on wet beans and 282 on dry beans) from 87 females (13 females did not lay any eggs) and from the no-choice test I kept 1970 eggs (984 on wet beans and 986 on dry beans) from 96 females (4 females did not lay any eggs). Eppendorf tubes were checked for 60 days after eggs were laid to ensure that all emerging beetles were recorded. Development time was calculated as the date new beetles emerged minus the date females laid their eggs. Methods and results regarding the measurement of body weight at emergence are presented in

Chapter 3.

Statistical Analysis

To look at how the water content of beans affected egg-laying preference of female seed beetles and their offspring's short term fitness, I analyzed my data with the glmmTMB package (Brooks et al., 2017) in R version 3.6.2 (R-Development-Core-Team, 2013). I used the 'summary' function to obtain parameter estimates and determine statistical significance.

Oviposition preference

I tested female egg-laying preferences for the two methods (two-choice tests and no-choice tests) separately. For both analyses the response variable was created using the 'cbind' function (number of eggs laid on wet beans; number of eggs laid on dry beans). This can be interpreted as the proportion of eggs laid on wet beans. I then used generalized linear mixed models (GLMMs) with a binomial error distribution to determine whether bean water content affects female oviposition preference. Prior to these analyses, I tested for an effect of marked beans (two-choice test) and the order females were presented with the beans (no-choice test) to see if these factors needed to be controlled for in my main analyses. These analyses were conducted in the same way as outlined above except that I used 'cbind' to create response variables that corresponded with the proportion of eggs laid on marked beans (two-choice test) and proportion of eggs laid on eggs presented to female first (no-choice test).

Offspring development

To investigate the effect of bean moisture content on offspring fitness, I modelled development time, survival to emergence, body weight at emergence and adult lifespan in separate LMMs/GLMMs (as appropriate). In all of these models, I included the water treatment of beans as a fixed effect and mother ID as a random effect to control for non-independence of siblings. Development time was analyzed using a gaussian error distribution after square root transformation to meet the assumption of normality. For survival to emergence, I used the 'cbind' function with a binomial error distribution to determine how this trait was affected by the water treatment of beans, I also included mother ID for non-independence of siblings.

2.4 Results

Oviposition preference

Marking the beans did not affect oviposition preference ($z = -1.083$, $p = 0.279$, Table 2.1) and neither did the order that females encountered the beans ($z = 0.18$, $p = 0.857$, Table 2.2). For this reason, these factors were not included in further analyses.

The water treatment of beans did not affect the oviposition preference of female seed beetles in either the two-choice test ($z = 0.5$, $p = 0.617$, Table 2.1) or the no-choice test ($z = -0.045$, $p = 0.964$, Table 2.2).

Offspring development

The water content of beans did not affect offspring development time (mean ± SE =

33.056 ± 0.074 day, z = 0.3, p=0.752, Table 2.3), nor their egg-to-adult survival (mean ± SE = 0.694 ± 0.017 day, z = 1.856, p = 0.063, Table 2.4).

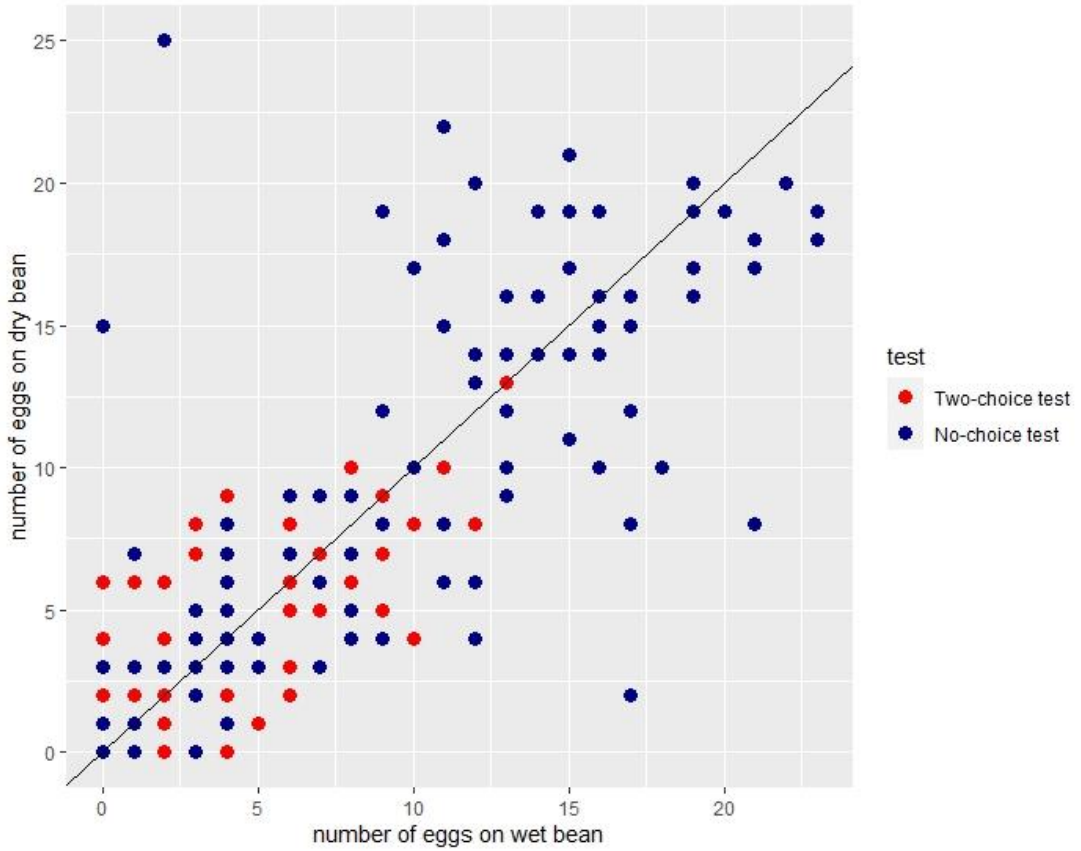

Figure 2.1 Number of eggs laid on wet or dry beans in the two preference tests. Red dots are number of eggs laid in the two-choice test and blue dots are number of eggs laid in the no-choice test. The line represents no preference for either wet or dry beans (i.e. where x=y).

2.5 Discussion

Recently, considerable research has focused on the maternal oviposition preferences of insects (Gripenberg et al., 2010). However, contrary to theoretical predictions, females do not always show a preference for the oviposition site that promotes the fitness of their larvae (Adar and Dor, 2018), and sometimes even prefer oviposition sites that are detrimental to the fitness of their larvae (Clark et al., 2011; Henry et al., 2005; Mayhew, 2001). Here, I manipulated the water content of beans, tested

whether female seed beetles preferred to lay their eggs on wet or dry beans and measured the fitness of offspring that developed on these beans. In contrast to expectation, I found that females showed no preference for wet or dry beans, and that the moisture content of beans had no effect on my measures of larval fitness.

I based my prediction of an oviposition preference for wet beans on the fact that previous studies have shown that water is a limited and precious resource for seed beetles (Edvardsson, 2007; Iglesias-Carrasco et al., 2018a; Ursprung et al., 2009; Vincent et al., 2020). One reason for the mismatch between my results and prediction could be that the treatment I used to wet beans was not enough to substantially alter bean physiology or morphology, in other words, the difference between wet and dry beans (which although significant was very small and may not have lasted the entire duration of development) may not have been enough to provide the cues that beetles need to elicit a response. An alternative explanation is that moisture causes physical or chemical changes in wet beans which deter females from laying eggs on them (Hudaib et al., 2010; Sulehrie et al., 2003) and that this could have counteracted a potential preference for wet beans. Although there is currently no direct evidence, the deterrent D-limonene which is found in black-eyed beans (Hudaib et al., 2010), may also occur in mung beans and could consequently mask a preference for wet beans in the seed beetle. Further research into the physiological changes that occur when beans get wet would be an interesting topic for future research and could help to provide a more comprehensive understanding of oviposition preferences in seed beetles.

A more general reason why I did not find an oviposition preference could be due to a lack of selection. I found no difference in offspring fitness traits (i.e. development time and egg to adult survival) between beetles that developed in wet or dry beans. A similar result has also been shown for beetles developing in wet and dry black-eyed beans (Hudaib et al., 2010). I also found few differences in adult fitness traits: there were no differences in body weight at emergence or lifespan between beetles that were reared in wet or dry beans, and differences in fecundity, ejaculate traits and mating behavior when present were context dependent (see Chapters 3 & 4). The fact that females do show preferences between beans that differ in their fitness value (e.g. due to seed size (Cope and Fox, 2003); presence of other larvae (Guedes and Yack, 2016); and bean variety (Kébé et al., 2020)) suggests that it is not that females cannot tell the difference between the beans but that there is simply no overall benefit in doing so on wet or dry beans.

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2.7 Tables

Table 2.1. Effects of marking beans and bean water content on **egg laying preference in the two-choice tests**. Model outputs obtained from generalized linear models using template model builder with binomial error distribution.

Terms	estimate	SE	Z	P
Marked beans				
Intercept	-0.090	0.083	-1.083	0.279
Water				
Intercept	0.042	0.083	0.500	0.617

Table 2.2. Effects of the order that beans were presented to females and bean water content on **egg laying preference in the no choice tests**. Model outputs obtained from generalized linear models using template model builder with binomial error distribution.

Terms	estimate	SE	Z	P
Order				
Intercept	0.008	0.045	0.18	0.857
Water				
Intercept	-0.002	0.045	-0.045	0.964

Table 2.3. The effect of bean water content on **development time**. Model output obtained from linear mixed models using template model builder.

Terms	estimate	SE	Z	P
Fixed effects				
Intercept	5.762	0.015	379.7	<0.001
Water (Wet)	0.003	0.011	0.300	0.752
Random effects	**Variance**	**SE**		
Mother	0.015	0.121		

Table 2.4. The effects of bean water content on **egg to adult survival**. Model outputs obtained from generalized linear mixed models using template model builder with binomial error distribution.

Terms	estimate	SE	Z	P
Fixed effects				
Intercept	0.852	0.108	7.884	<0.001
Water (Wet)	0.284	0.153	1.856	0.063
Random effects	**Variance**	**SE**		
Mother	<0.001	<0.001		

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

Zhuzhi Zhang & Megan Head. Does developmental environment affect sexual conflict? An experimental test in the seed beetle. Behavioral Ecology, in minor revision (22-Mar-2021).

3.1 Abstract

Sexual conflict and sexually antagonistic coevolution are driven by differences in reproductive interests between the sexes. There have been numerous studies focused on how both the social and physical environment that individuals experience as adults, or where mating occurs, mediate the intensity of sexual conflict. However, how the physical environment that juveniles experience, mediates their later mating interactions, is still poorly understood. In seed beetles, *Callosobruchus maculatus*, water is an important resource that can impact fitness and reproduction. Here, we manipulated the water content of beans that beetles were reared in and explored how this environmental variation affects mating interactions and subsequent male and female fitness. We measured the mass of ejaculate transferred, mating behavior, female fecundity and offspring production as well as male and female survival. We found that males reared in wet environments transferred a larger ejaculate to females, but only when females were reared in dry environments. We also found that

females mated to males reared in dry environments laid more eggs than those mated to males from wet environments. Additionally, eggs laid by females reared in dry conditions had greater survival when they had mated to males reared in dry than wet environments. Our results suggest that greater access to water during development enhances the ability of males to invest in their first mating, but also causes more severe sexual conflict and lowers egg quality. Our research provides evidence for the importance of the influence of developmental environment on sexual conflict and its reproductive consequences.

3.2 Introduction

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

sexes.

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

In addition to the effects of the social environment, resource availability experienced during development can play an important role in determining how adults invest in reproduction. Numerous studies across a broad range of taxa have demonstrated that developmental diet influences adult male and female reproductive traits. For instance, the effects of diet quality have been shown to affect reproductive traits in a range of species, like sperm quality and fecundity in flies (Klepsatel et al., 2020;

Macartney et al., 2018), remating frequency and resistance in ladybirds Perry et al., 2009, sexually responsiveness and mate preference in crickets (Hunt et al., 2005), mate preference in spiders (Hebets et al., 2008), sperm reserves and sperm replenishment rate in fish (Vega-Trejo et al., 2016) and sexual signals in birds (Naguib and Nemitz, 2007). However, all of these studies only manipulate diet in one sex or did not focus on the sexual interaction, and few studies explore how resource availability influences mating interactions and the resulting fitness of females involved in these matings.

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

Most previous research investigating how access to resources during development influences adult mating behavior has focused on manipulating juvenile diet (Vega-

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

consequences of these interactions.

In this study, we raise seed beetles in beans with low or high moisture content and then pair males and females emerging from these beans (using a 2×2 factorial design) to look at the effects of developmental water availability on ejaculate size, mating behavior and fitness. Given the findings of previous studies (described above) we hypothesize that water availability during development could mediate sexual conflict via two pathways. It could, as seen when manipulating adult access to water, affect the condition of males and females and hence lead to escalated persistence and resistance, i.e. longer kicking duration, in pairs where both sexes have had access to water. Alternatively, having greater access to water during development could increase the amount of water males are able to transfer to females in their ejaculates and/or decrease females need for water, which may result in changes to ejaculate mass and/or kicking latency and kicking duration.

3.3 Methods

Study species

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

1 °C.

Generating '*wet*' and '*dry*' beans

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

Generating experimental beetles

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

more than one egg on a bean, the extra eggs were scraped off to ensure each bean had only one egg. Each bean was then placed in an individual Eppendorf tube with a small hole in the lid for ventilation, and all Eppendorf tubes were allocated with a unique ID to keep track of each beetle. We kept a maximum of 5 beans from each female for each water treatment. Eppendorf tubes with beans were then incubated at 25°C ± 1°C for around 28 days, following which virgin adults started to emerge. When beetles emerged, they were sexed and left for one day prior to being used in mating trials to ensure they were sexually mature (Edvardsson, 2007; Jigisha et al., 2020). Further, as beetles do not require food or water as adults, adults were kept without food or water for the duration of the experiment. This is standard practice for studies on seed beetles (e.g. Edvardsson, 2007; Iglesias-Carrasco et al., 2018a).

Mating trials and measurement of fitness traits

To test how the rearing environment of males and females influenced mating behavior we paired one day old adult beetles emerging from wet and dry beans. We used a 2×2 factorial (wet and dry environment versus two sexes) design so that we could look at the effects of both male and female rearing environment independently, as well as their interaction. This resulted in four pair combinations: dry male with dry female (♂D×♀D, n=90); dry male with wet female (♂D×♀W, n=86); wet male with dry female (♂W×♀D, n=84); and wet male with wet female (♂W×♀W, n=82) and conducted over 30 days. On any given day males and females were paired randomly except that sibling beetles were never paired to avoid potential effects of inbreeding.

All beetles were weighed to 0.001 mg, using a Sartorius Cubis microbalance, before mating.

To begin a mating trial, a male was added to a female's Eppendorf tube and both beetles were knocked to the bottom of the tube to ensure they made contact with each other. We then observed the pairs' mating behavior. Latency to kicking was recorded as the time from when the mating started to when the female started kicking the male, this measure reflects the optimum mating duration of females (Edvardsson and Canal, 2006). Kicking duration was the time from when the female started kicking to when the copulation ended. This behavior is thought to reflect behavioral conflict between sexes (Wilson and Tomkins, 2014). And total copulation duration was the time from when mating started to when it ended, which may reflect the ejaculate mass transferred in the copulation (Edvardsson and Canal, 2006). If beetles did not start mating within 10 minutes, they were separated and the pair was recorded as not mating and excluded from subsequent analyses (Edvardsson, 2007). There were 5 pairs that did not mate (0 of ♂D×♀D, 3 of ♂D×♀W, 1 of ♂W×♀D and 1 of ♂W×♀W). After mating, males were weighed again so that we could calculate their ejaculate weight (weight before mating – weight after mating) and then they were transferred back to their original Eppendorf tube; females were placed in individual vials with 20 untreated beans (i.e. beans stored in the freezer that we use to rear stock beetles) to lay eggs for 24 hours.

To look at remating behavior and introduce different levels of multiple mating, two thirds of pairs were placed together again for a second mating trial. We set up two

thirds of the pairs to remate because we estimated based on previous work, that only around half of the remating pairs would actually mate (Savalli and Fox, 1998). This gave us three remating groups: pairs that were placed together and remated (remated group, N = 112), pairs that were placed together for 10 min to remate but they did not (fail group, N = 115), and pairs that were not allowed to remate (never meet group, N = 115). This second trial was conducted in the same way as outlined above for the first mating trial and the same behaviors were recorded. After the second mating trials were completed, all females were transferred into new vials, containing 40 untreated beans, to lay eggs until they died. After the mating trials both males and females were monitored daily to determine their adult lifespan. Once females died, we counted eggs in both of their vials. Once eggs were counted, these were placed back in the incubator, left until the tenth day after the first offspring emerged from each vial, and then frozen to allow us to count the number of emerged offspring in each vial. This gave us two measures of both female fecundity and offspring emergence success: “Day 1” which was the number of eggs/emerged offspring following the first mating (i.e., eggs laid over a 24hr period prior to the second mating trial and thus where females had only mated once), and “Rest of life” which was the number of eggs/emerged offspring following the second mating trial (including all three groups).

Statistical Analysis

To test how bean moisture affects mating traits and subsequent female fitness traits, we analyzed our data using linear mixed models (LMM) and generalized linear mixed models (GLMM) with the 'glmmTMB' package (Brooks et al., 2017) in R version 3.6.2 (R-Development-Core-Team, 2013), including mother ID as a random effect to control for potential non-independence of siblings. For all linear models (i.e. those with a Gaussian error distribution) we checked the residuals to ensure they met the assumptions of normality and homoscedasticity. We used the 'summary' function to obtain parameter estimates and determine statistical significance. In all models, when two-way interactions were significant, we conducted post hoc pairwise comparisons using Anova and using lsmeans in emmeans package (Searle et al., 1980) to determine which treatments differed. When two-way interactions were not significant, we dropped them from the models so that we could interpret the main effects (Engqvist, 2005), but we show both the full model including the interaction and the main effect model. Figures were plotted using the 'ggplot2' package (Wickham, 2016). Further model details are provided below. Sample sizes analyzed for all traits are shown in Tables 3.1, 3.2 and 3.3.

Body weight

Because the adult body weight of seed beetles can affect their mating behavior and fitness traits, we looked at whether males and females reared in wet and dry beans differed in body size prior to conducting further analyses. We modelled body weight

separately for males and females because large size differences between males and females are known in *C. maculatus*, and we did not want this variation to swamp potential within sex variation. Male and female body weight were both analyzed using LMM. In this model, we included the bean water treatment (wet or dry) as a fixed effect.

Mating traits

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

Fitness traits

The fecundity of females on day 1 and for the rest of their life (i.e. before and after the second mating trial) were analyzed separately using LMM. This was done so that

we could tease apart short-term and long-term treatment effects. For the number of eggs laid on day 1 we included the water treatment of both males and females, as well as their interaction as fixed effects. In addition, we included the body weight of both males and females as covariates, because body weight of both sexes is known from previous studies to affect fecundity (Savalli and Fox, 1999). For the number of eggs laid during the rest of life, we additionally included the remating status (i.e. remated, failed and never meet) as well as its interactions with male and female rearing treatment as fixed effects.

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

Male and female lifespan were modelled separately as the sexes are known to differ in lifespan, and we were not interested in comparing them. Lifespan was analyzed using a Cox proportional hazard model (function coxph, R package ‘survival,’ Therneau and Grambsch, 2000). In these models, we included the water treatment (wet or dry) of the focal beetle and their partner, as well as the remating status and all their two-way interactions as fixed effects. In addition, the focal beetle’s body

weight was included as a covariate, as this is known to influence lifespan (Fox et al., 2003).

3.4 Results

Body weight

The water content of beans that beetles were reared in did not significantly affect the adult body weight of males (mean ± SE = 5.897 ± 0.035 mg, z = 0.620, p = 0.534, Table 3.4) or females (mean ± SE = 3.669 ± 0.024 mg, z = -0.510, p = 0.608, Table 3.4).

Ejaculate weight

The rearing treatment of males and females interacted to affect male ejaculate weight (mean ± SE = 0.303 ± 0.008 mg, z = -2.607, p = 0.009, Table 3.5). Males reared in wet beans transferred larger ejaculates to females reared in dry beans, compared to all other male – female pair combinations (all pairwise: $p < 0.001$, Table 3.6, Fig. 3.1). In addition, larger males had larger ejaculates ($z = 6.578$, $p < 0.001$, Table 3.5).

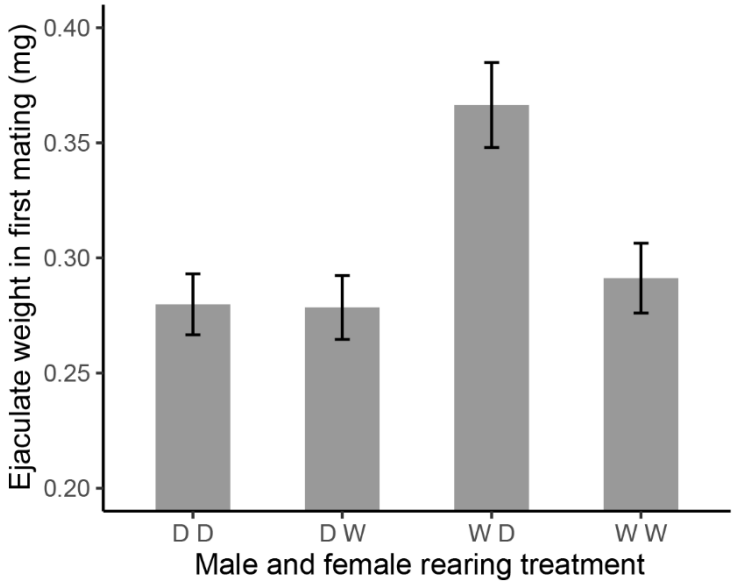

Figure 3.1 Effect of male and female rearing treatment on Ejaculate weight (male body weight before mating – male body weight after mating) in the first copulation. DD: dry males mating with dry females mating group; DW: dry males mating with wet females mating group; WD: wet males mating with dry females mating group; WW: wet males mating with wet females mating group). Raw means ± SE are presented.

Mating behavior

None of the mating behaviors recorded during the first mating trial were significantly affected by the male's or female's rearing treatment (latency to kicking: mean ± SE = 335.307 ± 6.853 s; kicking duration: mean ± SE = 202.030 ± 11.030 s; mating duration: mean ± SE = 537.337 ± 11.323 s; all interactions: $p > 0.611$, all main effects: $p > 0.259$, Table 3.7 to Table 3.9). Latency to kicking and the kicking duration were, however, associated with both male and female body weight (Table 3.7 and Table 3.9). Females started kicking sooner, and kicked for longer, when they were smaller (latency to kicking: $z = 2.404$, $p = 0.016$; kicking duration: $z = -2.443$, $p =$

0.015, Table 3.7 and Table 3.9) and when the male they were mating with was larger (latency to kicking: z = -3.284, p = 0.001; kicking duration: z = 2.972, p = 0.003, Table 3.7 and Table 3.9).

Remating behavior

Whether a pair remated or not was unaffected by the rearing treatment of males (in dry treatment, failed group/remated group = 48.696%; in wet treatment, failed group/remated group = 55.357%, z = -0.387, p = 0.698, Table 3.10) or females (in dry treatment, failed group/remated group = 49.091%; in wet treatment, failed group/remated group = 54.701%, z = -0.439, p = 0.661, Table 3.10), nor the interaction between them (z = -0.182, p = 0.856, Table 3.10). The likelihood of remating was, however, affected by male body weight (z = -3.070, p = 0.002, Table 3.10), with smaller males being more likely to remate than larger males. When looking at mating behavior during a pair's second mating (for those that mated), we found that none of the mating behaviors were significantly affected by either the male's or the female's rearing treatment (latency to kicking: mean ± SE = 478.141 ± 28.836 s; kicking duration: mean ± SE = 104.245 ± 15.296 s; mating duration: mean ± SE = 582.387 ± 30.986 s; all interactions: p > 0.168, all main effects: p > 0.053, Table 3.11 to Table 3.13). Latency to kicking and mating duration were, however, associated with male body weight. Females kicked sooner when they were mating with larger males (z = -2.822, p = 0.005, Table 3.11) and mating ended sooner when the male involved was larger (z = -2.470, p = 0.013, Table 3.12).

Fitness traits

In the first 24 hours after their first mating females mated with males reared in dry beans laid more eggs than those mated with males reared in wet beans (mean ± SE = 31.147 ± 0.377, $z = -2.216$, $p = 0.027$, Table 3.14, Fig. 3.2), but there was no effect of the females own rearing treatment ($z = -1.318$, $p = 0.188$, Table 3.14) nor an interaction between male and female rearing treatment ($z = -1.286$, $p = 0.198$, Table 3.14). In contrast, the likelihood that eggs laid after the first mating survived to adulthood was affected by the interaction between male and female rearing treatment (mean ± SE = 80.490 ± 0.789%, $z = 2.869$, $p = 0.004$, Table 3.15, Fig. 3.3). When we look more closely at this result, we see that eggs laid by wet females mated to dry males were less likely to survive until emergence than eggs laid by dry females mated to dry males ($p = 0.025$, Table 3.16). Further, the number of adult offspring that a female produced in the 24 hours following the first mating was not affected by the rearing treatment of males (mean ± SE = 25.003 ± 0.349, $z = -1.678$, $p = 0.093$, Table 3.17), females ($z = -1.788$, $p = 0.074$, Table 3.17) nor their interaction ($z = -0.121$, $p = 0.904$, Table 3.17). We also found that larger females laid more eggs ($z = 4.647$, $p < 0.001$, Table 3.17) and had more adult offspring emerging after the first mating ($z = 3.009$, $p = 0.003$, Table 3.17).

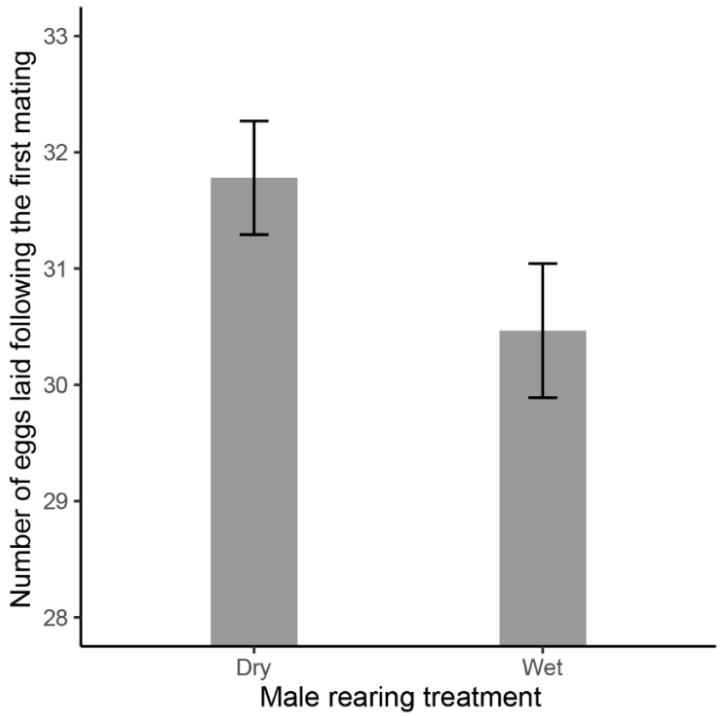

Figure 3.2 Effect of male rearing treatment on the number of eggs females laid following the first mating. Raw means ± SE are presented

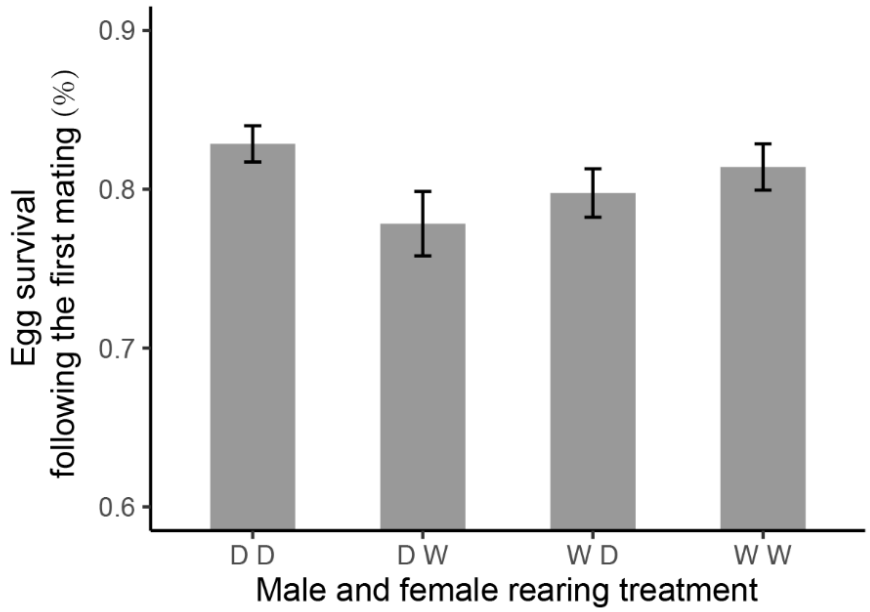

Figure 3.3 Effect of male and female rearing treatment on survival of eggs laid following the first mating. DD: dry males mating with dry females mating group; DW: dry males mating with wet females mating group; WD: wet males mating with dry females mating group; WW: wet males mating with wet females mating group. Raw means ± SE are presented

After the second mating assay, female fecundity was not affected by the rearing treatment of males (mean ± SE = 53.510 ± 0.641, z = -1.356, p = 0.175, Table 3.18), females (z = 0.219, p = 0.826, Table 3.18) nor their interaction (z = 0.702, p = 0.482, Table 3.18). It was also not affected by the remating status (χ^2 = 1.072, Df = 2, p = 0.585) or the two-way interactions between remating status and either the male or female water treatment (male rearing treatment and remating status: χ^2 = 2.076, Df = 2, p = 0.354; female rearing treatment and remating status: χ^2 = 3.223, Df = 2, p = 0.200). However, the likelihood that these eggs survived until adulthood was affected by the interaction between male water treatment and remating status (mean ± SE = 73.345 ± 0.649%, male water treatment and remating status: χ^2 = 12.143, Df = 2, p = 0.002, Fig. 4), but was not affected by the rearing treatment of males or females, their remating status or their interactions (all other interactions: p > 0.308, Table 3.19). When we looked more closely, our analysis showed that egg to adult survival of dry males is lower when they successfully remate compared to when they have the opportunity but failed to remate (dry males which successfully remated vs. dry males which failed to remate Df = 298, p= 0.035, Table 3.20). The number of adult offspring showed the same pattern as for the number of eggs laid (mean ± SE = 39.112 ± 0.528). It was not affected by either the male or female rearing treatment, the remating status, or any of their interactions (all interactions: p > 0.200; all main effects: p > 0.419), and larger females had more adult offspring (z = 8.345, p < 0.001, Table 3.21).

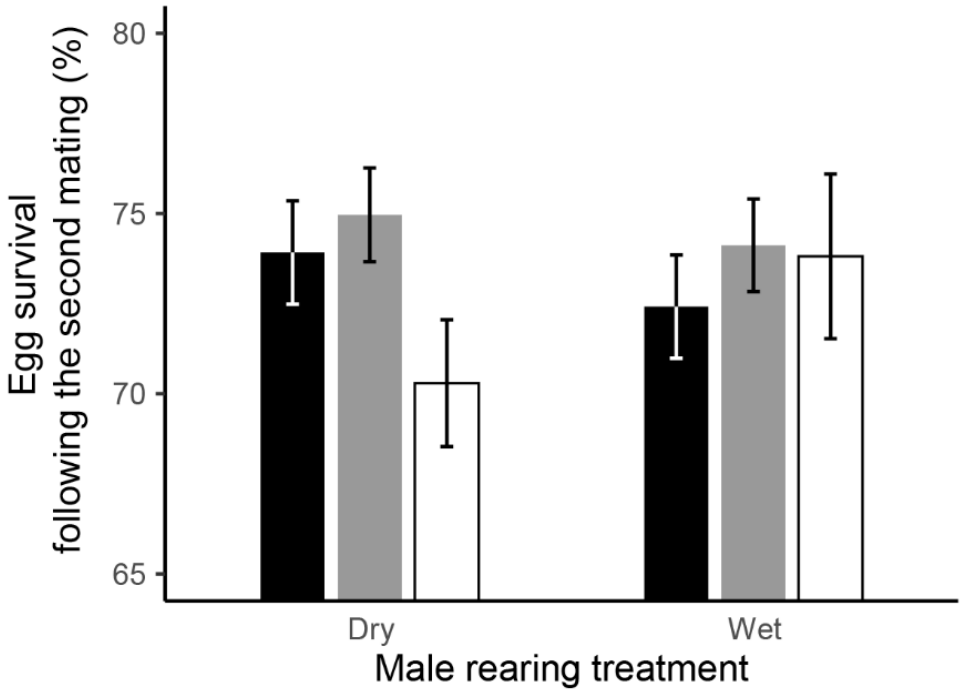

Figure 3.4 Effect of male rearing treatment and remating status on survival of eggs laid following the second mating (failed group in black; never meet again group in grey; remated group in white). Raw means ± SE are presented.

Lifespan of neither sex was affected by their own rearing treatment (males: mean ± SE = 17.624 ± 0.271 day, z = -0.423, p = 0.673, Table 3.22; females: mean ± SE = 10.129 ± 0.082 day, z = -0.680, p = 0.496, Table 3.23), the rearing treatment of their partner (partner of male: z = -1.012, p = 0.312, Table 3.22; partner of female: z = 0.587, p = 0.557, Table 3.23), nor the interaction between them (male: z = 0.166, p = 0.868, Table 3.22; female: z = -0.854, p = 0.393, Table 3.23). Female lifespan was also not affected by their remating status (female: χ^2 = 3.061, Df = 2, p = 0.216, Table 3.23), but males those mated twice lived shorter than those never met female again (male: χ^2 = 6.058, Df = 2, p = 0.048, Table 3.22). However, for both males and females, larger beetles lived longer (male: z = -9.815, p < 0.01, Table 3.22; female: z = -5.219, p < 0.01, Table 3.23).

3.5 Discussion

The degree of sexual conflict between males and females over reproduction is expected to vary depending on the environment (Fricke et al., 2009; Vincent et al., 2020; Yun et al., 2017). In seed beetles, water is thought to be a limiting resource which could change the economics of mating and thus the degree of sexual conflict (Iglesias-Carrasco et al., 2018a; Vincent et al., 2020). Previous studies have manipulated the availability of water for adult seed beetles, and concluded that it most likely influences mating behavior by altering male and female condition via effects of hydration (Edvardsson, 2007; Vincent et al., 2020). Here we manipulated the water content of beans (distinguished as wet or dry beans), to test whether water availability during development influences mating interactions and/or sexual conflict. We found that even though beetles emerged from the two types of bean at the same size, males reared in wet beans (wet males) mating with females reared in dry beans (dry females) transferred significantly larger ejaculates than any of the other three mating pair combinations. Mating behavior on the other hand was not affected by the rearing environment of either males or females. We also found in the day following the first mating, that females mated to wet males laid fewer eggs than females mated to dry males, and that eggs laid by dry females mated to wet males had lower survival to adulthood than eggs produced by dry-dry pairs. This meant that, overall, all pairs produced a similar number of adult offspring. When providing pairs with the opportunity to mate a second time, we found no effect of either male or female rearing treatment on the likelihood of remating, behavior during remating, or female

fecundity following the remating. However, the likelihood that eggs survived to adulthood was affected by an interaction between male water treatment and the remating status. Specifically, in the first mating, when mating with dry males, eggs produced by dry females were more likely to survive than those produced by wet females. But in the second mating assay, regardless of female treatment, eggs produced by females that remated with dry males were less likely to survive than eggs produced by those who only mated once. Over all though, the number of adult offspring produced by pairs after the second mating was not affected by either the male or female water treatment, or the remating status. As expected, the body weight of both males and females had important effects on almost all aspects of fitness and reproduction.

Effect of developmental environment on mating traits

As in many animals that exhibit multiple mating, antagonistic interactions in seed beetles are driven by competing interests of males and females (Arnqvist and Nilsson, 2000). In seed beetles, larger ejaculates and longer copulations have been shown to increase male reproductive success (Edvardsson and Canal, 2006; Savalli and Fox, 1999), but decrease female fitness (Edvardsson and Canal, 2006) because these traits are associated with the transfer of seminal toxins (Bayram et al., 2019) and damaging the female reproductive tract (Crudgington and Siva-Jothy, 2000). However, male and female mating traits as well as the benefits and costs of mating may differ depending on the environment (Fricke et al., 2009), and more specifically,

limited resources (Perry and Rowe, 2010). In seed beetles, water has been shown to influence female fitness and reproduction (Edvardsson, 2007; Ursprung et al., 2009; Vincent et al., 2020). In our experiment, we predicted that providing water during development could allow males to transfer larger (i.e. water rich) ejaculates and reduce female need for water. Our results are consistent with this prediction – wet males transferred larger ejaculates, but only when mated with dry females. This suggests that indeed wet males had the potential to provide larger ejaculates compared with dry males, and dry females were willing to receive larger ejaculates. The fact that wet males didn't transfer larger ejaculates to wet females, even though they were presumably capable of doing so, could suggest that wet females acquire all necessary water during development, and that when they do, the decreased value of water in the ejaculate means that it cannot offset the cost of receiving larger ejaculate (Crudgington and Siva-Jothy, 2000). Alternatively, this pattern could result from strategic ejaculation by males based on some aspect of female quality (i.e. males could be choosing not to transfer large ejaculates to wet females) (Kelly and Jennions, 2011; Lupold et al., 2011). Therefore, although ejaculate weight shows the predicted pattern, it is still unclear whether males or females drive the decreased ejaculate size when wet males mate with wet females.

We also predicted that access to water during development would alter male and female mating behavior. However, despite observing environmentally dependent differences in ejaculate mass, there were no effects of rearing environment on mating behavior (during either the first or second mating trial) nor the propensity of pairs to

remate. This differs from previous research which has manipulated male and female access to water during adulthood and found that females who have access to water are less willing to remate (Edvardsson, 2007), males who have access to water mate for longer than males that did not (Vincent et al., 2020), and that females who have access to water have shorter kicking durations, but only when they mated with males that had no access to water (Vincent et al., 2020). Differences between the results we present here and this previous research suggest that the effects of water intake on mating interactions differ depending on the life stage during which access to water is experienced (Boggs, 2009). To be specific, access to water during development affects ejaculate size, while access to water after emergence appears to mainly influence body condition (as evidenced by differences in body weight and longevity, Vincent et al., 2020), and this in turn alters the ability of males and females to persevere/resist during mating (Vincent et al., 2020).

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

Consequences of developmental environments for fitness and sexual conflict

To understand how environmentally mediated differences in mating behavior influence sexual conflict, it is necessary to look at the fitness consequences of mating interactions between individuals from different environments (Fricke et al., 2009). When considering the fitness outcomes of mating between beetles reared in wet and dry beans, if wet beans provide larvae with water and the effect lasts into adulthood, then wet-wet pairs might be expected to have greater reproductive output than other pairs. However, if females can obtain water via male ejaculates, then developing with differing access to water could affect the benefits and costs of mating and lead to increased sexual conflict (which might be reflected as longer kicking durations, earlier onset of kicking or decreased ejaculate size during mating) in wet-wet pairs (Iglesias-Carrasco et al., 2018b). In contrast to either of these predictions, our results show that females mated to dry males laid a slight, but significantly greater number of eggs than females mated to wet males just after the first mating. Previous research on a range of insects (e.g. seed beetles, Takashi Yamane, 2015; fruit flies, Chapman et al., 1995; Gillott, 2003; Herndon and Wolfner, 1995; and moths, Xu and Wang, 2011) has shown that accessory gland proteins increase female egg laying rate after mating. Thus, one explanation for our result could be that wet males have more dilute ejaculates and thus transfer fewer accessory gland proteins and are less able to stimulate female egg laying than dry males. Future studies looking at the composition of male ejaculates are needed to test this idea. To our knowledge this is the first study to show that water availability during development might influence ejaculate

composition, however, effects of developmental environment on ejaculate traits are common in studies that manipulate the diet or social environment of developing individuals (e.g. fruit flies, *Drosophila melanogaster*, Morimoto et al., 2016; Wigby et al., 2016; cockroach, *Nauphoeta cinerea*, Harris and Moore, 2004 and neriid fly, *Telostylinus angusticollis*, Macartney et al., 2018). The fact that the rearing treatment of males and females also interacted to affect egg survival, but that the number of offspring surviving to adulthood did not differ between the four pair types, means it is unclear what the long-term fitness consequences of these effects might be.

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

3.6 Conclusion

In seed beetles, the effect of water availability during development lasts into adulthood and affects the economics of mating. These effects seem to be primarily driven by differences in ejaculate size. These results are different to those that have been found when manipulating access to water during adulthood (Iglesias-Carrasco

et al., 2018a; Vincent et al., 2020), implying that how resources are allocated to different traits depends on the life stage during which they are acquired (Boggs, 2009). Our study provides evidence for the importance of the developmental environment in mediating mating interactions, and the fitness consequences of these interactions. However, further studies are needed to tease apart whether and how access to water during development mediates sexual conflict, and to test for a potential interaction between developmental and adult environment. It would be interesting in future studies to look at how access to resources during development affects ejaculate composition, and how the adult environment interacts with the juvenile environment to affect multiple mating, levels of sexual conflict and fitness.

3.7 References

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3.8 Tables

Table 3.1. Sample size of traits related to each individual.

Rearing treatment	Total males	Wet male	Dry male	Total females	Wet female	Dry female
Body weight	342	166	176	342	168	174

Table 3.2. Sample size of traits related to mating.

Mating pair	WM × WF	WM × DF	DM × WF	DM × DF
First mating trial*	82	84	86	90
Female fecundity of the first mating	82	78	85	88
Offspring survival rate of the first mating	79	75	74	84
Emerged offspring number of the first mating	79	75	74	84

WM: males reared in wet beans; DM: males reared in dry beans; WF: females reared in wet beans; DF: females reared in dry beans.

* Traits measured during first mating trial include ejaculate weight and mating behaviour (Latency to kick, mating duration and kicking duration).

Table 3.3. Sample size of traits related to remating.

Mating pair	WM × WF			WM × DF			DM × WF			DM × DF		
Remate	F	R	N	F	R	N	F	R	N	F	R	N
Remate likelihood	34	26	-	26	25	-	28	27	-	28	30	-
Remating behaviour*	-	24	-	-	24	-	-	27	-	-	30	-
Reproductive consequence**	33	25	21	22	23	30	23	23	28	27	28	29
Lifespan of males	29	24	19	21	20	29	26	24	30	25	27	21
Lifespan of females	34	25	23	24	25	30	23	22	30	27	31	31

WM: males reared in wet beans; DM: males reared in dry beans; WF: females reared in wet beans; DF: females reared in dry beans.

F: failed remating group; R: remated group; N: never meet again group.

*Remating behaviors include latency to kick, mating duration and kicking duration in the second mating trial.

** Reproductive consequences include female fecundity, offspring survival rate and emerged offspring number.

Table 3.4. Effects of bean water content on **adult male and female body weight**. Model outputs obtained from linear mixed models using template model builder.

Terms	estimate	SE	Z	P
Females				
Fixed effects				
Intercept	5.916	0.059	100.660	<0.001
Water (Wet)	-0.032	0.063	-0.510	0.608
Random effects	**Variance**	**SD**		
Mother	0.109	0.330		
Males				
Fixed effects				
Intercept	3.648	0.040	91.860	<0.001
Water (Wet)	0.027	0.043	0.620	0.534
Random effects	**Variance**	**SD**		
Mother	0.057	0.239		

Significant p-values are highlighted in bold

Table 3.5. Effects of bean water content on **male ejaculate weight** (Male body weight before mating - male body weight after mating). Model outputs obtained from linear mixed models using template model builder.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	-0.222	0.089	-2.480	0.013
Male Rearing Treatment (Wet)	0.085	0.020	4.213	**<0.001**
Female Rearing Treatment (Wet)	-0.002	0.020	-0.089	0.929
Male Weight	0.106	0.016	6.578	**<0.001**
Female Weight	0.019	0.011	1.756	0.079
Interaction Water	-0.074	0.028	-2.607	**0.009**
Random effects	**Variance**	**SD**		
Mother of male	6.836e-04	2.615e-02		
Mother of female	1.527e-11	3.907e-06		

Significant p-values are highlighted in bold

Table 3.6. Post-hoc pairwise comparisons showing the effect of male and female rearing treatment on **male ejaculate weight**. Model outputs obtained using lsmeans with Tukey adjustment for multiple testing.

Contrast	estimate	SE	df	t.ratio	P
D D - W D	-0.085	0.020	332	-4.213	**<0.001**
D D - D W	0.002	0.020	332	0.089	1.000
D D - W W	-0.009	0.020	332	-0.433	0.973
W D - D W	0.086	0.020	332	4.290	**<0.001**
W D - W W	0.076	0.020	332	3.710	**0.001**
D W - W W	-0.010	0.020	332	-0.517	0.955

Significant p-values are highlighted in bold

The first letter in contrast represents male rearing water treatment, the second represents the female water treatment.

Table 3.7. Effects of bean water content on **latency to kicking in the first mating**. Model outputs obtained from linear mixed models using template model builder. Parameter estimates and significance values are presented for main effects in the full model for transparency (grey text) but these should only be interpreted from the main effects model. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	360.360	84.094	4.285	<0.001
Male Rearing Treatment (Wet)	-2.232	18.278	-0.122	0.903
Female Rearing Treatment (Wet)	19.395	18.460	1.051	0.293
Male Weight	-49.027	14.965	-3.276	**0.001**
Female Weight	24.987	10.708	2.334	**0.020**
Interaction Water	-9.605	26.328	-0.365	0.715
Random effects	**Variance**	**SD**		
Mother of males	889.2	29.82		
Mother of females	1463.4	38.25		
Main effects model				
Fixed effects				
Intercept	360.065	84.147	4.279	<0.001
Male Rearing Treatment (Wet)	-6.991	12.776	-0.547	0.584
Female Rearing Treatment (Wet)	14.597	12.954	1.127	0.260
Male Weight	-49.166	14.971	-3.284	**0.001**
Female Weight	25.519	10.616	2.404	**0.016**
Random effects	**Variance**	**SD**		
Mother of males	916.6	30.27		
Mother of females	1491.2	38.62		

Significant p-values are highlighted in bold

Table 3.8. Effects of bean water content on **mating duration in the first mating**. Model outputs obtained from linear mixed models using template model builder. Parameter estimates and significance values are presented for main effects in the full model for transparency (grey text), but these should only be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	555.611	142.872	3.889	<0.001
Male Rearing Treatment (Wet)	-3.638	31.733	-0.115	0.909
Female Rearing Treatment (Wet)	17.878	31.310	0.571	0.568
Male Weight	20.583	25.901	0.795	0.427
Female Weight	-16.506	17.646	-0.935	0.350
Interaction Water	-23.048	45.276	-0.509	0.611
Random effects	**Variance**	**SD**		
Mother of males	2.681e+03	5.178e+01		
Mother of females	5.241e-32	2.289e-16		
Main effects model				
Fixed effects				
Intercept	523.109	140.414	3.725	<0.001
Male Rearing Treatment (Wet)	-15.424	22.587	-0.683	0.495
Female Rearing Treatment (Wet)	7.376	22.544	0.327	0.744
Male Weight	24.952	25.085	0.995	0.320
Female Weight	-12.459	17.489	-0.712	0.476
Random effects	**Variance**	**SD**		
Mother of males	3.219e-03	5.673e-02		
Mother of females	7.285e-30	2.699e-15		

Table 3.9. Effects of bean water content on **kicking duration in the first mating**. Model outputs obtained from linear mixed models using template model builder. Parameter estimates and significance values are presented for main effects in the full model for transparency (grey text) but these should only be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	180.109	136.566	1.319	0.187
Male Rearing Treatment (Wet)	-6.146	30.348	-0.202	0.840
Female Rearing Treatment (Wet)	-8.506	30.044	-0.283	0.777
Male Weight	72.639	24.726	2.938	**0.003**
Female Weight	-40.236	16.873	-2.385	**0.017**
Interaction Water	-4.824	43.389	-0.111	0.911
Random effects	**Variance**	**SD**		
Mother of males	2.281e+03	47.765		
Mother of females	2.433e-02	0.156		
Main effects model				
Fixed effects				
Intercept	186.618	137.716	1.355	0.175
Male Rearing Treatment (Wet)	-8.525	21.175	-0.403	0.687
Female Rearing Treatment (Wet)	-10.521	21.347	-0.493	0.622
Male Weight	73.531	24.738	2.972	**0.003**
Female Weight	-41.835	17.121	-2.443	**0.015**
Random effects	**Variance**	**SD**		
Mother of males	2431	49.31		
Mother of females	1203	34.69		

Significant p-values are highlighted in bold

Table 3.10. Effects of bean water content on **likelihood of remating**. Model outputs obtained from generalized linear mixed models using template model builder with binomial error distribution. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	6.428	2.033	3.162	0.002
Male Rearing Treatment (Wet)	-0.057	0.408	-0.141	0.888
Female Rearing Treatment (Wet)	-0.075	0.398	-0.189	0.850
Male Weight	-1.030	0.336	-3.063	**0.002**
Female Weight	-0.449	0.255	-1.759	0.079
Interaction Water	-0.104	0.570	-0.182	0.856
Random effects	**Variance**	**SD**		
Mother of males	3.576e-08	1.891e-04		
Mother of females	1.325e-01	0.364		
Main effects model				
Fixed effects				
Intercept	6.450	2.031	3.176	0.001
Male Rearing Treatment (Wet)	-0.111	0.285	-0.387	0.698
Female Rearing Treatment (Wet)	-0.125	0.286	-0.439	0.661
Male Weight	-1.032	0.336	-3.070	**0.002**
Female Weight	-0.448	0.255	-1.753	0.080
Random effects	**Variance**	**SD**		
Mother of males	2.644e-08	1.626e-04		
Mother of females	1.336e-01	0.365		

Significant p-values are highlighted in bold

Table 3.11. Effects of bean water content on **latency to kicking in the second mating assay**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	1092.05	381.87	2.860	0.004
Male Rearing Treatment (Wet)	130.46	77.31	1.687	0.092
Female Rearing Treatment (Wet)	72.27	75.22	0.961	0.337
Male Weight	-170.92	62.35	-2.741	**0.006**
Female Weight	-10.64	46.12	-0.231	0.818
Interaction Water	-152.94	111.06	-1.377	0.168
Main effects model				
Fixed effects				
Intercept	1155.645	383.620	3.013	0.003
Male Rearing Treatment (Wet)	56.532	56.113	1.008	0.314
Female Rearing Treatment (Wet)	2.163	56.449	0.038	0.969
Male Weight	-178.360	63.206	-2.822	**0.005**
Female Weight	-11.424	46.499	-0.246	0.806
Random effects	**Variance**	**SD**		
Mother of males	934	30.56		

Significant p-values are highlighted in bold

Table 3.12. Effects of bean water content on **mating duration in the second mating assay**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	967.42	407.57	2.374	0.018
Male Rearing Treatment (Wet)	190.40	82.52	2.308	**0.021**
Female Rearing Treatment (Wet)	42.22	80.28	0.526	0.599
Male Weight	-156.60	66.55	-2.353	**0.019**
Female Weight	17.61	49.22	0.358	0.720
Interaction Water	-157.84	118.53	-1.332	0.183
Main effects model				
Fixed effects				
Intercept	1035.94	407.71	2.541	0.011
Male Rearing Treatment (Wet)	114.16	59.92	1.905	0.057
Female Rearing Treatment (Wet)	-29.53	60.01	-0.492	0.623
Male Weight	-165.01	66.80	-2.470	**0.013**
Female Weight	16.76	49.63	0.338	0.736

Significant p-values are highlighted in bold

Table 3.13. Effects of bean water content on **kicking duration in the second mating assay**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	-124.638	204.354	-0.610	0.542
Male Rearing Treatment (Wet)	59.941	41.373	1.449	0.147
Female Rearing Treatment (Wet)	-30.044	40.251	-0.746	0.455
Male Weight	14.320	33.366	0.429	0.668
Female Weight	28.247	24.679	1.145	0.252
Interaction Water	-4.903	59.432	-0.082	0.934
Random effects	**Variance**	**SD**		
Mother of males	6.943e-04	0.026		
Main effects model				
Fixed effects				
Intercept	-122.48	202.75	-0.604	0.546
Male Rearing Treatment (Wet)	57.58	29.79	1.933	0.053
Female Rearing Treatment (Wet)	-32.22	29.92	-1.077	0.281
Male Weight	13.99	33.39	0.419	0.675
Female Weight	28.26	24.76	1.141	0.254
Random effects	**Variance**	**SD**		
Mother of males	0.129	0.359		
Mother of females	25.671	5.067		

Table 3.14. Effects of bean water content on **female fecundity after the first mating**. Model outputs obtained from linear mixed model using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	11.227	4.535	2.476	0.013
Male Rearing Treatment (Wet)	-0.627	0.989	-0.634	0.526
Female Rearing Treatment (Wet)	-0.032	0.974	-0.033	0.973
Male Weight	1.510	0.787	1.919	0.055
Female Weight	2.539	0.578	4.391	**<0.001**
Interaction Water	-1.8127	1.409	-1.286	0.198
Random effects	**Variance**	**SD**		
Mother of Male	5.382e-04	0.023		
Mother of Female	5.225	2.286		
Main effects model				
Fixed effects				
Intercept	11.053	4.540	2.435	0.015
Male Rearing Treatment (Wet)	-1.537	0.693	-2.216	**0.027**
Female Rearing Treatment (Wet)	-0.915	0.694	-1.318	0.188
Male Weight	1.492	0.789	1.890	0.059
Female Weight	2.655	0.571	4.647	**<0.001**
Random effects	**Variance**	**SD**		
Mother of Male	2.039e-07	4.516e-04		
Mother of Female	4.894	2.212		

Significant p-values are highlighted in bold

Table 3.15. Effects of bean water content on **offspring survival rate of eggs laid in the first mating**. Model outputs obtained from generalized linear mixed models using template model builder with binomial error distribution.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	2.605	0.466	5.587	<0.001
Male Rearing Treatment (Wet)	-0.130	0.084	-1.540	0.124
Female Rearing Treatment (Wet)	-0.250	0.088	-2.833	**0.005**
Male Weight	-0.112	0.080	-1.405	0.160
Female Weight	-0.093	0.058	-1.593	0.111
Interaction Water	0.358	0.125	2.869	**0.004**
Random effects	**Variance**	**SD**		
Mother of Male	0.126	0.355		
Mother of Female	0.241	0.491		

Significant p-values are highlighted in bold

Table 3.16. Post-hoc pairwise comparisons showing the effect of male rearing treatment and female rearing treatment on offspring survival rate of eggs laid in the first mating. Model outputs obtained using lsmeans with Tukey adjustment for multiple testing.

Contrast	estimate	SE	df	t.ratio	P
DD - DW	0.050	0.022	325	2.295	0.022
DD - WD	0.030	0.022	325	1.344	0.180
DD - WW	0.014	0.022	325	0.647	0.518
DW - WD	-0.020	0.023	325	-0.885	0.377
DW - WW	-0.036	0.022	325	-1.610	0.108
WD - WW	-0.016	0.023	325	-0.697	0.486

Significant p-values are highlighted in bold

The first letter in contrast represents male treatment, the second is for female.

Table 3.17. Effects of bean water content on **emerged offspring number of eggs laid after the first mating**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	12.968	4.115	3.151	0.002
Male Rearing Treatment (Wet)	-0.940	0.863	-1.089	0.276
Female Rearing Treatment (Wet)	-1.025	0.864	-1.186	0.235
Male Weight	1.032	0.709	1.454	0.146
Female Weight	1.560	0.527	2.959	**0.003**
Interaction Water	-0.149	1.239	-0.121	0.904
Random effects	**Variance**	**SD**		
Mother of Male	1.934	1.391		
Mother of Female	9.499	3.082		
Main effects model				
Fixed effects				
Intercept	12.956	4.114	3.149	0.002
Male Rearing Treatment (Wet)	-1.014	0.604	-1.678	0.093
Female Rearing Treatment (Wet)	-1.098	0.614	-1.788	0.074
Male Weight	1.030	0.709	1.452	0.146
Female Weight	1.570	0.522	3.009	**0.003**
Random effects	**Variance**	**SD**		
Mother of Male	1.928	1.389		
Mother of Female	9.478	3.079		

Significant p-values are highlighted in bold

Table 3.18. Effects of bean water content on **female fecundity after the second mating**. Model outputs obtained from linear mixed model using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	-8.728	6.822	-1.279	0.201
Male Rearing treatment (Wet)	-0.611	2.013	-0.304	0.761
Female Rearing treatment (Wet)	-1.696	2.034	-0.834	0.404
Male Body Weight	2.028	1.193	1.700	0.089
Female Body Weight	9.419	0.827	11.390	**<0.001**
Remate (Failed)	-0.243	2.174	-0.112	0.911
Remate (Remated)	1.635	2.197	0.744	0.457
Interaction Water	1.460	2.079	0.702	0.482
Interaction Male Rearing Treatment (Wet) & Remate (Failed)	-2.230	2.519	-0.885	0.376
Interaction Male Rearing Treatment (Wet) & Remate (Remated)	-2.783	2.540	-1.096	0.273
Interaction Female Rearing Treatment (Wet) & Remate (Failed)	3.771	2.525	1.493	0.135
Interaction Female Rearing Treatment (Wet) & Remate (Remated)	0.097	2.547	0.038	0.970
Random effects	**Variance**	**SD**		
Mother of Males	10.775	3.282		
Mother of Females	1.119	1.058		
Main effects model				
Fixed effects				
Intercept	-8.427	6.730	-1.252	0.210
Male Rearing	-1.400	1.032	-1.356	0.175

treatment (Wet)				
Female Rearing treatment (Wet)	0.226	1.032	0.219	0.826
Male Body Weight	1.931	1.196	1.615	0.106
Female Body Weight	9.390	0.824	11.399	**<0.001**
Remate (Failed)	0.695	1.314	0.529	0.597
Remate (Remated)	0.368	1.315	0.280	0.779

Random effects	**Variance**	**SD**
Mother of Males	0.637	0.798
Mother of Females	11.272	3.357

Significant p-values are highlighted in bold

Table 3.19. Effects of bean water content on **offspring survival rate of eggs laid after the second mating assay**. Model outputs obtained from generalized linear mixed models using template model builder with binomial error distribution. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	2.164	0.332	6.529	<0.001
Male Rearing treatment (Wet)	-0.168	0.084	-1.997	**0.046**
Female Rearing treatment (Wet)	-0.094	0.090	-1.047	0.295
Male Body Weight	-0.061	0.055	-1.105	0.269
Female Body Weight	-0.127	0.040	-3.144	0.002
Remate (Never)	-0.134	0.097	-1.383	0.167
Remate (Remated)	-0.292	0.091	-3.222	**0.001**
Interaction Water Interaction	0.020	0.087	0.223	0.823
Male Rearing Treatment (Wet) & Remate (Never) Interaction	0.169	0.104	1.625	0.104
Male Rearing Treatment (Wet) & Remate (Remated) Interaction	0.365	0.105	3.481	**<0.001**
Female Rearing Treatment (Wet) & Remate (Never) Interaction	-0.019	0.112	-0.170	0.865
Female Rearing Treatment (Wet) & Remate (Remated)	0.131	0.107	1.224	0.221
Random effects	**Variance**	**SD**		
Mother of Males	0.050	0.223		
Mother of Females	0.078	0.280		

Significant p-values are highlighted in bold

Table 3.20. Post-hoc pairwise comparisons showing the effect of male rearing treatment and remate treatment on **offspring survival rate of eggs laid after the second mating assay**. Model outputs obtained using lsmeans with Tukey adjustment for multiple testing.

Contrast	estimate	SE	df	t.ratio	P
D F - W F	0.159	0.073	298	2.169	0.256
D F - D N	0.143	0.078	298	1.845	0.439
D F - W N	0.133	0.081	298	1.647	0.568
D F - D R	0.226	0.076	298	2.993	**0.035**
D F - W R	0.020	0.076	298	0.258	1.000
W F - D N	-0.015	0.076	298	-0.201	1.000
W F - W N	-0.025	0.078	298	-0.326	0.999
W F - D R	0.068	0.073	298	0.926	0.939
W F - W R	-0.139	0.075	298	-1.850	0.435
D N - W N	-0.010	0.074	298	-0.139	1.000
D N - D R	0.083	0.076	298	1.094	0.884
D N - W R	-0.124	0.076	298	-1.632	0.578
W N - D R	0.093	0.080	298	1.161	0.855
W N - W R	-0.113	0.079	298	-1.431	0.708
D R - W R	-0.207	0.074	298	-2.804	0.060

Significant p-values are highlighted in bold

The first letter in contrast represents male rearing water treatment, the second is for remate consequence.

Table 3.21. Effects of bean water content on **emerged offspring number of eggs laid after the second mating**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	1.808	6.148	0.294	0.769
Male Rearing treatment (Wet)	-3.600	1.877	-1.928	0.055
Female Rearing treatment (Wet)	1.295	1.869	0.693	0.488
Male Body Weight	0.385	1.074	0.359	0.720
Female Body Weight	6.270	0.750	8.364	**<0.001**
Remate (Never)	0.364	1.980	0.184	0.854
Remate (Remated)	-1.048	1.996	-0.525	0.600
Interaction Water Interaction	2.186	1.850	1.182	0.237
Male Rearing Treatment (Wet) & Remate (Never) Interaction	2.653	2.270	1.169	0.243
Male Rearing Treatment (Wet) & Remate (Remated) Interaction	3.070	2.316	1.326	0.185
Female Rearing Treatment (Wet) & Remate (Never) Interaction	-3.967	2.255	-1.759	0.079
Female Rearing Treatment (Wet) & Remate (Remated)	-2.720	2.305	-1.180	0.238
Random effects	**Variance**	**SD**		
Mother of Males	0.710	0.843		
Mother of Females	6.357	2.521		
Main effects model				
Fixed effects				
Intercept	1.829	6.101	0.300	0.764
Male Rearing	-0.490	0.927	-0.529	0.597

treatment (Wet)				
Female Rearing treatment (Wet)	0.076	0.933	0.081	0.935
Male Body Weight	0.355	1.079	0.329	0.742
Female Body Weight	6.237	0.747	8.345	**<0.001**
Remate (Never)	-0.393	1.183	-0.332	0.740
Remate (Remated)	-0.943	1.166	-0.809	0.419

Random effects	**Variance**	**SD**
Mother of Males	0.719	0.848
Mother of Females	5.744	2.397

Significant p-values are highlighted in bold

Table 3.22. Effects of bean water content on **lifespan of males**. Model outputs obtained from a survival model. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	coef	exp(coef)	se(coef)	Z	P
Full model					
Fixed effects					
Male Rearing treatment (Wet)	-0.070	0.932	0.170	-0.412	0.680
Female Rearing treatment (Wet)	-0.139	0.870	0.163	-0.853	0.394
Male Body Weight	-1.622	0.198	0.166	-9.759	**<0.001**
Female Body Weight	0.114	1.121	0.093	1.219	0.223
Remate (Never)	-0.228	0.797	0.145	-1.565	0.118
Remate (Remated)	0.125	1.133	0.149	0.838	0.402
Interaction Water	0.040	1.041	0.240	0.166	0.868
Main effects model					
Fixed effects					
Male Rearing treatment (Wet)	-0.050	0.951	0.118	-0.423	0.673
Female Rearing treatment (Wet)	-0.121	0.886	0.119	-1.012	0.312
Male Body Weight	-1.618	0.198	0.165	-9.815	**<0.001**
Female Body Weight	0.113	1.120	0.093	1.210	0.226
Remate (Never)	-0.230	0.794	0.144	-1.597	0.110
Remate (Remated)	0.124	1.132	0.149	0.829	0.407

Significant p-values are highlighted in bold

Table 3.23. Effects of bean water content on **lifespan of females**. Model outputs obtained from a survival model. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	coef	exp(coef)	se(coef)	Z	P
Full model					
Fixed effects					
Female Rearing treatment (Wet)	0.015	1.015	0.156	0.098	0.922
Male Rearing treatment (Wet)	0.162	1.176	0.159	1.021	0.307
Female Body Weight	-0.538	0.584	0.102	-5.278	**<0.001**
Male Body Weight	0.095	1.100	0.123	0.772	0.440
Remate (Never)	0.019	1.019	0.136	0.138	0.890
Remate (Remated)	-0.200	0.819	0.143	-1.401	0.161
Interaction Water	-0.193	0.825	0.226	-0.854	0.393
Main effects model					
Fixed effects					
Female Rearing treatment (Wet)	-0.077	0.926	0.113	-0.680	0.496
Male Rearing treatment (Wet)	0.066	1.069	0.113	0.587	0.557
Female Body Weight	-0.530	0.589	0.101	-5.219	**<0.001**
Male Body Weight	0.086	1.090	0.122	0.702	0.482
Remate (Never)	0.024	1.025	0.136	0.179	0.858
Remate (Remated)	-0.202	0.817	0.143	-1.417	0.156

Significant p-values are highlighted in bold

4 How does access to water at different life stages affect male investment in reproduction and survival?

4.1 Abstract

Holometabolous insects have four distinct life-stages – eggs, larvae, pupae and adults. Active resource acquisition generally occurs during either or both the larval and adult stages. Previous research on the acquisition of food resources in holometabolous insects, has shown that resources acquired during each of these life-stages can differ in how they are allocated to different traits, and how they affect fitness. In addition to food, water is also an essential resource needed for a range of biological processes that enhance fitness. Yet, how water acquired at different life-stages affects key fitness traits is still not clear. Here, I manipulated both developmental and adult water availability of male seed beetles to explore how water acquired at each life-stage affects a range of traits. I measured male body mass, ejaculate weight, mating behavior and lifespan to explore the effect of water on fitness. My results indicate that the juvenile environment had little effect on body weight, ejaculate weight, ejaculate replenishment or lifespan, but did influence male mating behavior in both early and late adult life. This contrasts with the adult environment which had strong effects on weight loss, ejaculate size and lifespan but little effect on mating behavior. Males with access to water during adulthood

transferred larger ejaculates during their first mating, lost less weight as they aged, and lived longer. These results suggest that water acquired during development and adulthood are allocated differently to a variety of fitness traits across life.

4.2 Introduction

Nutrient intake from the environment is essential for animal growth, reproduction and survival (Simpson and Raubenheimer, 2012). However, resources may be allocated differently to various traits depending on when they are acquired (Boggs, 2009; Nestel et al., 2016) and the breeding strategy of the species (i.e. whether they are capital or income breeders, Jönsson, 1997). The timing of when resources are acquired during an animals' life can therefore be important in determining how animals resolve resource allocation trade-offs between different fitness traits. For instance, in holometabolous insects resources acquired during the larval stage are generally allocated to growth and storage, whereas resources acquired as adults are generally allocated to foraging, maintenance and reproduction (Boggs, 2009). Such differences in nutrient function and allocation may also select for differences in resource requirements between larvae and adults which can be reflected in the expression of functional traits (Aguila et al., 2013; Boggs, 2009).

Investment in fitness traits, such as reproduction, longevity and foraging, is important in determining an individual's fitness, and may be affected by both developmental and adult environments as well as their interaction. For instance, limited nutrition in scorpionfly larvae (*Panorpa vulgaris*) not only restricts male adult body weight, but

also interacts with their adult diet to influence their ability to invest in nuptial gifts, which have a strong effect on mating success (Engels and Sauer, 2007). Similarly, in fruit fly, *Drosophila melanogaster*, although both larval and adult diet affect adult body size, poor larval diet causes a more severe reduction in adult body size than a poor adult diet. Even so, a good adult diet is able to mitigate the effect of a poor larval diet (Poças et al., 2020). Effects like these are generally thought to result from variation in how individuals allocate resources to storage versus development (Boggs, 2009; Fischer et al., 2004).

Most research looking at how nutrient intake during different life stages affects different fitness traits has focused on food quality and quantity, but water can also be important (Chown et al., 2011; Danks, 2000). Access to water has been shown to affect both lifespan and fecundity in several insect species. For instance, in seed beetles (*Callosobruchus maculatus*, Coleoptera), access to water as larvae increases fecundity and reduces egg quality (Chapter 3), and having access to water during adulthood can increase body weight and prolong lifespan, but can also negatively affect their offspring (Vincent et al., 2020). In the almond moth (*Ephestia cautella*, Lepidoptera), on the other hand, access to water as larvae prolongs male and female lifespan and reduces female fecundity, while the effects of access to water during adulthood depend on the sex – for males there is no effect on lifespan, but for females the effect of water on lifespan depends on whether their mates had access to water as adults, or not (Ryne et al., 2004). In general, the relative importance of water availability during development and during adulthood for determining male

investment in mating, the difference between the effect of food and water in male capital breeders, or whether and how variation in resource availability across a male's life interact is unclear in most insects.

To explore the effects of water during development and adulthood, as well as their interaction, I used the seed beetle, *Callosobruchus maculatus*, as a model species. Seed beetles naturally inhabit stored, dried beans (Cope and Fox, 2003), which contain limited water - an important resource that may mediate sexual interactions (Edvardsson, 2007; Iglesias-Carrasco et al., 2018; Ursprung et al., 2009; Vincent et al., 2020). Even though seed beetles are facultatively aphagous capital breeders (i.e. they do not need to eat or drink as adults, Edvardsson, 2007; Messina and Fry, 2003), access to water during both development and adulthood has been shown to affect sexual interactions and fitness in this species (Edvardsson, 2007; Iglesias-Carrasco et al., 2018; Ursprung et al., 2009; Vincent et al., 2020; Chapter 3).

To investigate the effect of larval and adult water environment, and their potential interaction, male beetles were reared in wet or dry beans, and then allocated to an adult treatment either with or without access to water. To look at the effect of water acquired in different life-stages on capital breeders, male seed beetles mate frequently with multiple females (Ofuya, 1995). However, as has been reported in other species (e.g. bedbugs, *Cimex lectularius* (Reinhardt et al., 2011), limited water could affect a male's ability to replenish seminal fluids and thus restrict his capacity for multiple mating. To get a more complete picture of how developmental and adult water environments affect male allocation decisions and fitness I measured male

investment in reproductive traits (i.e. mating behavior, ejaculate mass, ejaculate replenishment) across multiple matings spread over three days. I also noted changes in male body weight throughout their life, and their total lifespan. If seed beetles acquire water across their lives, and are able to allocate it to mating and it affects their fitness, then several predictions can be made. First, I expect access to water during development will increase male ability to invest in some traits (and thus lead to larger ejaculates, faster ejaculate replenishment, later onset of kicking and longer mating duration), but that this effect will diminish over time. For other traits (i.e. body weight, lifespan) I expect there may be more lasting effects and affected mainly by adult water access. Second, I expect access to water during adulthood may initially have weak effects but its importance may increase with time as water stored during development is gradually depleted. For instance, access to water as an adult may help males to maintain their body weight, prolong their lifespan, replenish their ejaculates, and affect their mating behavior later in life. Third, I expect if adults increase their water intake to compensate for a dry developmental environment, then developmental and adult water availability will have an interactive effect. Whereby, males that develop in dry beans, but subsequently have access to water as adults perform better than those that spend their entire life in dry conditions.

4.3 Methods

Origin and maintenance of laboratory stock

Seed beetles (*Callosobruchus maculatus*) used in my study were from a large stock

population kept at The Australian National University. This stock has been maintained on dry black-eyed beans, *Vigna unguiculata*, for 38 generations and incubated at 25 ± 1 °C.

Experimental design

To test how juvenile and adult environments affect male reproduction and lifespan, I manipulated both juvenile and adult water environments. I then gave males the opportunity to mate two times a day for three days and measured their mating behavior, ejaculate size, ejaculate replenishment and lifespan. My 2×2 factorial design resulted in four treatment combinations: dry rearing environment and dry adult environment (DD, n=36); dry rearing environment and wet adult environment (DW, n=52); wet rearing environment and dry adult environment (WD, n=50); wet rearing environment and wet adult environment (WW, n=40). This design allowed us to tease apart the effect of the water environment during development and adulthood, and to explore their interaction.

Generating '*wet*' and '*dry*' beans

To manipulate the water content of beans, I placed 20 mung beans into individual 70 ml plastic vials. Wet beans were created by placing open vials of beans in an enclosed box with wet cotton wool on the bottom for 10 days at 4±1°C to prevent mildew. Dry beans were treated in the same way except that the box did not contain wet cotton wool and they were kept at 25±1°C. To evaluate the water content of the beans, the beans contained in each vial were weighed (to 0.1 mg, using an electronic balance, Mettler Toledo AG135) before treatment and just before being allocated to females for

egg laying. Beans in the wet treatment gained 1.96 ± 0.85% (mean ± SE) of their original weight and beans in the dry treatment lost 0.04 ± 0.24% (mean ± SE) of their original weight.

Manipulating male developmental and adult environment

To generate experimental males, I randomly picked two female beetles from our lab stock daily (for 30 days for a total of 60 females) and placed them individually in a 70ml vial with 20 wet and 20 dry beans at same time for 1 day to lay eggs. By placing females with both types of beans I was able to control for variation due to female identity in later analyses. For each female, either the wet or dry beans were marked (which type of bean was marked was decided by coin toss for each female) with a black marker, so that I knew the rearing treatment of the emerging males. Marking beans has previously been shown not to affect oviposition preference in seed beetles (Chapter 2). Once females had laid their eggs they were removed, and beans with a single egg were placed in individually labelled Eppendorf tubes with a small hole in the lid for ventilation. If there was more than one egg on a bean, extra eggs were scraped off to ensure each beetle developed without competition. The Eppendorf tubes were then placed in incubators at 25°C ± 1°C until adults emerged. Newly emerged adults were sexed, females were discarded, and males were weighed and then allocated to one of two adult treatments. Males in the wet treatment were kept in a 70 ml vial with a piece of water-soaked cotton wool placed in a small lid. Males in the dry treatment were treated in exactly the same way but without the water-soaked cotton wool.

Mating trials and measurement of mating and male lifespan

Females used in mating trials were bred separately, from stock beetles so that their emergence corresponded with that of the experimental males. To reduce variation between females, they were all reared individually in “dry” beans at 25 ± 1°C. Every female beetle was used only once to eliminate effects of their willingness to remate on the measured traits (Savalli and Fox, 1998).

To test how male treatment group affected male investment in reproduction, I mated the males twice a day for three consecutive days. This allowed us to look not only at how the treatment affects their first mating but also how their behavior and ejaculate mass change with subsequent matings. For each mating, males were weighed to 0.001mg using a Sartorius Cubis microbalance before and after mating to calculate their ejaculate weight (weight before mating – weight after mating). The females that participated in the mating trials were also weighed to control for potential effects of female body weight in my analyses. To begin a mating trial, males were placed into the female’s Eppendorf tube and both beetles were knocked to the bottom to ensure they made contact with each other. I then observed their mating behavior and timed each stage of mating.

Latency to kicking was recorded as the time from when the male climbed onto the female’s back and inserted his aedeagus into her reproductive tract (i.e. when the mating started) to the time when the female started kicking the male with her hind legs, to estimate the optimum mating duration of females (Edvardsson and Canal, 2006).

Kicking duration was recorded as the time from when the female started kicking until the copulation ended (i.e. when the male removed his aedeagus from the female reproductive tract), which reflects the directly behavioral conflict between sexes. Mating duration was recorded as the time from when mating started to when it ended. If a pair did not start mating within 10 minutes, they were separated, recorded as not mating and the male was discarded from the experiment (number of males discarded in each treatment combination: dry juvenile/dry adult = 4; dry juvenile/wet adult = 1; wet juvenile/dry adult = 2; wet juvenile/wet adult = 0). The first mating trial for each male was conducted at approximately the same time each day and males were returned to their own vial between trials. The second mating trial of the day for each male was conducted 30 minutes after the first. After the last mating (second mating on the third day), males were placed in their own vial and monitored daily until they died to determine their lifespan.

Statistical Analysis

To test how bean moisture during development and water availability during adulthood affect mating traits, I analyzed my data using the ‘glmmTMB’ package (Brooks et al., 2017) in R version 3.6.2 (R-Development-Core-Team, 2013). For all models using a Gaussian distribution I checked the residuals to ensure they met the assumptions of normality and homoscedasticity. For models that included interaction terms, I first ran a full model (i.e. with the interaction terms). If the interactions were nonsignificant I dropped them from the models so that I could interpret the main

effects (Engqvist, 2005). For models with a three-way interaction, I first ran the full model with the three-way interaction, if it was nonsignificant, I removed it to look at the two-way interactions and then the main effect model. However, if the interactions were significant, I conducted post hoc pairwise comparisons using 'lsmeans' function in 'emmeans' package (Searle et al., 1980) to determine which treatments differed. For all models, significance of effects presented in text were obtained using the 'Anova' function in the 'car' package (Fox and Weisberg, 2018). Full model summaries are provided in the appendix. Figures were plotted using the 'ggplot2' package (Wickham, 2016). Further model details are provided below.

Body weight

To explore the effect of bean moisture content on male emergence weight, I used a linear mixed model (LMM) in which I specified the juvenile water treatment (wet or dry beans) as a fixed effect and mother ID as a random effect to control for potential non-independence of siblings. To determine whether males acquired water provided to them during adulthood I analyzed their change in body weight between emergence and their first mating (i.e. body weight before first mating – body weight at emergence), as well as between the last and first mating of each day (i.e. body weight before the first mating of the day – body weight after the second mating of the previous day). To analyze the change in body weight prior to mating I used a LMM including juvenile and adult water treatment (wet or dry), as well as their interaction as fixed effects. I also included male body weight at emergence as a covariate and

mother ID as a random effect. To analyze the change in weight between mating days I again used an LMM. In this model the fixed effects were the same as for the previous model except that I also included the mating day. For the random effects I included male ID to account for multiple measures per male instead of mother ID. Mother ID was removed because including both random effects led to overparameterization of the model and because it explained less variation than male ID. However, the results remain the same regardless of which random effect I include in the model.

Mating traits

To evaluate the effect of rearing and adult water treatment on male ability to invest in mating, I analyzed three ejaculate measures: ejaculate weight of the first mating, ejaculate replenishment between days (i.e. ejaculate weight during the first mating of one mating day - ejaculate weight during the second mating of the previous mating day) and the change in ejaculate weight between matings on the same day (i.e. ejaculate weight during the first mating of a day – ejaculate weight during the second mating of the same day). Ejaculate weight of the first mating was analyzed using an LMM, including the rearing and adult water treatment, as well as their interaction as fixed effects. I also included male and female body weight at emergence as covariates, as body size of both sexes is known from previous studies to influence ejaculate weight (Edvardsson and Canal, 2006; Savalli and Fox, 1998). In addition, mother ID was included as a random effect to control for potential non-independence

of siblings. Ejaculate replenishment across days and change in ejaculate weight between matings on the same day were analyzed in the same way except I also included the day of mating as well as its three- and two-way interactions with rearing and adult water treatment in the model. Female body weight was removed as different females were used in each mating. I also included male ID as a random effect.

To explore the immediate effects of juvenile and adult water treatment on mating behavior, I analyzed the effect of juvenile and adult water on three aspects of mating behavior (i.e. latency to kicking, kicking duration and mating duration) for the males' first mating. For each of the three mating behavior measures I performed LMMs, including the juvenile water treatment, adult water treatment and all two and three way interactions as fixed effects. Male weight at emergence and female body weight before mating were both included as covariates. In addition, mother ID was included as a random effect. For kicking duration the data were transformed using the 'powerTransform' function in 'car' package (Fox and Weisberg, 2018) to ensure model residuals met the assumption of normality.

To explore the effect of juvenile and adult water treatment on a male's ability to sustain mating effort over multiple matings I analyzed the effects of juvenile and adult water on the same three aspects of mating behavior across the first and second mating of the first mating day. This model was set up in the same way as described above, except I also included the order of each mating, and interactions with it, as fixed effects in the model. Kicking duration was, again, transformed to ensure

normality of model residuals using the same method as above. Male ID was included in these models to control for potential non-independence of each male's mating behavior over the successive matings.

To explore the effect of water on mating behavior over a longer time period, I also looked at the effect of juvenile and adult water treatment on mating behavior during the first mating of each mating day. All three behavioral measures were analyzed in the same way as described for the analysis of sustained mating behavior except that the order of mating was replaced by the mating day. As in previous analyses, kicking duration was transformed to ensure normality of model residuals.

Lifespan

The effect of juvenile and adult water on male lifespan was analyzed using a Cox proportional hazard model (function 'coxph', R package 'survival', Therneau and Grambsch, 2000). I included the juvenile water treatment, adult water treatment and male body weight at emergence as fixed effects, and mother ID as a random effect.

4.4 Results

Body weight

The water treatment experienced during development did not affect male body weight at emergence (mean ± SE = 3.863 ± 0.030 mg, $\chi^2 = 0.003$, Df = 1, p = 0.959, Table 4.1). Male change in body weight before mating was not affected by the interaction between juvenile and adult water treatment (mean ± SE = -0.289 ± 0.013 mg, χ^2 =

1.285, Df = 1, p = 0.257, Table 4.2). However, both wet juvenile environments and wet adult environments caused greater weight loss before mating (juvenile: χ^2 = 4.405, Df = 1, p = 0.036; adult: χ^2 = 25.842, Df = 1, p < 0.001, Fig. 4.1, Table 4.2), and males that were larger at emergence lost more weight (χ^2 = 138.428, Df = 1, p < 0.001, Table 4.2).

The change in male weight between mating days was affected by an interaction between juvenile and adult water treatments (mean ± SE = -0.060 ± 0.005 mg, χ^2 = 4.933, Df = 1, p = 0.026, Table 4.3). However, post hoc tests revealed that the dominant pattern in the data is driven by male access to water during adulthood (Fig. 4.2, Table 4.4). Furthermore, larger males lost more weight between matings (χ^2 = 5.705, Df = 1, p = 0.017, Table 4.3) and males lost less weight between the second and third mating day than they did between the first and second mating day (χ^2 = 29.484, Df = 1, p < 0.001, Table 4.3).

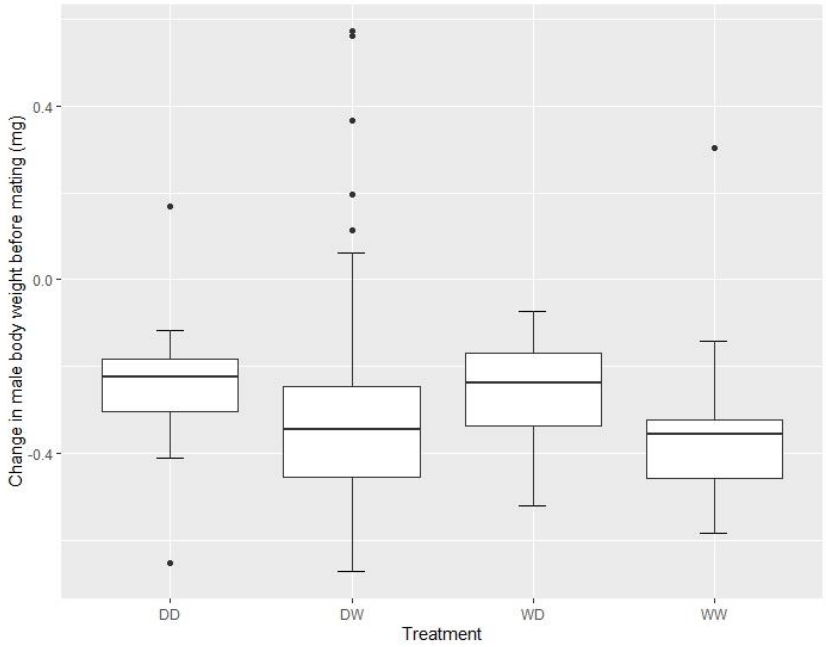

Figure 4.1 Effect of juvenile and adult water treatment on change in male body weight before mating (male body weight at emergence – male body weight before the first mating). DD: dry juvenile and dry adult treatment; DW: dry juvenile and wet adult treatment; WD: wet juvenile and dry adult treatment; WW: wet juvenile and wet adult treatment. The box plots show the median and range of dispersion (lower and upper quartiles, and outliers).

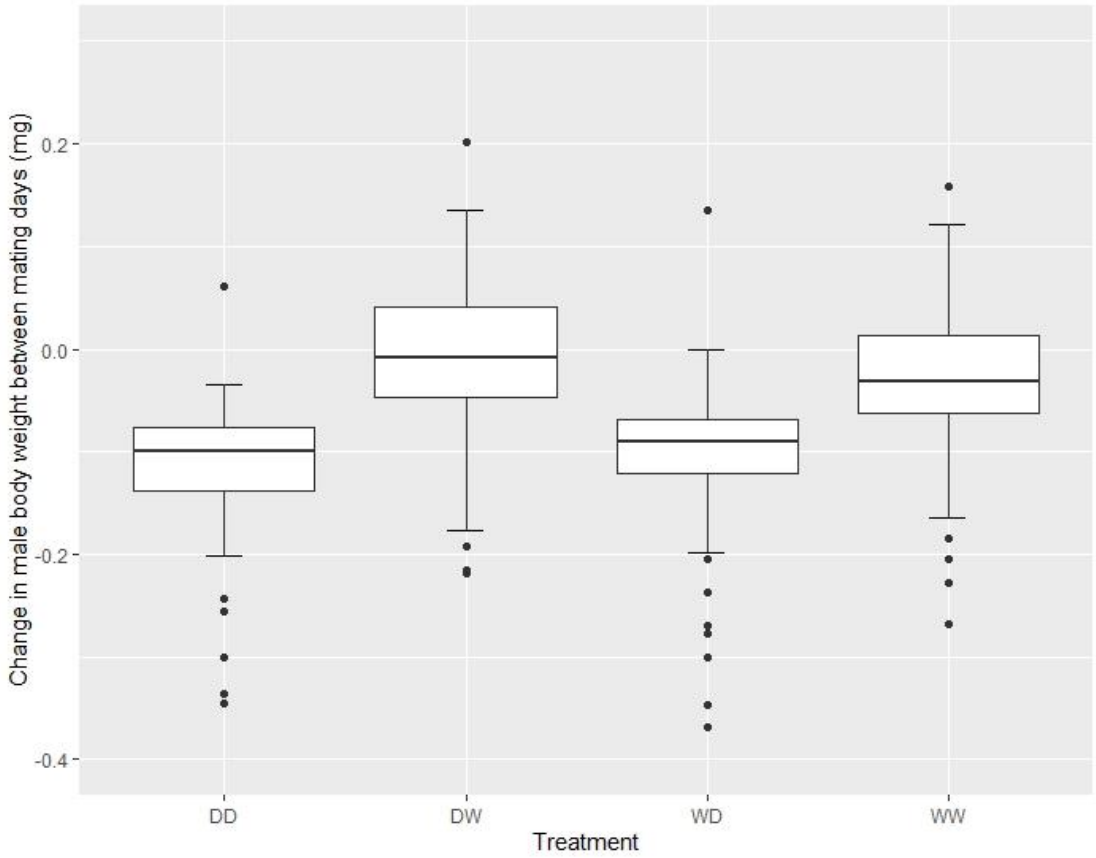

Figure 4.2 Effect of juvenile and adult water treatment on change in male body weight between mating days (male body weight before the first mating of the day – male body weight after the second mating of the previous day). DD: dry juvenile and dry adult treatment; DW: dry juvenile and wet adult treatment; WD: wet juvenile and dry adult treatment; WW: wet juvenile and wet adult treatment. The box plots show the median and range of dispersion (lower and upper quartiles, and outliers).

Ejaculate traits

Neither juvenile water treatment, nor the interaction between juvenile and adult water treatment affected male ejaculate weight in their first mating (mean ± SE = 0.227 ± 0.004 mg, juvenile: $\chi^2 = 0.635$, Df = 1, p = 0.426, juvenile*adult interaction: $\chi^2 = 0.009$, Df = 1, p = 0.926, Table 4.5). However, males with access to water during adulthood had a larger ejaculate in their first mating than those without ($\chi^2 = 4.344$,

Df = 1, p = 0.037, Fig. 4.3, Table 4.5), and males who were larger at emergence had larger ejaculates in their first mating (χ^2 = 9.613, Df = 1, p = 0.002, Table 4.5).

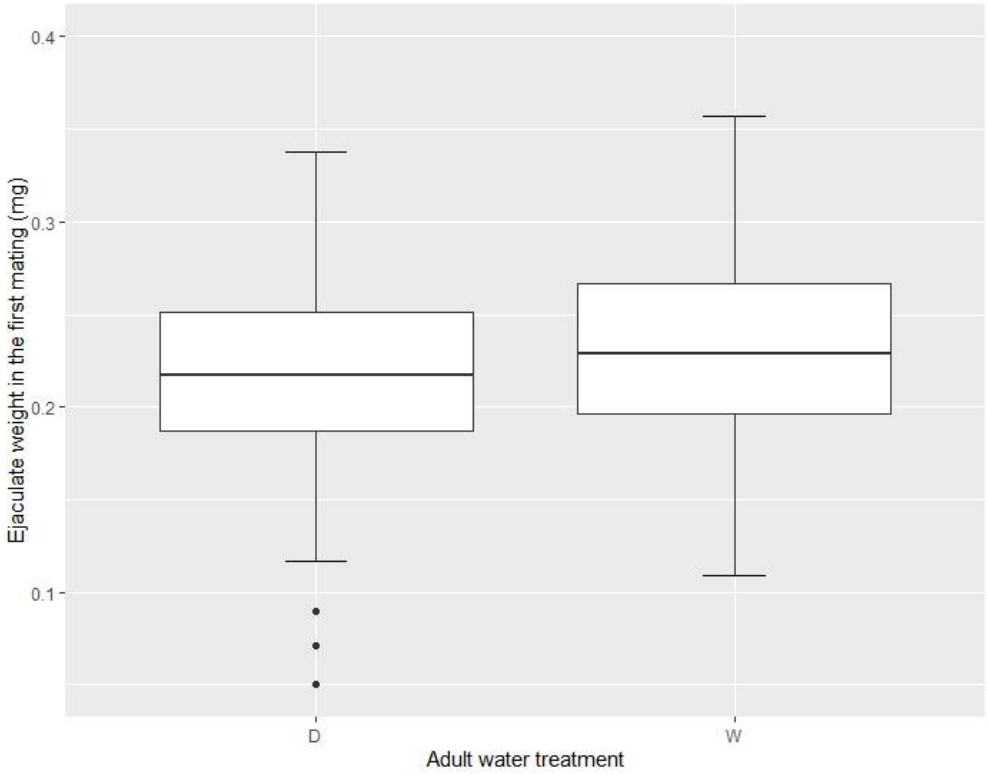

Figure 4.3 Effect of adult water treatment on male ejaculate weight in the first mating (male body weight before the first mating – male body weight after the first mating). D: dry adult water treatment; W: wet adult water treatment. The box plots show the median and range of dispersion (lower and upper quartiles, and outliers).

Ejaculate replenishment across days was not affected by the three-way nor two-way interactions involving juvenile water treatment, adult water treatment or mating day (all p > 0.090, Table 4.6), nor was it affected by juvenile or adult water treatments (juvenile: χ^2 = 0.084, Df = 1, p = 0.771; adult: χ^2 = 0.068, Df = 1, p = 0.794, Table 4.6). Ejaculate replenishment was also not affected by male body weight at emergence (χ^2 = 0.701, Df = 1, p = 0.402, Table 4.6). However, the difference in ejaculate weight between the first and second day of mating was greater than between the second and third day (χ^2 = 267.236, Df = 1, p < 0.001, Fig. 4.4, Table 4.6).

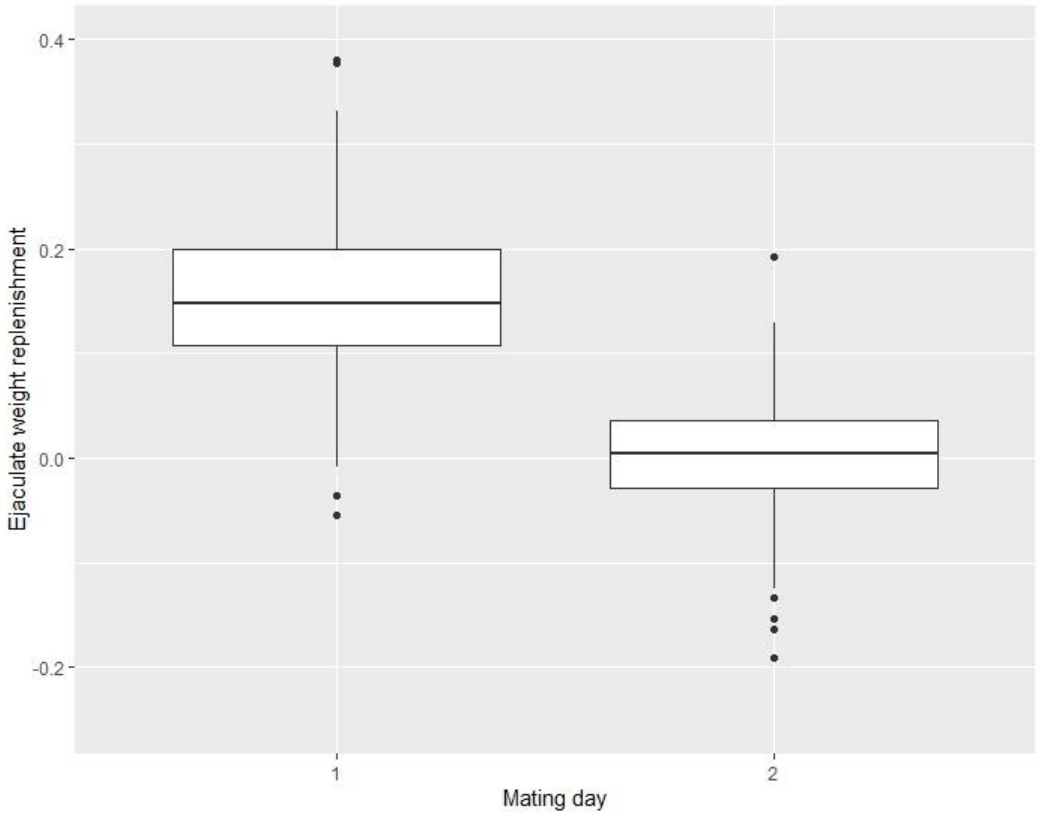

Figure 4.4 Effect of mating day on the ejaculate replenishment between days. 1: ejaculate weight during the first mating of day two – ejaculate weight during the second mating of day one; 2: ejaculate weight during the first mating of day three – ejaculate weight during the second mating of day two. Larger values indicate that ejaculate replenishment was greater between two mating days. The box plots show the median and range of dispersion (lower and upper quartiles, and outliers).

The change in ejaculate weight between matings on the same day was not affected by the three-way or the two-way interactions involving juvenile water treatment, adult water treatment and mating day (all $p > 0.095$, Table 4.7). There was also no effect of the juvenile water treatment ($\chi^2 = 1.611$, Df = 1, $p = 0.204$, Table 4.7), adult water treatment ($\chi^2 = 3.244$, Df = 1, $p = 0.072$, Table 4.7), or male body weight at emergence ($\chi^2 = 0.368$, Df = 1, $p = 0.544$, Table 4.7). However, there was a greater decrease in ejaculate weight on the first mating day (Fig. 4.5, Table 4.8).

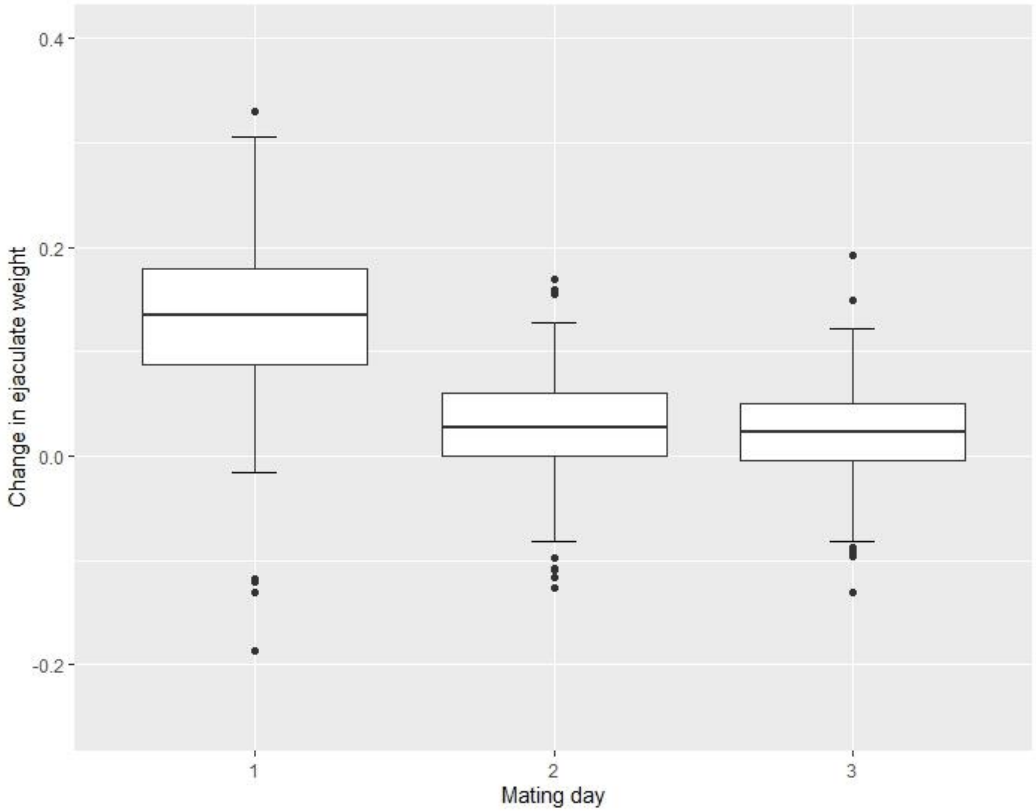

Figure 4.5 Effect of mating day on the change in ejaculate weight on a given day, i.e. ejaculate weight during the first mating of a day – ejaculate weight during the second mating of the same day 1: mating day one; 2: mating day two; 3: mating day three. Positive numbers indicate that ejaculate weight decreased across subsequent matings. The box plots show the median and range of dispersion (lower and upper quartiles, and outliers).

Mating behavior

When comparing mating behavior in the first mating, mating duration was not affected by the interaction between juvenile and adult water treatment (mean ± SE = 456.882 ± 9.152 s, $\chi^2 = 2.689$, Df = 1, p = 0.101, Table 4.9) or the adult water treatment ($\chi^2 = 0.018$, Df = 1, p = 0.893, Table 4.9). But males reared in wet beans had shorter matings than those from dry beans ($\chi^2 = 7.158$, Df = 1, p = 0.007, Fig. 4.6, Table 4.9). Also, larger males mated for a shorter duration than smaller males (χ^2

= 3.995, Df = 1, p = 0.046, Table 4.9), but female body weight had no effect on mating duration (χ^2 = 1.493, Df = 1, p = 0.222, Table 4.9).

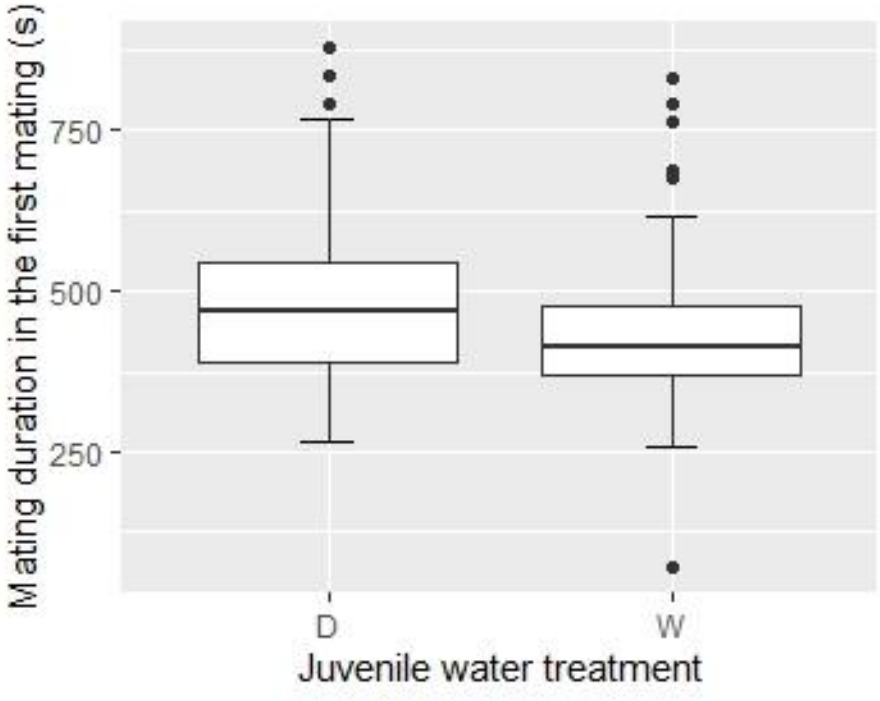

Figure 4.6 Effect of juvenile water treatment on the mating duration in the first mating. D: males reared from dry beans; W: males reared from wet beans. The box plots show the median and range of dispersion (lower and upper quartiles, and outliers).

Kicking latency, on the other hand, was affected by the interaction between juvenile and adult water treatment (mean ± SE = 344.353 ± 7.843 s, χ^2 = 8.044, Df = 1, p = 0.005, Table 4.10). Males reared in dry beans and without access to water during adulthood were kicked later than males reared in wet beans and without access to water (t ratio = 3.395, p = 0.005, Fig. 4.7, Table 4.11) or reared in dry beans and with access to water during adulthood (t ratio = 3.115, p = 0.011, Fig. 4.7, Table 4.11). Finally, kicking duration in the first mating was only affected by adult water accessibility (mean ± SE = 112.529 ± 6.640 s, χ^2 = 6.384, Df = 1, p = 0.012, Fig. 4.8, others: p > 0.133, Table 4.12).

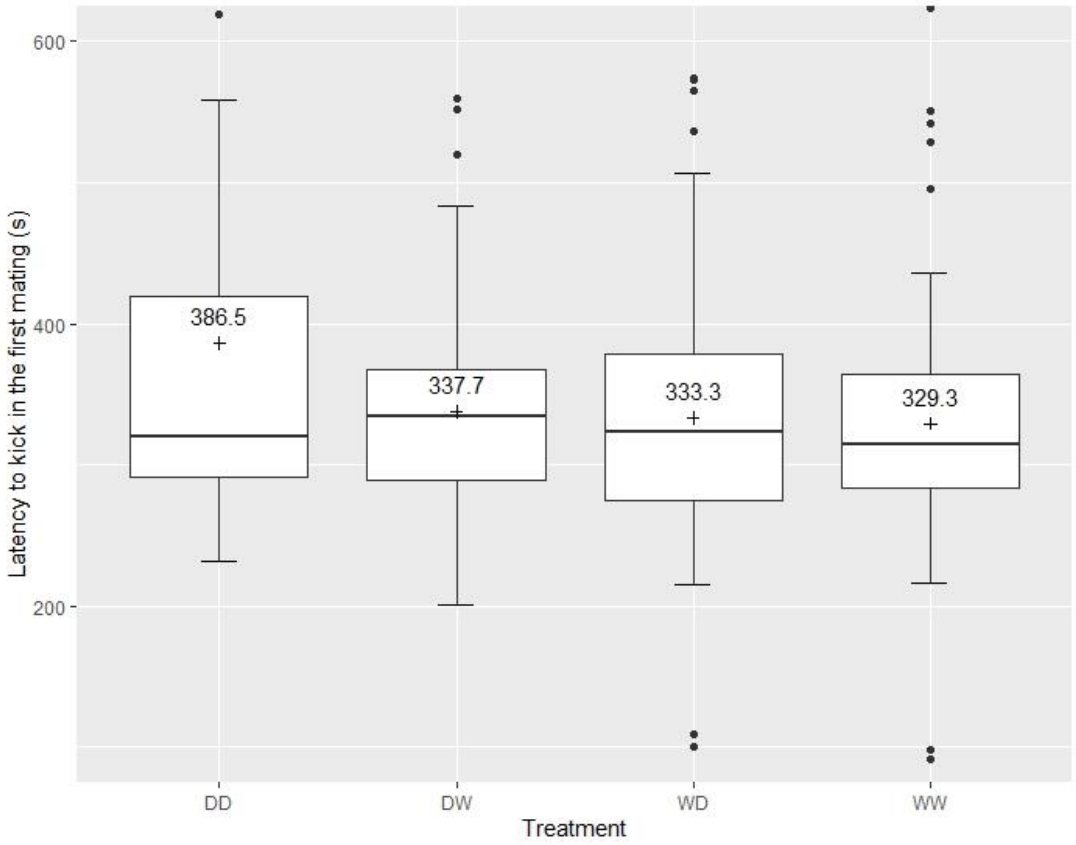

Figure 4.7 Effect of juvenile and adult water treatment on the kicking latency in the first mating. DD: dry juvenile and dry adult treatment; DW: dry juvenile and wet adult treatment; WD: wet juvenile and dry adult treatment; WW: wet juvenile and wet adult treatment. The box plots show the median (solid line), mean (cross point) and range of dispersion (lower and upper quartiles, and outliers).

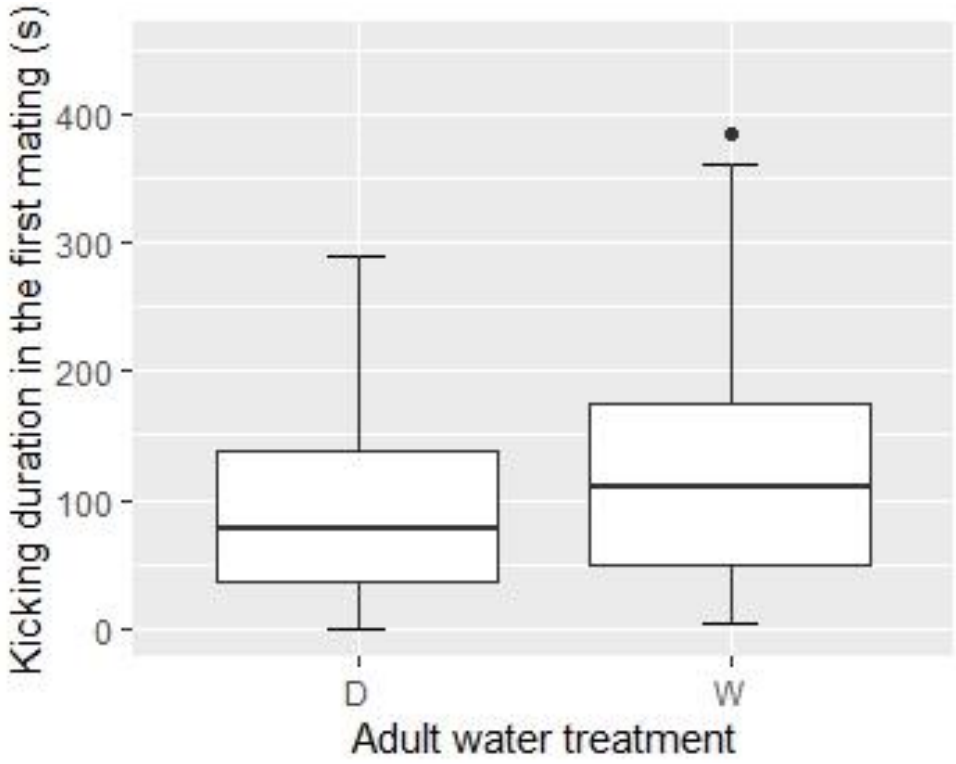

Figure 4.8 Effect of adult water treatment on the kicking duration in the first mating. D: males without access to water; W: males can access to water. The box plots show the median and range of dispersion (lower and upper quartiles, and outliers).

When comparing mating behavior across the first and second mating of the first mating day, none of the interactions involving order were significant for any of the behaviors (mating duration - all $p > 0.398$, Table 4.13; latency to kick - all $p > 369$, Table 4.14; kicking duration - all $p > 0.250$, Table 4.15). Nor were there any significant effects of adult water treatments (mating duration: $\chi^2 = 0.041$, Df = 1, $p = 0.840$, Table 4.13; latency to kick: $\chi^2 = 1.019$, Df = 1, $p = 0.313$, Table 4.14; kicking durations: $\chi^2 = 0.623$, Df = 1, $p = 0.430$, Table 4.15). Further, juvenile water treatment had no effect on latency to kicking ($\chi^2 = 0.852$, Df = 1, $p = 0.356$, Table 4.14), but males reared in wet beans had a shorter mating duration ($\chi^2 = 4.630$, Df = 1, $p = 0.031$, Table 4.13) and kicking durations ($\chi^2 = 3.869$, Df = 1, $p = 0.049$, Table 4.15) than males reared in

dry beans. Finally, all behaviors prolonged in the second mating (i.e. effect of order: mating duration - χ^2 = 105.946, Df = 1, p < 0.001, Table 4.13; latency to kick - χ^2 = 135.005, Df = 1, p < 0.001, Table 4.14; kicking duration - χ^2 = 7.297, Df = 1, p = 0.007, Table 4.15).

When comparing mating behavior across the first mating of each day, although mating duration decreased across days (χ^2 = 172.191, Df = 2, p < 0.001) this decline was similar regardless of the males' treatment (all interactions: p > 0.057, Table 4.16; see also Table 4.17). Latency to kicking during the first mating of each day was affected by none of the interactions involving mating day (all p> 0.346, Table 4.18) except that with the juvenile water treatment (χ^2 = 18.644, Df = 2, p < 0.001, Fig. 4.9, Table 4.18).

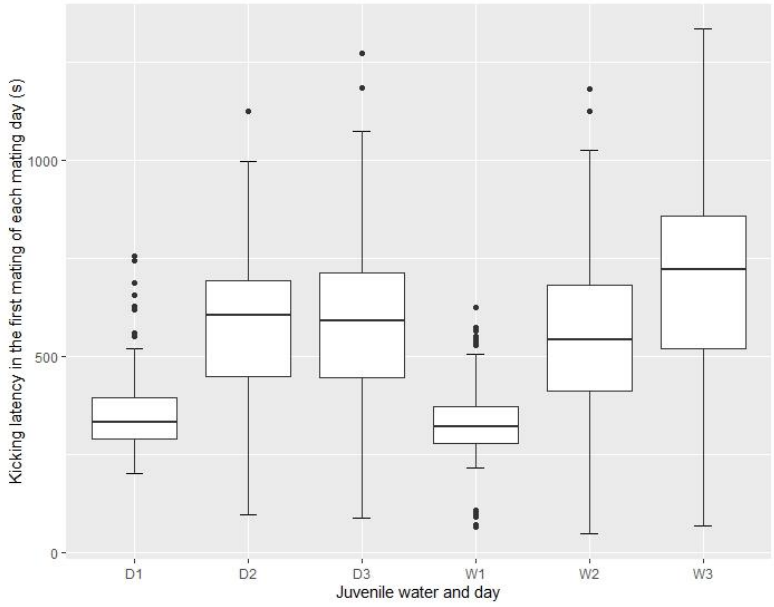

Figure 4.9 Effect of juvenile water treatment and mating day on the kicking latency in the first mating of each mating day. D: dry juvenile water treatment; W: wet juvenile water treatment. 1: the first mating day; 2: the second mating day; 3: the third mating day. The box plots show the median and range of dispersion (lower and upper quartiles, and outliers).

Finally, for the kicking duration during the first mating trial of each day I found that the effect of juvenile and adult water treatment depended on the day of the mating trial (three-way interaction: $\chi^2 = 6.065$, Df = 2, p = 0.048, Table 4.21). On the first mating day, males who were reared in dry beans and then had access to water as adults were kicked significantly later than those reared in wet beans without access to water as adults. On the second mating day all males had similar kicking durations regardless of their treatment. And on the third mating day, males who were reared in dry beans and then had access to water as an adult, had longer kicking durations than those reared in wet beans that had access to water as an adult (Fig. 4.10, and see Table 4.21 for pairwise comparisons).

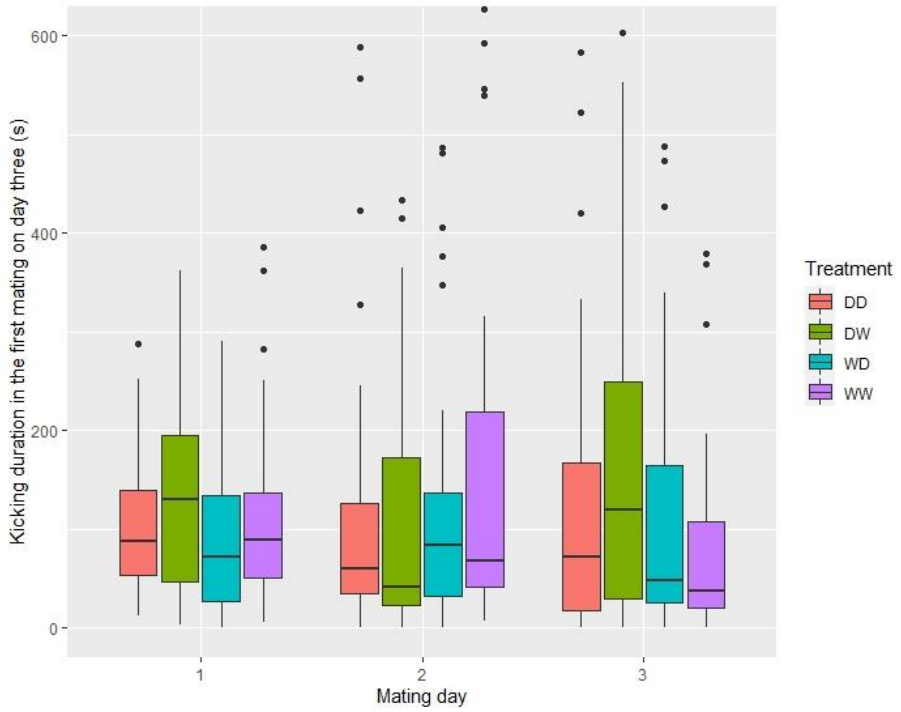

Figure 4.10 Effect of juvenile and adult water treatment on the kicking duration of the third mating day. DD: dry juvenile and dry adult treatment; DW: dry juvenile and wet adult treatment; WD: wet juvenile and dry adult treatment; WW: wet juvenile and wet adult treatment. The box plots show the median and range of dispersion (lower and upper quartiles, and outliers).

Lifespan

Male lifespan was not affected by an interaction between juvenile and adult water treatment, nor the juvenile water environment on its own (mean ± SE = 13.987 ± 0.320 day, Table 4.22). However, males with access to water during adulthood lived significantly longer than those without (z = -3.118, p = 0.002, Fig. 4.11, Table 4.22), as did males that were larger at emergence (z = -4.214, p < 0.001, Table 4.22).

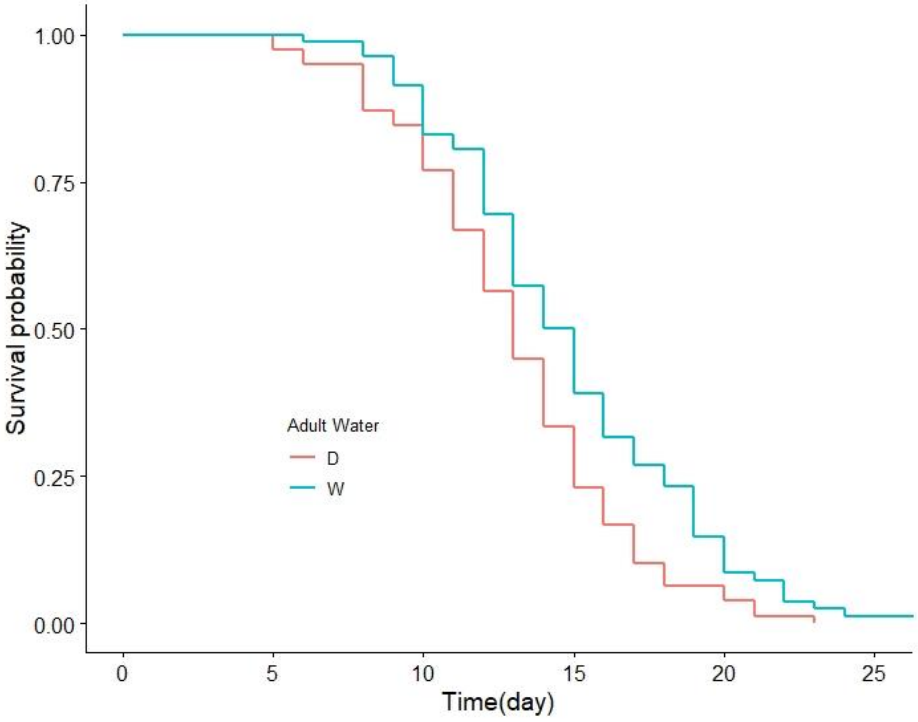

Figure 4.11 Kaplane – Meier survival plots for males maintained in different adult water treatments. D: dry adult treatment; W: wet adult treatment.

4.5 Discussion

Adult fitness is expected to vary depending on both juvenile and adult environments (Boggs, 2009; Engels and Sauer, 2007). In seed beetles, water is a limited resource which affects fitness and the economics of mating and reproduction (Edvardsson, 2007; Iglesias-Carrasco et al., 2018; Ursprung et al., 2009; Vincent et al., 2020; Chapter 3). Previous studies have manipulated the availability of water for juvenile or adult seed beetles, and found that both affect adult life-histories and reproduction (Vincent et al., 2020; Chapter 3), albeit in different ways. Greater moisture content of beans during development increases male ejaculate size but reduces female fecundity (Chapter 3). In contrast, access to water during adulthood increases male body size and mating duration, and leads females to produce low quality eggs rather than fewer eggs (Vincent et al., 2020). Here, I manipulated the water condition, during both development (i.e., water content of beans) and adulthood (i.e., water availability), of males to test: 1) whether water acquired in different life stages is allocated differently to reproduction throughout their life; and 2) whether beetles are able to compensate for dry developmental environments if they have greater access to water as adults. My results show that water acquired during development and during adulthood is allocated towards different traits. For instance, males developing in wet and dry environments showed differences in mating behavior, particularly early in adult life, whereas males with or without access to water as adults showed differences in their ejaculate weight as well as mating behavior during their first mating. Access to water as adults also had longer lasting effects – influencing both

weight loss with age, and lifespan. Furthermore, I found little evidence for interactive effects between juvenile and adult water treatments, indicating that males do not compensate for dry juvenile environments by increasing water intake during adulthood, or that if they do, they do not allocate it to the same traits in the same way.

Body weight of male seed beetles often reflects their developmental conditions (Fox, 1994) and is related to their mating behavior and reproductive performance (Edvardsson and Canal, 2006; Savalli and Fox, 1998). Here, consistent with previous research (Chapter 3), I found that male body weight at emergence was not affected by their developmental water environment. However, after emerging, although all males lost weight, those with access to water as adults lost more than those without on the first day of the adulthood, a result which contrasts with previous research (Edvardsson, 2007). The reason for this initial loss of weight is unclear, and over a longer time period it appears that males with access to water were better able to maintain their body weight than those without. I also found that males with access to water as adults lived longer - a result that has previously been shown for female seed beetles (Ursprung et al., 2009; Vincent et al., 2020). These results along with observations of beetle drinking (Edvardsson, 2007) suggest that males are able to acquire water from their environment as adults and that this water is allocated to somatic maintenance. However, increased lifespan is only expected to increase fitness if males are able to continue investing in ejaculates in later life. Even though males can mate till the end of life (Ofuya, 1995), if ejaculates are not replenished then increased longevity will have little effect on overall fitness.

Mating behavior in seed beetles is thought to reflect sexual conflict (Johnstone and Keller, 2000). Males prefer longer copulations that increase their reproductive success (Savalli and Fox, 1999), while females deploy kicking behavior to terminate mating sooner (Crudgington and Siva-Jothy, 2000; Edvardsson and Canal, 2006; Van Lieshout et al., 2014) probably because longer matings cause more severe genital tract damage (Crudgington and Siva-Jothy, 2000) and reduce female receptivity to mating (Van Lieshout et al., 2014). However, there is also evidence that kicking duration is partly under male control (Wilson and Tomkins, 2014), and so the function of kicking is being questioned (Dougherty and Simmons, 2017; McNamara et al., 2020). Here, I found that mating behavior during the first mating was affected by both juvenile and adult water treatment as well as their interaction. Males reared in dry beans had longer mating durations than males from wet beans, and males living without water throughout their life started to be kicked later than those with access to water during only one life-stage. These results could reflect that males reared in dry environments take longer to transfer their ejaculate and those with access to water as adults can resist female kicking for a longer time due to their better body condition because of the water they acquired. However, how copulation duration relates to fitness in this species is still under debate, some studies show that longer copulations increase female fecundity (Edvardsson and Canal, 2006; Vincent et al., 2020). Thus, it is difficult to say how selection would act on such differences. For the kicking latency, which was shortened by access to water as either larvae or adults, however, the reason for these difference is still unclear. In addition, I also found that the time it

took for females to start kicking males increased across consecutive days. For males reared in dry beans the kicking latency plateaued after two days, but for males reared in wet beans it continued to increase from day two to day three. The fact that all females used for mating were virgin stock females of a similar age indicates that the effects here are the result of differences in the way that the rearing environment affects male reproductive investment over time or aging. Given I found no effect of developmental environment on male ejaculates, body weight or lifespan it is unclear precisely what drives this effect. Recent research suggests, however, that developmental environments may be important for determining rates of senescence for some traits (Cooper and Kruuk, 2018; Sanghvi et al., 2021), thus investigating the potential for developmental water to influence senescence could be an interesting avenue for future research.

Male seed beetles transfer large ejaculates (Fox, 1993; Fox et al., 1995; Paukku and Kotiaho, 2005), which stimulate oviposition and delay remating by their partners (Edvardsson, 2007; Harano, 2012; Moya-Laraño and Fox, 2006; Takashi Yamane, 2015). Here, male ejaculate mass did not differ between males from wet and dry rearing environments, but males with access to water during adulthood transferred larger ejaculates. This is different from previous studies which showed wet developmental environments increase male ejaculate mass (Chapter 3), but that access to water during adulthood does not (Vincent et al., 2020). The lack of effect of developmental water on ejaculate mass could occur if the treatment of beans in the current experiment was ineffective. However, the beans used here gained more

weight than in my previous experiment (Chapter 3), and the developmental water treatment did influence behavior, thus this is unlikely to be the cause. Another possible explanation is that the method used to manipulate bean wetness in this study differed from previous studies. Here bean wetting involved keeping beans at four degrees centigrade, which could have affected beans in unintended ways that impact on nutrition intake or development of larvae and masked the effects of water itself. When it comes to differences between studies in the effect of adult water, however, I used exact the same method as previous research (Vincent et al., 2020), so the reason for the differences in results between the studies is unclear.

Following the first mating, male access to water during adulthood did not influence the ability to replenish ejaculates either within or between days. This suggests that although male seed beetles can replenish their ejaculate (Fox et al., 1995), access to water may only be allocated into ejaculates when received early in adult life (i.e. prior to reaching sexual maturity) and that excess water received after this time is unable to be stored for investment in later ejaculates. Further, the fact that ejaculate replenishment in early life (mating day two and one) was larger than in later life (mating day three and two) implies a decrease in their ejaculate replenishment capacity. Whether this decrease is due to aging or depletion of stored nutrients, however, is still unclear.

I predicted that access to water during both juvenile and adult life stages would influence adult male investment in mating and survival, but that the effects of the adult environment would continue later into life. My results partly support this

expectation. Both larval and adult water treatment affect male mating behavior, but only developmental water continued to affect mating behavior in later life. Also, male access to water during adulthood initially increased ejaculate size and later allowed males to maintain body weight and prolong their lifespan. Finally, I found little evidence that males compensate for dry developmental environments through increased water consumption as adults. The only significant interaction between juvenile and adult water environments was for latency to kicking and the pattern seen here was inconsistent with compensation.

4.6 Reference

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4.7 Tables

Table 4.1. Effects of juvenile water treatment on **male body weight at emergence**. Model outputs obtained from a linear mixed model using template model builder.

Terms	estimate	SE	Z	P
Fixed effects				
Intercept	3.873	0.049	78.54	<0.001
Juvenile Water (Wet)	-0.003	0.059	-0.05	0.959
Random effects	**Variance**	**SD**		
Mother	0.035	0.188		

Table 4.2. Effects of juvenile water treatment and adult water treatment on **change in male body weight before mating**. Model outputs obtained from linear mixed models using template model builder. For completeness main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	0.670	0.080	8.312	<0.001
Juvenile Water (Wet)	-0.018	0.027	-0.644	0.519
Adult Water (Wet)	-0.074	0.027	-2.713	0.007
Emerged body weight	-0.231	0.020	-11.743	**<0.001**
Juvenile Water * Adult Water	-0.043	0.038	-1.133	0.257
Random effects	**Variance**	**SD**		
Mother	0.0003	0.016		
Main effects model				
Fixed effects				
Intercept	0.687	0.080	8.629	<0.001
Juvenile Water (Wet)	-0.040	0.019	-2.099	**0.036**
Adult Water (Wet)	-0.096	0.019	-5.084	**<0.001**
Emerged body weight	-0.232	0.020	-11.766	**<0.001**
Random effects	**Variance**	**SD**		
Mother	0.0002	0.014		

Significant p-values are highlighted in bold

Table 4.3. Effects of juvenile water treatment and adult water treatment on **change in male body weight between mating days**. Model outputs obtained from linear mixed models using template model builder. For completeness main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	-0.055	0.034	-1.630	0.103
Juvenile Water (Wet)	0.016	0.011	1.363	0.173
Adult Water (Wet)	0.101	0.012	8.737	**<0.001**
Emerged body weight	-0.020	0.008	-2.389	**0.017**
Day 2	0.043	0.008	5.430	**<0.001**
Juvenile Water * Adult water	-0.035	0.016	-2.221	**0.026**
Random effects	**Variance**	**SD**		
Male	9.505e-11	9.749e-06		

Significant p-values are highlighted in bold

Table 4.4. Post-hoc pairwise comparisons showing the effect of juvenile water treatment and adult water treatment on **change in male body weight between days**. Model outputs obtained using lsmeans with Tukey adjustment for multiple testing.

Contrast	estimate	SE	df	t.ratio	P
D D - W D	-0.016	0.012	343	-1.358	0.527
D D - D W	-0.101	0.012	343	-8.718	**<0.001**
D D - W W	-0.081	0.012	343	-6.709	**<0.001**
W D - D W	-0.085	0.011	343	-8.035	**<0.001**
W D - W W	-0.065	0.011	343	-5.869	**<0.001**
D W - W W	0.020	0.011	343	1.785	0.282

Significant p-values are highlighted in bold

Table 4.5. Effects of juvenile water treatment and adult water treatment on **ejaculate weight in the first mating**. Model outputs obtained from a linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Fixed effects				
Intercept	0.164	-0.006	2.699	0.007
Juvenile Water (Wet)	-0.006	0.012	-0.478	0.632
Adult Water (Wet)	0.017	0.012	1.475	0.140
Emerged body weight	0.028	0.009	3.101	**0.002**
Female body weight	-0.008	0.007	-1.092	0.275
Juvenile Water * Adult water	-0.002	0.017	-0.093	0.926
Random effects	**Variance**	**SD**		
Mother	0.001	0.025		
Main effects model				
Fixed effects				
Intercept	0.164	0.061	2.700	0.007
Juvenile Water (Wet)	-0.006	0.008	-0.797	0.426
Adult Water (Wet)	0.017	0.008	2.084	**0.037**
Emerged body weight	0.028	0.009	3.100	**0.002**
Female body weight	-0.008	0.007	-1.088	0.277
Random effects	**Variance**	**SD**		
Mother	0.001	0.025		

Significant p-values are highlighted in bold

Table 4.6. Effects of juvenile water treatment, adult water treatment and mating day on **ejaculate replenishment** (i.e. ejaculate weight during the first mating of one mating day - ejaculate weight during the second mating of the previous mating day). Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Three-way interactions				
Fixed effects				
Intercept	0.126	0.039	3.238	0.001
Juvenile Water (Wet)	-0.008	0.018	-0.439	0.661
Adult Water (Wet)	0.005	0.018	0.267	0.789
Day 2	-0.155	0.020	-7.880	**<0.001**
Emerged body weight	0.008	0.009	0.836	0.403
Juvenile Water * Adult water	-0.019	0.025	-0.736	0.462
Juvenile Water * Day	0.008	0.026	0.317	0.751
Adult Water * Day	-0.008	0.026	-0.305	0.761
Three-way interaction	0.042	0.036	1.188	0.235
Random effects	**Variance**	**SD**		
Mother	9.174e-13	9.578e-07		
Mating	1.327e-12	1.152e-06		
Two-way interactions				
Fixed effects				
Intercept	0.132	0.039	3.426	0.001
Juvenile Water (Wet)	-0.019	0.016	-1.220	0.222
Adult Water (Wet)	-0.006	0.015	-0.385	0.700
Day 2	-0.168	0.016	-10.200	**<0.001**
Emerged body weight	0.008	0.009	0.840	0.401
Juvenile Water * Adult water	0.003	0.018	0.148	0.883
Juvenile Water * Day	0.030	0.018	1.702	0.089
Adult Water * Day	0.014	0.018	0.774	0.439
Random effects	**Variance**	**SD**		

Mother	1.227e-12	1.108e-06		
Mating	1.761e-12	1.327e-06		
Main effect model				
Fixed effects				
Intercept	0.120	0.038	3.192	0.001
Juvenile Water (Wet)	-0.003	0.009	-0.291	0.771
Adult Water (Wet)	0.002	0.009	0.261	0.794
Day 2	-0.145	0.009	-16.347	**<0.001**
Emerged body weight	0.008	0.009	0.837	0.402
Random effects	**Variance**	**SD**		
Mother	1.674e-12	1.294e-06		
Mating	4.500e-13	6.708e-07		

Significant p-values are highlighted in bold

Table 4.7. Effects of juvenile water treatment, adult water treatment and mating day on **the change in ejaculate weight between matings** on the same day. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Three-way interactions				
Fixed effects				
Intercept	0.120	0.029	4.100	<0.001
Juvenile Water (Wet)	-0.037	0.016	-2.263	**0.024**
Adult Water (Wet)	0.008	0.016	0.531	0.595
Day 2	-0.102	0.018	-5.835	**<0.001**
Day 3	-0.111	0.018	-6.335	**<0.001**
Emerged body weight	0.004	0.007	0.589	0.556
Juvenile Water * Adult water	0.037	0.022	1.624	0.104
Juvenile Water * Day	0.020	0.023	0.892	0.373
Adult Water * Day	-0.011	0.023	-0.480	0.631
Three-way interaction (Day 2)	-0.003	0.032	-0.098	0.922
Three-way interaction (Day 3)	-0.048	0.032	-1.530	0.126
Random effects	**Variance**	**SD**		
Mother	5.099e-05	7.141e-03		
Mating	2.975e-11	5.454e-06		
Two-way interactions				
Fixed effects				
Intercept	0.115	0.029	3.983	<0.001
Juvenile Water (Wet)	-0.028	0.013	-2.110	**0.035**
Adult Water (Wet)	0.017	0.013	1.319	0.187
Day 2	-0.101	0.015	-6.934	**<0.001**
Day 3	-0.096	0.015	-6.579	**<0.001**
Emerged body weight	0.004	0.007	0.589	0.556
Juvenile Water * Adult water	0.019	0.013	1.471	0.141
Juvenile Water * Day 2	0.019	0.016	1.187	0.235

Juvenile Water * Day 3	0.009	0.016	0.574	0.566
Adult Water * Day 2	-0.012	0.016	-0.786	0.432
Adult Water * Day 3	-0.034	0.016	-2.145	**0.032**
Random effects	**Variance**	**SD**		
Mother	4.892e-05	6.994e-03		
Mating	3.041e-11	5.515e-06		
Main effect model				
Fixed effects				
Intercept	0.112	0.028	4.001	<0.001
Juvenile Water (Wet)	-0.008	0.007	-1.269	0.204
Adult Water (Wet)	0.012	0.007	1.801	0.072
Day 2	-0.098	0.008	-12.529	**<0.001**
Day 3	-0.109	0.008	-13.922	**<0.001**
Emerged body weight	0.004	0.007	0.607	0.544
Random effects	**Variance**	**SD**		
Mother	6.629e-05	8.142e-03		
Mating	2.084e-11	4.565e-06		

Significant p-values are highlighted in bold

Table 4.8. Post-hoc pairwise comparisons showing the effect of mating day on **the change in ejaculate weight between matings**. Model outputs obtained using lsmeans with Tukey adjustment for multiple testing.

Contrast	estimate	SE	df	t.ratio	P
1 – 2	0.098	0.008	525	12.529	**<0.001**
1 – 3	0.109	0.008	525	13.922	**<0.001**
2 – 3	0.011	0.008	525	1.393	0.345

Significant p-values are highlighted in bold

Table 4.9. Effects of juvenile water treatment and adult water treatment on **mating duration for the first mating**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Three-way interaction				
Fixed effects				
Intercept	530.86	133.19	3.986	<0.001
Juvenile Water (Wet)	-76.54	25.07	-3.054	**0.002**
Adult Water (Wet)	-31.98	25.22	-1.268	0.205
Male emerged body weight	-44.55	22.31	-1.997	**0.046**
Female weight	20.83	14.82	1.406	0.160
Juvenile Water * Adult Water	57.42	35.02	1.640	0.101
Random effects	**Variance**	**SD**		
Mother	2.649e-57	5.147e-29		
Main effects model				
Fixed effects				
Intercept	531.603	134.063	3.965	<0.001
Juvenile Water (Wet)	-47.103	17.605	-2.676	**0.007**
Adult Water (Wet)	-2.374	17.717	-0.134	0.893
Male emerged body weight	-44.874	22.451	-1.999	**0.046**
Female weight	18.115	14.824	1.222	0.222
Random effects	**Variance**	**SD**		
Mother	2.706e-52	1.645e-26		

Significant p-values are highlighted in bold

Table 4.10. Effects of juvenile water treatment and adult water treatment on **latency to kick for the first mating**. Model outputs obtained from linear mixed models using template model builder.

Terms	estimate	SE	Z	P
Full model				
Three-way interaction				
Fixed effects				
Intercept	129.47	119.35	1.085	0.278
Juvenile Water (Wet)	-73.02	21.51	-3.395	**<0.001**
Adult Water (Wet)	-67.21	21.58	-3.115	**0.002**
Male emerged body weight	-24.96	19.64	-1.271	0.204
Female weight	57.25	13.08	4.379	**<0.001**
Juvenile Water * Adult Water	85.56	30.17	2.836	**0.005**
Random effects	**Variance**	**SD**		
Mother	2.738e+02	1.655e+01		
Mating	1.077e+04	1.038e+02		

Significant p-values are highlighted in bold

Table 4.11. Post-hoc pairwise comparisons showing the effect of juvenile and adult water treatment on **latency to kick in the first mating**. Model outputs obtained using lsmeans with Tukey adjustment for multiple testing.

Contrast	estimate	SE	df	t.ratio	P
D D – W D	73.02	21.5	195	3.395	**0.005**
D D – D W	67.21	21.6	195	3.115	**0.011**
D D – W W	54.67	22.8	195	2.399	0.080
W D – D W	-5.82	19.5	195	-0.299	0.991
W D – W W	-18.35	21.0	195	-0.872	0.819
D W – W W	-12.54	20.9	195	-0.599	0.932

Significant p-values are highlighted in bold

Table 4.12. Effects of juvenile water treatment and adult water treatment on **kicking duration for the first mating**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Three-way interaction				
Fixed effects				
Intercept	39.018	97.975	0.398	0.690
Juvenile Water (Wet)	-20.275	18.439	-1.100	0.272
Adult Water (Wet)	29.980	18.549	1.616	0.106
Male emerged body weight	24.699	16.408	1.505	0.132
Female weight	-3.535	10.902	-0.324	0.746
Juvenile Water * Adult Water	5.306	25.759	0.206	0.837
Random effects	**Variance**	**SD**		
Mother	2.989e-02	0.173		
Mating	8.155e+03	90.307		
Main effects model				
Fixed effects				
Intercept	39.086	97.985	0.399	0.690
Juvenile Water (Wet)	-17.554	12.867	-1.364	0.172
Adult Water (Wet)	32.717	12.949	2.527	**0.012**
Male emerged body weight	24.670	16.409	1.503	0.133
Female weight	-3.786	10.835	-0.350	0.727
Random effects	**Variance**	**SD**		
Mother	2.987e-02	0.173		
Mating	8.157e+03	90.317		

Significant p-values are highlighted in bold

Table 4.13. Effects of juvenile water treatment, adult water treatment and mating order on **mating duration for two matings on the first mating day**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Three-way interaction				
Fixed effects				
Intercept	751.21	111.93	6.712	<0.001
Juvenile Water (Wet)	-71.61	42.04	-1.703	0.088
Adult Water (Wet)	-31.28	41.93	-0.746	0.456
Order (2)	198.63	42.62	4.661	**<0.001**
Male emerged body weight	-69.67	28.88	-2.413	**0.016**
Juvenile Water * Adult Water	43.20	58.26	0.741	0.458
Juvenile Water * Order	15.03	55.01	0.273	0.785
Adult Water * Order	15.49	54.77	0.283	0.777
Three-way interaction	-11.82	76.20	-0.155	0.877
Random effects	**Variance**	**SD**		
Mother	2914	53.98		
Mating	3450	58.73		
Two-way interaction				
Fixed effects				
Intercept	749.453	111.351	6.731	<0.001
Juvenile Water (Wet)	-68.512	37.011	-1.851	0.064
Adult Water (Wet)	-28.225	37.022	-0.762	0.446
Order (2)	202.241	35.679	5.668	**<0.001**
Male emerged body weight	69.686	28.875	-2.413	0.016
Juvenile Water * Adult Water	37.291	44.105	0.845	0.398
Juvenile Water * Order	8.866	38.041	0.233	0.816
Adult Water * Order	9.384	38.083	0.246	0.805
Random effects	**Variance**	**SD**		
Mother	2911	53.95		
Mating	3448	58.72		

Main effects model				
Fixed effects				
Intercept	734.242	105.508	6.959	<0.001
Juvenile Water (Wet)	-44.552	20.705	-2.152	**0.031**
Adult Water (Wet)	-4.235	20.979	-0.202	0.840
Order (2)	211.630	20.561	10.293	**<0.001**
Male emerged body weight	-70.086	27.867	-2.515	**0.012**
Random effects	**Variance**	**SD**		
Mother	3.501e+03	5.917e+01		
Mating	1.676e-04	1.295e-02		

Significant p-values are highlighted in bold

Table 4.14. Effects of juvenile water treatment, adult water treatment and mating order on **latency to kick for two matings on the first mating day**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Three-way interaction				
Fixed effects				
Intercept	667.95	98.24	6.799	<0.001
Juvenile Water (Wet)	-48.34	37.91	-1.275	0.202
Adult Water (Wet)	-54.25	37.82	-1.435	0.151
Order (2)	182.57	39.97	4.568	**<0.001**
Male emerged body weight	-77.58	25.22	-3.076	**0.002**
Juvenile Water * Adult Water	36.00	52.48	0.686	0.493
Juvenile Water * Order	31.31	51.69	0.606	0.545
Adult Water * Order	39.43	51.44	0.766	0.443
Three-way interaction	-14.09	71.59	-0.197	0.844
Random effects	**Variance**	**SD**		
Mother	3.308e+03	57.513		
Mating	4.817e-03	0.069		
Two-way interaction				
Fixed effects				
Intercept	665.86	97.67	6.817	<0.001
Juvenile Water (Wet)	-44.66	32.97	-1.355	0.176
Adult Water (Wet)	-50.62	33.01	-1.534	0.125
Order (2)	186.88	33.45	5.587	**<0.001**
Male emerged body weight	-77.60	25.22	-3.077	0.002
Juvenile Water * Adult Water	28.97	38.43	0.754	0.451
Juvenile Water * Order	23.96	35.75	0.670	0.503
Adult Water * Order	32.15	35.78	0.899	0.369
Random effects	**Variance**	**SD**		
Mother	3.304e+03	57.481		
Mating	1.685e-03	0.041		

Main effects model				
Fixed effects				
Intercept	640.46	95.76	6.688	<0.001
Juvenile Water (Wet)	-17.35	18.80	-0.923	0.356
Adult Water (Wet)	-19.21	19.03	-1.009	0.313
Order (2)	215.92	18.58	11.619	**<0.001**
Male emerged body weight	-77.15	25.28	-3.052	**0.002**
Random effects	**Variance**	**SD**		
Mother	3.379e+03	5.813e+01		
Mating	1.464e-05	3.826e-03		

Significant p-values are highlighted in bold

Table 4.15. Effects of juvenile water treatment, adult water treatment and mating order on **kicking duration for two matings on the first mating day**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Three-way interaction				
Fixed effects				
Intercept	8.884	1.754	5.066	<0.001
Juvenile Water (Wet)	-0.899	0.645	-1.394	0.163
Adult Water (Wet)	0.314	0.643	0.488	0.626
Order (2)	-0.346	0.644	-0.538	0.591
Male emerged body weight	-0.129	0.453	-0.286	0.775
Juvenile Water * Adult Water	0.418	0.894	0.468	0.640
Juvenile Water * Order	-0.345	0.830	-0.416	0.678
Adult Water * Order	-0.909	0.827	-1.100	0.271
Three-way interaction	0.739	1.150	0.642	0.521
Random effects	**Variance**	**SD**		
Mother	0.530	0.728		
Mating	1.167	1.080		
Two-way interaction				
Fixed effects				
Intercept	9.003	1.749	5.147	<0001
Juvenile Water (Wet)	-1.094	0.571	-1.916	0.055
Adult Water (Wet)	0.123	0.572	0.216	0.829
Order (2)	-0.573	0.541	-1.059	0.289
Male emerged body weight	-0.128	0.454	-0.282	0.778
Juvenile Water * Adult Water	0.788	0.685	1.151	0.250
Juvenile Water * Order	0.040	0.576	0.069	0.945

Adult Water * Order	-0.529	0.576	-0.918	0.359
Random effects	**Variance**	**SD**		
Mother	0.532	0.730		
Mating	1.166	1.080		
Main effects model				
Fixed effects				
Intercept	8.896	1.744	5.102	<0.001
Juvenile Water (Wet)	-0.673	0.342	-1.967	**0.049**
Adult Water (Wet)	0.273	0.347	0.789	0.430
Order (2)	-0.827	0.306	-2.701	**0.007**
Male emerged body weight	-0.112	0.460	-0.244	0.807
Random effects	**Variance**	**SD**		
Mother	0.586	0.766		
Mating	1.183	1.088		

Significant p-values are highlighted in bold

Table 4.16. Effects of juvenile water treatment, adult water treatment and mating day on **mating duration for the first mating of each day**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Three-way interactions				
Fixed effects				
Intercept	752.931	96.544	7.799	<0.001
Juvenile Water (Wet)	-74.430	39.826	-1.869	0.062
Adult Water (Wet)	-32.459	39.802	-0.816	0.415
Day 2	226.488	44.036	5.143	**<0.001**
Day 3	222.045	45.787	4.850	**<0.001**
Male body weight before mating	-70.280	24.691	-2.846	**0.004**
Juvenile Water * Adult Water	55.841	55.395	1.008	0.313
Juvenile Water * Day 2	1.241	56.134	0.022	0.982
Juvenile Water * Day 3	147.446	56.512	2.609	**0.009**
Adult Water * Day 2	-15.898	55.878	-0.285	0.776
Adult Water * Day 3	32.034	56.250	0.569	0.569
Three-way interaction (Day 2)	20.083	78.020	0.257	0.797
Three-way interaction (Day 3)	-114.816	78.434	-1.464	0.143
Random effects	**Variance**	**SD**		
Mother	3.981e+03	6.310e+01		
Mating	5.851e-13	7.649e-07		
Two-way interactions				
Fixed effects				
Intercept	741.601	95.780	7.743	<0.001
Juvenile Water (Wet)	-59.046	33.153	-1.781	0.075
Adult Water (Wet)	-17.095	33.232	-0.514	0.607
Day 2	220.984	37.392	5.910	**<0.001**
Day 3	256.969	39.216	6.553	**<0.001**

Male body weight before mating	-69.639	24.746	-2.814	**0.005**
Juvenile Water * Adult Water	26.160	35.244	0.742	0.458
Juvenile Water * Day 2	11.681	39.090	0.299	0.765
Juvenile Water * Day 3	87.728	39.291	2.233	**0.026**
Adult Water * Day 2	-5.848	39.156	-0.149	0.881
Adult Water * Day 3	-26.653	39.494	-0.675	0.500
Random effects	**Variance**	**SD**		
Mother	3.929e+03	6.268e+01		
Mating	1.295e-05	3.599e-03		
Main effect model				
Fixed effects				
Intercept	640.57	86.08	7.442	<0.001
Juvenile Water (Wet)	-15.92	17.04	-0.934	0.350
Adult Water (Wet)	-13.61	17.09	-0.797	0.426
Day 2	232.76	22.29	10.442	**<0.001**
Day 3	299.61	23.95	12.512	**<0.001**
Male body weight before mating	-46.69	23.01	-2.029	**0.042**
Random effects	**Variance**	**SD**		
Mother	1.936e-02	0.139		
Mating	5.878e-04	0.024		

Significant p-values are highlighted in bold

Table 4.17. Post-hoc pairwise comparisons showing the effect of mating day on **mating duration**. Model outputs obtained using lsmeans with Tukey adjustment for multiple testing.

Contrast	estimate	SE	df	t.ratio	P
1 – 2	-232.8	22.3	560	-10.442	**< 0.001**
1 – 3	-299.6	23.9	560	-12.512	**< 0.001**
2 – 3	-66.8	21.3	560	-3.133	**0.005**

Significant p-values are highlighted in bold

Table 4.18. Effects of juvenile water treatment, adult water treatment and mating day on **latency to kick for the first mating of each day**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model (grey text), but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Three-way interaction				
Fixed effects				
Intercept	558.062	90.812	6.145	<0.001
Juvenile Water (Wet)	-55.418	38.037	-1.457	0.145
Adult Water (Wet)	-57.223	38.025	-1.505	0.132
Day 2	212.493	42.276	5.026	**<0.001**
Day 3	194.550	43.901	4.432	**<0.001**
Male body weight before mating	-46.483	23.203	-2.003	**0.045**
Juvenile Water * Adult Water	48.799	52.907	0.922	0.356
Juvenile Water * Day 2	-0.255	53.972	-0.005	0.996
Juvenile Water * Day 3	158.454	54.332	2.916	0.003
Adult Water * Day 2	10.262	53.714	0.191	0.848
Adult Water * Day 3	25.726	54.064	0.476	0.634
Three-way interaction Day 2	-17.105	75.007	-0.228	0.820
Three-way interaction Day 3	-38.159	75.401	-0.506	0.613
Random effects	**Variance**	**SD**		
Mother	2.324e+03	48.204		
Mating	1.183e-03	0.034		
Two-way interaction				
Fixed effects				
Intercept	552.226	89.855	6.146	<0.001
Juvenile Water (Wet)	-46.299	31.454	-1.472	0.141
Adult Water (Wet)	-48.156	31.541	-1.527	0.127
Day 2	217.749	35.778	6.086	**<0.001**
Day 3	206.141	37.469	5.502	**<0.001**

Male body weight before mating	-46.346	23.201	-1.998	**0.046**
Juvenile Water * Adult Water	31.226	33.150	0.942	0.346
Juvenile Water * Day 2	-9.110	37.471	-0.243	0.808
Juvenile Water * Day 3	138.63	37.660	3.681	**<0.001**
Adult Water * Day 2	1.439	37.528	0.038	0.969
Adult Water * Day 3	6.182	37.848	0.163	0.870

Random effects	**Variance**	**SD**
Mother	2.311e+03	4.808e+01
Mating	2.804e-05	5.295e-03

Significant p-values are highlighted in bold

Table 4.19. Post-hoc pairwise comparisons showing the effect of juvenile water treatment and mating day on **latency to kick of the first mating of a day**. Model outputs obtained using lsmeans with Tukey adjustment for multiple testing.

Contrast	estimate	SE	df	t.ratio	P
D 1 - W 1	28.19	26.5	557	1.063	0.896
D 1 - D 2	-228.05	28.6	557	-7.962	**<0.001**
D 1 - W 2	-188.30	28.6	557	-6.594	**<0.001**
D 1 - D 3	-223.13	29.8	557	-7.478	**<0.001**
D 1 - W 3	-330.55	29.9	557	-11.040	**<0.001**
W 1 - D 2	-256.23	28.3	557	-9.058	**<0.001**
W 1 - W 2	-216.49	28.1	557	-7.712	**<0.001**
W 1 - D 3	-251.32	29.4	557	-8.541	**<0.001**
W 1 - W 3	-358.73	29.4	557	-12.210	**<0.001**
D 2 - W 2	39.74	27.8	557	1.427	0.710
D 2 - D 3	4.91	28.2	557	0.174	1.000
D 2 - W 3	-102.50	28.2	557	-3.635	**0.004**
W 2 - D 3	-34.83	28.1	557	-1.238	0.818
W 2 - W 3	-142.24	28.0	557	-5.085	**<0.001**
D 3 - W 3	-107.41	28.1	557	-3.825	**0.002**

Significant p-values are highlighted in bold

Table 4.20. Effects of juvenile water treatment, adult water treatment and mating day on **kick duration for the first mating of each day**. Model outputs obtained from linear mixed models using template model builder. For completeness, main effects are shown for the full model, but they should be interpreted from the main effects model.

Terms	estimate	SE	Z	P
Full model				
Three-way interactions				
Fixed effects				
Intercept	7.861	1.430	5.497	<0.001
Juvenile Water (Wet)	-0.821	0.604	-1.359	0.174
Adult Water (Wet)	0.308	0.604	0.510	0.610
Day 2	-0.473	0.675	-0.701	0.483
Day 3	-0.771	0.700	-1.102	0.270
Male body weight before mating	-0.091	0.365	-0.250	0.802
Juvenile Water * Adult Water	0.544	0.841	0.648	0.517
Juvenile Water * Day 2	0.763	0.862	0.885	0.376
Juvenile Water * Day 3	0.726	0.868	0.837	0.403
Adult Water * Day 2	-0.686	0.858	-0.800	0.424
Adult Water * Day 3	0.699	0.863	0.810	0.418
Three-way interaction Day 2	0.495	1.198	0.414	0.679
Three-way interaction Day 3	-2.338	1.204	-1.942	0.052
Random effects	**Variance**	**SD**		
Mother	3.867e-01	6.219e-01		
Mating	6.422e-07	8.014e-04		

Significant p-values are highlighted in bold

Table 4.21. Post-hoc pairwise comparisons showing the effect of juvenile and adult water treatment on **kicking duration** for the first mating within each mating day. Model outputs obtained using lsmeans with Tukey adjustment for multiple testing.

Contrast	estimate	SE	df	t.ratio	P
Day 1					
D D – W D	1.221	0.774	196	1.577	0.394
D D – D W	-0.840	0.776	196	-1.083	0.700
D D – W W	-0.268	0.819	196	-0.327	0.988
W D – D W	-2.061	0.696	196	-2.962	**0.018**
W D – W W	-1.489	0.748	196	-1.989	0.196
D W – W W	0.572	0.743	196	0.771	0.868
Day 2					
D D – W D	0.072	0.592	176	0.121	0.999
D D – D W	0.471	0.589	176	0.800	0.854
D D – W W	-0.436	0.620	176	-0.704	0.895
W D – D W	0.399	0.543	176	0.735	0.883
W D – W W	-0.508	0.576	176	-0.881	0.815
D W – W W	-0.907	0.571	176	-1.589	0.388
Day 3					
D D – W D	0.081	0.635	173	0.127	0.999
D D – D W	-0.786	0.627	173	-1.254	0.594
D D – W W	0.809	0.664	173	1.217	0.617
W D – D W	-0.866	0.570	173	-1.520	0.428
W D – W W	0.728	0.612	173	1.190	0.634
D W – W W	1.595	0.605	173	2.635	**0.045**

Significant p-values are highlighted in bold

Table A22. Effects of bean water content on **lifespan**. Model outputs obtained from a survival model. For completeness, main effects are shown for the full model, but they should be interpreted from the main effects model.

Terms	coef	exp(coef)	se(coef)	Z	Pr(>|z|)
Full model					
Fixed effects					
Juvenile Water (Wet)	0.076	1.079	0.231	0.328	0.743
Adult Water (Wet)	-0.331	0.718	0.231	-1.433	0.152
Male emerged body weight	-0.812	0.444	0.191	-4.243	**<0.001**
Juvenile Water * Adult Water	-0.361	0.697	0.32432	-1.115	0.265
Main effects model					
Fixed effects					
Juvenile Water (Wet)	-0.107	0.898	0.160	-0.670	0.503
Adult Water (Wet)	-0.510	0.600	0.1635	-3.118	**0.002**
Male emerged body weight	-0.8079	0.446	0.192	-4.214	**<0.001**

Significant p-values are highlighted in bold

5 Discussion

In this thesis I present three experiments on seed beetles, looking at how water affects female oviposition preferences, development, adult life history traits, and mating traits. In the first experiment, I manipulated the water content of mung beans and tested whether females preferred to lay their eggs on beans with a high or low moisture content. I found no preference for wet or dry beans, and the moisture content of beans had no effect on seed beetle development time or the likelihood of survival to adulthood. In the second experiment, I mated male and female offspring from the preference experiment to measure the effect of developmental water on their fitness and mating traits. I found that more water during development increases a male's ability to invest in his first mating, but does not increase body weight or lifespan. I also found that access to water during development has the potential to lower female egg quality and to strengthen sexual conflict. Finally, in the last experiment, I manipulated the water availability during both development and adulthood of males (although the method to generate wet beans was slightly different to the previous chapter), I then carried out multiple mating trials for each male over three days to explore the effect of water on their investment in reproduction throughout life. I found that developing in beans with a greater water content affects offspring mating behavior rather than ejaculate weight, and when males had access to water as adults, they initially lost more weight, but were better able to maintain their body weight throughout adult life and had longer lifespans compared to males

with no access to water as adults. Results summarized in tables 5.1 and 5.2.

Table 5.1. Result summary table of Chapter 3 where developmental water was manipulated for both males and females and then they were mated with each other. " √ ": significant effect; "×": nonsignificant effect (in grey); "-": not analyzed (in grey); "*": interaction with remating status.

Trait	Male water	Female water	Male Water * Female Water	Male body weight	Female body weight
Male emerged body weight	×	-	-	-	-
Female emerged body weight	-	×	-	-	-
Male ejaculate weight	-	-	√	√	×
Kicking latency in the first mating	×	×	×	√	√
Mating duration in the first mating	×	×	×	×	×
Kicking duration in the first mating	×	×	×	√	√
Likelihood of remating	×	×	×	√	×
Kicking latency in second mating	×	×	×	√	×
Mating duration in second mating	×	×	×	√	×
Kicking duration in second mating	×	×	×	×	×
Female fecundity after the first mating	√	×	×	×	√
Offspring survival after the first mating	-	-	√	×	×
Offspring number after the first mating	×	×	×	×	√
Female fecundity after second mating	×	×	×	×	√
Offspring survival after second mating	*	×	×	×	√
Offspring number after second mating	×	×	×	×	√
Male lifespan	×	×	×	√	×
Female lifespan	×	×	×	×	√

Table 5.2. Result summary table of Chapter 4 where male developmental and adult water were manipulated and then males were mated to stock (dry) females. “ √ ”: significant effect; “×”: nonsignificant effect (in grey); “-”: not analyzed (in grey); “*”: interaction with other “*” factors.

Trait	Juvenile water	Adult water	Juvenile Water * Adult Water	Male Emerged body weight	Female body weight	Mating day	Mating order
Male body weight at emergence	×	-	-	-	-	-	-
Body weight change before mating	√	√	×	√	-	-	-
Body weight change between mating days	×	×	√	√	-	√	-
Ejaculate weight in the first mating	×	√	×	√	×	-	-
Ejaculate replenishment between days	×	×	×	×	-	√	-
The change in ejaculate weight between matings on a given day	×	×	×	×	-	√	-
Mating duration for the first mating	√	×	×	√	×	-	-
Kicking latency for the first mating	×	×	√	×	√	-	-
Kicking duration for the first mating	×	√	×	×	×	-	-
Mating duration for two matings on the first mating day	√	×	×	√	-	-	√
Kicking latency for two matings on the first mating day	×	×	×	√	-	-	√
Kicking duration for two matings on the first mating day	√	×	×	×	-	-	√
Mating duration for the first mating of each day	×	×	×	√	-	√	-
Kicking latency for the first mating of each day	*	×	×	×	-	*	-
Kicking duration for the first mating of each day	-	-	*	×	-	*	-
Lifespan	×	√	×	√	-	-	-

Consistent with previous studies my findings demonstrate the importance of water availability during both larval and adult stages for reproduction in seed beetles (Edvardsson, 2007; Iglesias-Carrasco et al., 2018a; Ursprung et al., 2009; Vincent et al., 2020). Although males do not invest directly in their offspring, their mating activity was also profoundly affected by their breeding strategy. Consistent with a previous study (Soulsbury, 2019), my results indicate that, as capital breeders, male seed beetles with access to water during development increase their reproductive potential. However, in addition to increasing their reproductive potential, access to water in adulthood also helps them cope with the physiological stress associated with extended fasting. However, for females, mating with males, who had been able to acquire extra water as juveniles or adults, is not always beneficial (Chapter 3 and Vincent et al., 2020 respectively). Especially, for capital breeders, when females themselves have access to water. Similarly to what happens with access to additional food, although additional food can promote body weight and fecundity (Gu and Danthanarayana, 1990), the level of sexual conflict will change when the availability of water changes, and the reproductive consequences will be driven towards a new balance (Rostant et al., 2020), which may need to be considered in further studies.

The ultimate measure of fitness is how many offspring an individual produces, but how individuals invest in somatic maintenance and survival can also be important in affecting fitness. This is especially the case when individuals reproduce till the end of their life. My results show that the juvenile water condition did not affect the lifespan of either males or females. Access to water during adulthood can prolong lifespan of

both male and female seed beetles (Chapter 4, Vincent et al., 2020). Given that male seed beetles have the ability to keep mating till the end of their life (Ofuya, 1995), a longer lifespan may provide an overall fitness benefit because it allows them to mate more often. However, this would only be the case if males are able to produce viable ejaculates up until the end of life. My results suggest that male ejaculate weight decreased dramatically over the first three days of their adult life, a pattern that has been shown previously (Jones et al., 2007), so the relationship between lifespan and fitness is unclear. For females, on the other hand, longer lifespan does not always correspond with higher fecundity (Messina and Fry, 2003; Savalli and Fox, 1999; Vincent et al., 2020). If there were differences between males and females in how water affects overall fitness this could possibly influence how selection acts on ecological traits and potentially lead to sex specific expression of traits like habitat preferences and sexual segregation, which has been broadly reported in vertebrates (Alves et al., 2013; Stokke and Du Toit, 2002).

Future research focused on exploring what happens to the ejaculate in different water environments would be fruitful. How does water accessibility during development and adulthood affect ejaculate composition? How do resulting changes in ejaculate composition affect the copulation process and reproduction? and also how do the costs of transferring or receiving ejaculates differ between different environments? Further, while there is a substantial amount of research focused on how diet influences mating interactions and sexual conflict (Hebets et al., 2008; Hunt et al., 2005; Iglesias-Carrasco et al., 2018b; Klepsatel et al., 2020; Macartney et al., 2018;

Naguib and Nemitz, 2007; Perry et al., 2009; Vega-Trejo et al., 2016), and an increasing interest in the effects of water (Chown et al., 2011; Danks, 2000; Edvardsson, 2007; Iglesias-Carrasco et al., 2018a; Ryne et al., 2004; Ursprung et al., 2009; Vincent et al., 2020), there is little research looking at the interaction between these two resources. Ursprung et al. (2009) reported that sugar water prolongs lifespan compared with fresh water in female seed beetles, which suggests the food and water may have some interactive effect on fitness.

In summary, I investigated the effect of water at two life-stages of seed beetles and compared my findings with the existing research to reveal a complicated interactive effect of water acquisition on reproduction, and that water allocation differed depending on the life-stages at which water was acquired. Finally, the physiological mechanism behind the benefits and costs for reproduction caused by changes in ejaculate size are not clear and needs further research.

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