



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

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Received: 6 February 2022 / Accepted: 4 July 2022 / Published online: 29 October 2022
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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, we manipulated both developmental and adult water availability of male seed beetles (*Callosobruchus maculatus*) to explore how water acquired at each life-stage affects a range of fitness related traits, including body mass, ejaculate weight, mating behavior and lifespan. Our 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.

Keywords *Callosobruchus maculatus* · Environment · Fitness · Sexual conflict · Water

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)

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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 (Zhang and Head 2021), and having access to water during adulthood can increase body weight and prolong lifespan, but can also negatively affect the development of their offspring (Vincent et al. 2020). Access to water as larvae for the almond moth (*Ephestia cautella*, Lepidoptera), on the other hand prolongs male and female lifespan and reduces female fecundity. However, the effects of access to water during adulthood depends on the sex. In summary, there is no effect on lifespan for males, 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, we used the seed beetle, *Callosobruchus maculatus*, as a model species. In this species, larvae hatch from eggs laid on the surface of host beans. They then burrow into the bean to feed on the endosperm, pupate, and then emerge as adults 21 to 29 days later (depending on the ambient temperature and humidity, Fox et al. 2003). 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; Zhang and Head 2021).

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. Male seed beetles usually mate 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 comprehensive picture of how developmental and adult water environments affect male allocation decisions and fitness we measured male investment in reproductive traits (i.e. mating behavior, ejaculate mass, ejaculate replenishment) across multiple matings spread over three days. We also recorded 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, we expect access to water during development will increase male ability to invest in reproductive traits, and thus lead to larger ejaculates, faster ejaculate replenishment, later onset of kicking (a behavior performed by females in order to dislodge males when they are ready to finish mating Crudginton and Siva-Jothy 2000; Wilson et al. 2014) and longer mating duration, but that this effect will diminish over time. For other traits (i.e. body weight, lifespan) we expect the effects of developmental water availability to be longer lasting. Second, we 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, we 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.

Materials and methods

Origin and maintenance of laboratory stock

Seed beetles (*Callosobruchus maculatus*) used in our study were sourced in 2018 from stock kept at the University of Western Australia since 2005 (Dougherty and Simmons 2017). Since 2018 this stock has been maintained at the Australian National University, 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, we manipulated both juvenile and adult water environments. We 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. This 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, we 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 $1.96 \pm 0.85\%$ (mean \pm SE) of their original weight and beans in the dry treatment lost $0.04 \pm 0.24\%$ (mean \pm SE) of their original weight.

Manipulating male developmental and adult environment

To generate experimental males, we 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 we were 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 we knew the rearing treatment of the emerging males. Marking beans has previously been shown not to affect oviposition preference in seed beetles (Vrtílek et al. 2021). 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 \pm 1 °C until adults emerged. Newly emerged adults were sexed, females were discarded, and males were weighed and then randomly 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, we 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 at how their behavior and ejaculate mass

change with subsequent mating. For each mating, males were weighed to 0.001 mg 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 before mating to control for potential effects of female body weight in 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. We 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), this measure may reflect behavioral conflict between sexes (see discussion). *Mating duration* was recorded as the time from when mating started to when it ended. If a pair did not start mating within 10 min, 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, and final sample size for each treatment is: dry juvenile/dry adult=36; dry juvenile/wet adult=21; wet juvenile/dry adult=23; wet juvenile/wet adult=40). 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 min after the first. After the last mating (second mating on the third day), males were placed in their own vial and monitored daily to determine their lifespan.

Statistical analysis

To test how bean moisture during development and water availability during adulthood affect male mating traits, data were analyzed 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 we checked model residuals to ensure they met the assumptions of normality and homoscedasticity. For models that included interaction terms, we first ran a full model (i.e. with the interaction terms). If the interactions were nonsignificant, they were removed from the model to allow interpretation of lower level interactions and main effects (Engqvist 2005). If interactions were significant, we conducted post hoc pairwise comparisons using 'lsmeans' function in 'emmeans' package (Searle et al. 1980) to determine which treatments differed. For all models, the 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 online supplement (Tables A1 – A20). Figures were plotted using 'ggplot2' (Wickham 2016). Further model details are provided below.

Body weight

To explore the effect of bean moisture content on male emergence weight, we used a linear mixed model (LMM) in which we 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 during adulthood we 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). The change in body weight prior to mating was analyzed using a LMM including juvenile and adult water treatment (wet or dry), as well as their interaction as fixed effects, male body weight at emergence as a covariate and mother ID as a random effect. The change in weight between mating days was analyzed in the same way except that this model additionally included the mating day as a fixed effect and male ID was included as a random effect, instead of mother ID, to account for multiple measures per male. 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 was included in the model.

Mating traits

To evaluate the effect of juvenile and adult water treatment on male ability to invest in mating, we 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 juvenile and adult water treatment, as well as their interaction as fixed effects. We 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 we also included the mating day as well as its two- and three-way interactions with juvenile and adult water treatment in the model. Female body weight was removed as different females were used in each mating and male ID as well as mother ID was included as a random effect.

To explore the effect of juvenile and adult water treatment on mating behavior, we looked at three aspects of mating behavior (i.e. latency to kicking, kicking duration and mating duration) using LMMs. All models included the juvenile water treatment, adult water treatment, mating day and mating order on each day as fixed effects. In addition, all two- and three-way interactions between juvenile water treatment, adult water treatment and mating day or mating order were included and removed (or not) as described above. Male weight at emergence and female body weight before mating were both included as covariates. In addition, mother and mating ID were included as random effects. 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. In addition to this analysis of mating behavior across all matings, we also analysed the effects of juvenile

and adult water treatment on just the first mating. This allowed us to see potential effects more clearly at this stage.

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). We included juvenile water treatment, adult water treatment and male body weight at emergence as fixed effects, and mother ID as a random effect.

Results

Body weight

The water treatment experienced during development did not affect male body weight at emergence (mean \pm SE = 3.863 ± 0.030 mg, $\chi^2 = 0.003$, Df = 1, $p = 0.959$, Table A1). Males’ change in body weight prior to mating was not affected by the interaction between juvenile and adult water treatment ($\chi^2 = 1.285$, Df = 1, $p = 0.257$, Table A2). However, both wet juvenile environments and wet adult environments caused greater weight loss before mating (juvenile: $\chi^2 = 4.405$, Df = 1, $p = 0.036$; adult: $\chi^2 = 25.842$, Df = 1, $p < 0.001$, Fig. 1a, Table A2), and males that were larger at emergence lost more weight ($\chi^2 = 138.428$, Df = 1, $p < 0.001$, Table A2).

The change in male weight between mating days was affected by an interaction between juvenile and adult water treatments ($\chi^2 = 4.933$, Df = 1, $p = 0.026$, Table A3). However, post hoc tests revealed that the dominant pattern in the data is driven by male access to water during adulthood (Fig. 1b, Table A4). Furthermore, larger males lost more weight between matings ($\chi^2 = 5.705$, Df = 1, $p = 0.017$, Table A3) and males lost less weight between the second and third mating day than they did between the first and second mating day ($\chi^2 = 29.484$, Df = 1, $p < 0.001$, Table A3).

Ejaculate traits

Neither juvenile water treatment, nor the interaction between juvenile and adult water treatment affected male ejaculate weight in their first mating (juvenile: $\chi^2 = 0.635$, Df = 1, $p = 0.426$, juvenile*adult interaction: $\chi^2 = 0.009$, Df = 1, $p = 0.926$, Table A5). 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. 2, Table A5), and males who were larger at emergence had larger ejaculates in their first mating ($\chi^2 = 9.613$, Df = 1, $p = 0.002$, Table A5).

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 $p > 0.089$, Table A6), nor was it affected by juvenile or adult water treatments (juvenile: $\chi^2 = 0.084$, Df = 1, $p = 0.771$; adult: $\chi^2 = 0.068$, Df = 1, $p = 0.794$, Table A6). Ejaculate replenishment was also not affected by male body weight at emergence ($\chi^2 = 0.701$, Df = 1, $p = 0.402$, Table A6). However, the difference in ejaculate weight between the first and sec-

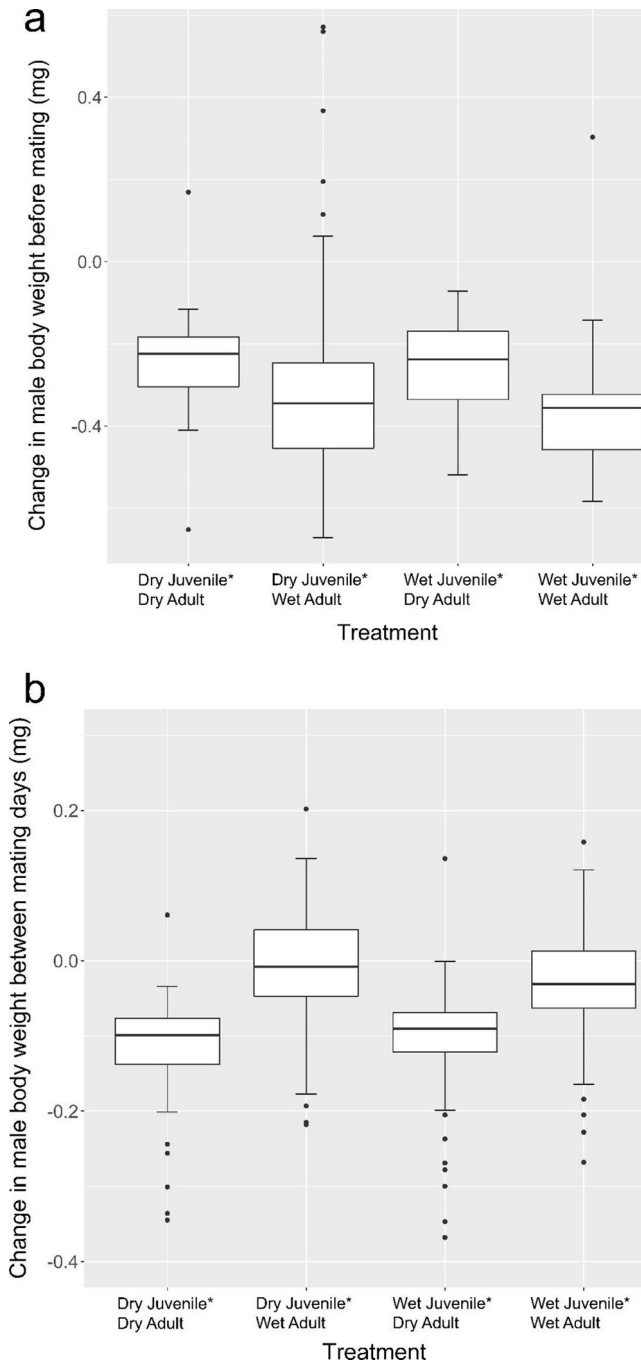
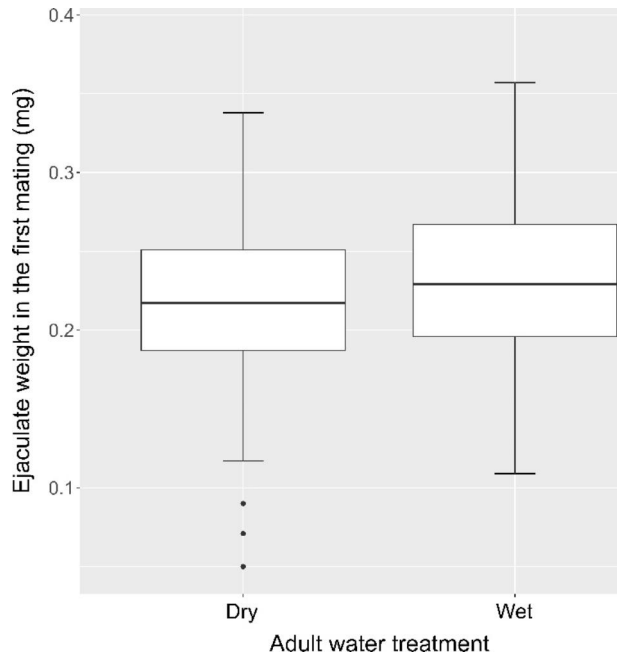


Fig. 1 The effect of male juvenile and adult water treatment on: (a) change in male body weight before mating (male body weight at emergence – male body weight before the first mating) and (b) change in male body weight between mating days (male body weight before the first mating of the day – male body weight after the second mating of the previous day). The box plots show the median and range of dispersion (lower and upper quartiles, and outliers)

Fig. 2 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). The box plots show the median and range of dispersion (lower and upper quartiles, and outliers)



ond day of mating was greater than between the second and third day ($\chi^2=267.236$, Df=1, $p<0.001$, Table A6).

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 A7). There was also no effect of the juvenile water treatment ($\chi^2=1.611$, Df=1, $p=0.204$, Table A7), adult water treatment ($\chi^2=3.244$, Df=1, $p=0.072$, Table A7), or male body weight at emergence ($\chi^2=0.368$, Df=1, $p=0.544$, Table A7). However, there was a greater decrease in ejaculate weight on the first mating day (Table A9).

Mating behavior

When looking at mating behavior during the first mating, mating duration was not affected by the interaction between juvenile and adult water treatment ($\chi^2=2.689$, Df=1, $p=0.101$, Table A10) or the adult water treatment ($\chi^2=0.018$, Df=1, $p=0.893$, Table A10). However, males reared in wet beans had shorter matings than those reared in dry beans ($\chi^2=7.158$, Df=1, $p=0.007$, Fig. 3a, Table A10). Also, larger males had shorter mating durations than smaller males ($\chi^2=3.995$, Df=1, $p=0.046$, Table A10), but female body weight had no effect ($\chi^2=1.493$, Df=1, $p=0.222$, Table A10).

Kicking latency, in the first mating, on the other hand, was affected by the interaction between juvenile and adult water treatment ($\chi^2=8.044$, Df=1, $p=0.005$, Table A11). Males reared in dry beans, without access to water during adulthood were kicked later than males reared in dry beans, *with* access to water during adulthood (t ratio=3.115, $p=0.011$, Fig. 3b, Table A12) as well as those reared in *wet* beans, without access to water (t ratio=3.395, $p=0.005$, Fig. 3b, Table A12). Finally, matings involving males with access to water as

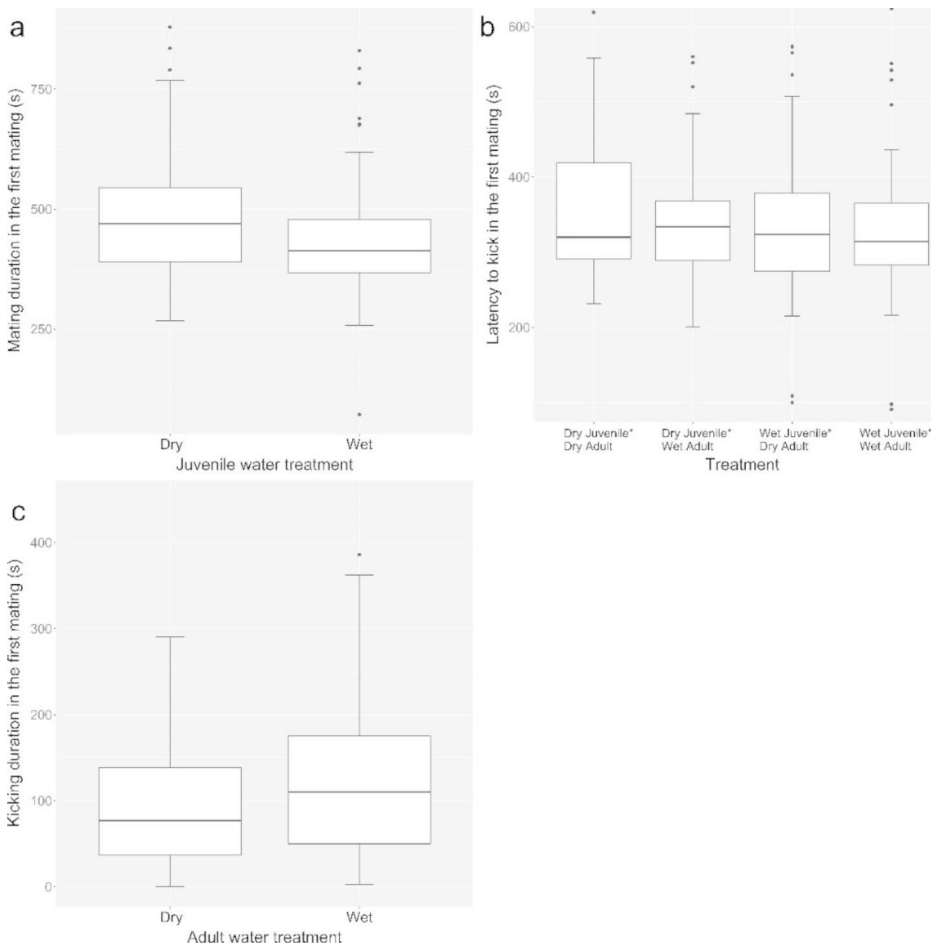


Fig. 3 Effect of juvenile water treatment on the first mating: (a) the effect of male juvenile water treatment on mating duration; (b) the effect of male juvenile and adult water treatment interaction on kicking latency and (c) the effect of male adult water treatment on kicking duration. The box plots show the median and range of dispersion (lower and upper quartiles, and outliers)

adults had longer kicking duration ($\chi^2=6.384$, $Df=1$, $p=0.012$, others: $p>0.133$, Fig. 3c, Table A13).

When analyzing mating duration across the entire experiment, there were no two or three-way interactions (all $p>0.065$, Table A14). Further, neither juvenile nor adult water treatment had any overall effects on mating duration (juvenile: $\chi^2=0.284$, $Df=1$, $p=0.593$; adult: $\chi^2=0.098$, $Df=1$, $p=0.754$, Table A14). However, mating duration tended to increase with subsequent matings both within and across days. Specifically, mating duration was longer on mating days two and three than it was on mating day one ($\chi^2=119.591$, $Df=2$, $p<0.001$, Table A15), and mating duration was longer for the second mating than the first mating of each mating day ($\chi^2=81.546$, $Df=1$, $p<0.001$, Table A14). Finally, although larger males had longer matings ($\chi^2=18.755$, $Df=1$, $p<0.001$, Table A14), larger females had shorter matings ($\chi^2=4.671$, $Df=1$, $p=0.031$, Table A14).

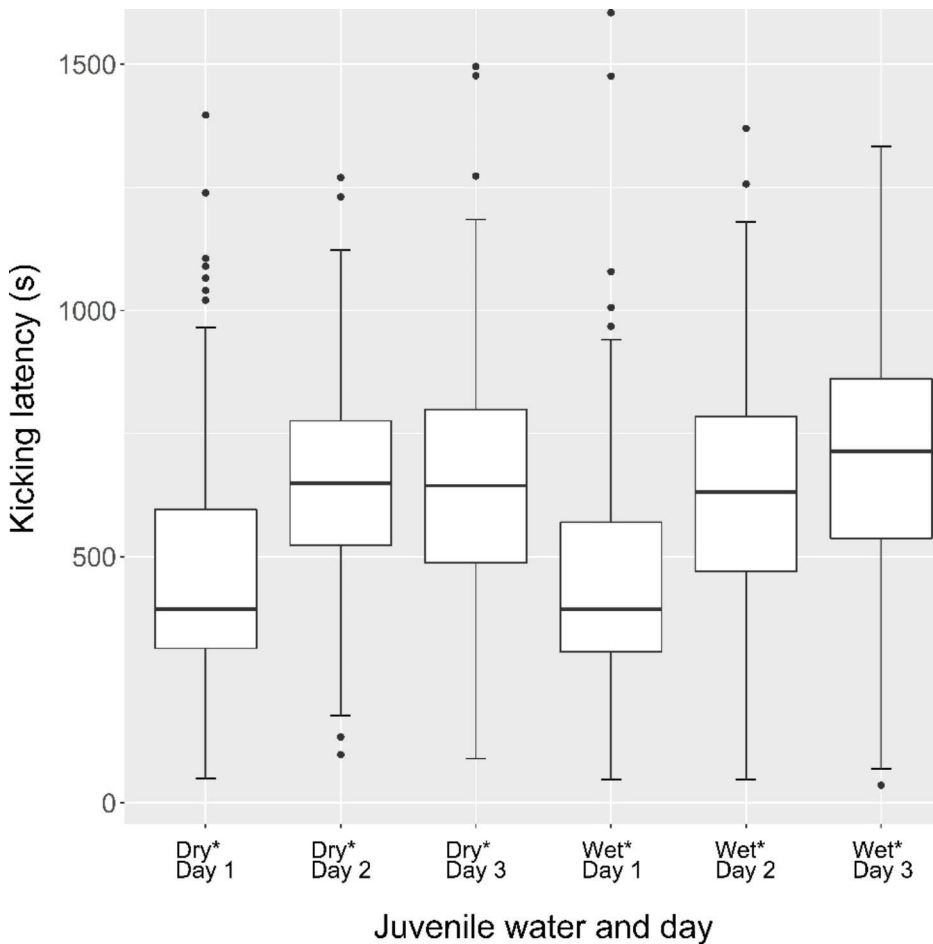


Fig. 4 Effect of juvenile water treatment and mating day on the kicking latency on each mating day. Dry: dry juvenile water treatment; Wet: wet juvenile water treatment. Day 1: the first mating day; Day 2: the second mating day; Day 3: the third mating day. The box plots show the median and range of dispersion (lower and upper quartiles, and outliers)

Our analysis of kicking latency, across the entire experiment, also showed no significant two or three-way interactions (all $p > 0.078$, Table A16), apart from a significant interaction between juvenile water treatment and mating day ($\chi^2 = 7.694$, $Df = 2$, $p = 0.021$, Table A16). This interaction reflects that for males reared in dry beans kicking latency initially increases from day one to day two and then plateaus (day 1 vs. day 2: t ratio = -7.141 , $p < 0.001$; day 2 vs. day 3: t ratio = 0.170 , $p = 1.000$, Fig. 4, Table A17), whereas for males reared in wet beans kicking latency increase across all three days (day 1 vs. day 2: t ratio = -7.405 , $p < 0.001$; day 2 vs. day 3: t ratio = -3.153 , $p = 0.021$, Fig. 4, Table A17). Further, as for mating duration, adult water treatment had no overall effect on kicking latency ($\chi^2 = 0.621$, $Df = 1$, $p = 0.431$, Table A16), but there were significant effects of mating order within each day as well as male and female size. Males were kicked later during the second mating of each day ($\chi^2 = 120.766$, $Df = 1$, $p < 0.001$, Table A16), larger males were kicked sooner than

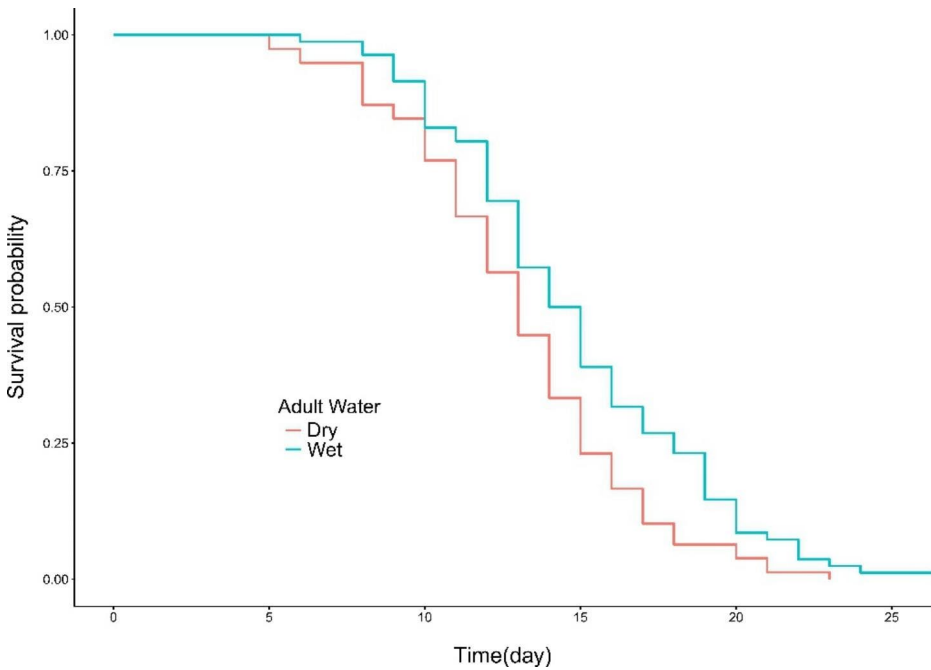


Fig. 5 Kaplan–Meier survival plots for males maintained in different adult water treatments

smaller males ($\chi^2 = 11.081$, $Df = 1$, $p < 0.001$, Table A16) and larger females started kicking later than smaller females ($\chi^2 = 10.581$, $Df = 1$, $p = 0.001$, Table A16).

For kicking duration, none of two or three-way interactions had a significant effect (all $p > 0.068$, Table A19), and there were no significant main effects (all $p > 0.050$, Table A19) apart from effects of mating order and male size. These showed that, males were kicked for a shorter duration in the second mating of each mating day ($\chi^2 = 7.129$, $Df = 1$, $p = 0.008$, Table A19) and that larger males generally experienced longer kicking durations than smaller males ($\chi^2 = 5.056$, $Df = 1$, $p = 0.025$, Table A19).

Lifespan

Male lifespan was not affected by an interaction between juvenile and adult water treatment, nor the juvenile water environment on its own (mean \pm SE = 13.987 ± 0.320 day, Table A20). However, males with access to water during adulthood lived significantly longer than those without ($z = -3.118$, $p = 0.002$, Fig. 5, Table A20), as did males that were larger at emergence ($z = -4.214$, $p < 0.001$, Table A20).

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 as well as the economics of mating and reproduction (Edvardsson 2007; Iglesias-

Carrasco et al. 2018; Ursprung et al. 2009; Vincent et al. 2020; Zhang and Head 2021). 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; Zhang and Head 2021), albeit in different ways. Greater moisture content of beans during development increases male ejaculate size but reduces female fecundity (Zhang and Head 2021). 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, we 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 seed beetles are able to compensate for dry developmental environments if they have greater access to water as adults. Our results show that water acquired during different life-stages is allocated towards different traits. For instance, males developing in wet versus dry environments showed differences in mating behavior, particularly in later adult life, whereas males with or without access to water as adults showed differences in their ejaculate weight during their first mating. Access to water as adults also had longer lasting effects – influencing both weight loss with age, and lifespan. Furthermore, we 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 ways.

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 (Zhang and Head 2021), we 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. We 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 beetles 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), our results showed the ejaculate replenishment after the second mating day is around zero and was not promoted by access to water, which suggests that access to water is unlikely to increase male fitness.

Mating behavior in seed beetles is thought to be driven by 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 (Crudginton and Siva-Jothy 2000; Edvardsson and Canal 2006; Van Lieshout et al. 2014) possibly because longer matings cause more severe genital tract damage (Crudginton 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 has been questioned (Dougherty and Sim-

mons 2017; McNamara et al. 2020). Here, we found that mating behavior during the first mating was affected by both juvenile and adult water treatments 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 indicate that males reared in dry environments take longer to transfer their ejaculate and/or males with access to water as adults can resist female kicking for a longer time - if, for instance, water acquisition during adulthood increases male body condition. Another possibility is that due to the short life cycle of seed beetles, and the fact that stored grain environments likely present homogenous habitats, male seed beetles may be more likely to encounter females that have experienced the same environment as themselves. If this is the case then differences in male behavior may reflect adaptations to expected environmentally induced changes in female behavior (Edvardsson 2007; Fox and Moya-Laraño 2009; Ursprung et al. 2009). Either way, how such results relate to male fitness is still unclear, as studies relating male mating behavior to reproductive success often have conflicting results (e.g. Edvardsson and Canal 2006; Vincent et al. 2020). Thus, it is difficult to say how selection would act on such differences.

When looking at effects on behavior over multiple matings, we found the only behavior to be affected by access to water was the latency to kick. 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. As all females used in mating trials were virgin stock females of a similar age, these effects are likely due to differences in the way that the rearing environment affects male reproductive investment over time, or aging. Given we 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 Koti-aho 2005), which stimulate oviposition and delay remating by their partners (Edvardsson 2007; Harano 2012; Moya-Laraño and Fox 2006; Takashi et al., 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 contrasts with previous studies which showed wet developmental environments increase male ejaculate mass (Zhang and Head 2021), 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 previous experiments (Zhang and Head 2021), where developmental water treatment did influence behavior. Thus, this is unlikely to be the cause. When it comes to differences between studies in the effect of adult water, however, we used the same method as previous research (Vincent et al. 2020), so the reason for the differences in results between the studies is unclear. Research to determine the generality of the effects of adult and juvenile water supplementation is necessary before we can identify reasons that the results between these studies differ. We also suggest that further studies look at ejaculate composition, as this may give a more comprehensive view of how ejaculates are affected by differences in water availability. For instance, it may be possible that water affects the acquisition of other nutrients from beans (Kibar and Kibar 2019).

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 (between mating day one and two) was larger than in later life (between mating day two and three) implies a decrease in male ejaculate replenishment capacity. Whether this decrease is due to aging or depletion of stored nutrients, however, is still unclear.

We 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 be more likely to continue later into life. Our results partly support this expectation. Both larval and adult water treatment affected 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, we 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.

Acknowledgements We thank Michael Jennions for thoughtful discussions, and Jiayu Zhang and Zhenyan Luo for assistance collecting data. We also thank Australian Research Council for funding this work.

Authors' contributions ZZ and MH conceived and designed research. ZZ conducted experiments, analyzed data and wrote the manuscript. MH supervised and directed all the work. All authors read and approved the manuscript.

Funding This work was supported by Australian Research Council (M.L.H., Future Fellowship FT160100149). Open Access funding enabled and organized by CAUL and its Member Institutions

Data Availability All material will be made available on a repository.

Code Availability All codes will be made available upon request.

Declarations

Conflicts of interest/Competing interests The authors declare that they have no conflict of interest.

Ethics approval All experimental procedures carried out complied with Australian laws.

Consent to participate Not applicable.

Consent for publication All authors consent to this research being published.

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References

- Aguila JR, Hoshizaki DK, Gibbs AG (2013) Contribution of larval nutrition to adult reproduction in *Drosophila melanogaster*. *J Exp Biol* 216:399–406
- Boggs CL (2009) Understanding insect life histories and senescence through a resource allocation lens. *Funct Ecol* 23:27–37
- Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Machler M, Bolker BM (2017) GlimmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J* 9:378–400
- Chown SL, Sørensen JG, Terblanche JS (2011) Water loss in insects: an environmental change perspective. *J Insect Physiol* 57:1070–1084
- Cooper EB, Kruuk LE (2018) Ageing with a silver-spoon: a meta-analysis of the effect of developmental environment on senescence. *Evol Lett* 2:460–471
- Cope JM, Fox CW (2003) Oviposition decisions in the seed beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae): effects of seed size on superparasitism. *J Stored Prod Res* 39:355–365
- Crudgington HS, Siva-Jothy MT (2000) Genital damage, kicking and early death. *Nature* 407:855–856
- Danks HV (2000) Dehydration in dormant insects. *J Insect Physiol* 46:837–852
- Dougherty LR, Simmons LW (2017) X-ray micro-CT scanning reveals temporal separation of male harm and female kicking during traumatic mating in seed beetles. *Proceedings of the Royal Society B: Biological Sciences* 284, 20170550
- Edvardsson M (2007) Female *Callosobruchus maculatus* mate when they are thirsty: resource-rich ejaculates as mating effort in a beetle. *Anim Behav* 74:183–188
- Edvardsson M, Canal D (2006) The effects of copulation duration in the bruchid beetle *Callosobruchus maculatus*. *Behav Ecol* 17:430–434
- Engels S, Sauer KP (2007) Energy beyond the pupal stage: larval nutrition and its long-time consequences for male mating performance in a scorpionfly. *J Insect Physiol* 53:633–638
- Engqvist L (2005) The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Anim Behav* 70:967–971
- Fischer K, O'Brien DM, Boggs CL (2004) Allocation of larval and adult resources to reproduction in a fruit-feeding butterfly. *Funct Ecol* 18:656–663
- Fox CW (1993) Multiple mating, lifetime fecundity and female mortality of the bruchid beetle, *Callosobruchus maculatus* (Coleoptera, Bruchidae). *Funct Ecol* 7:203–208
- Fox CW (1994) The influence of egg size on offspring performance in the seed beetle, *Callosobruchus maculatus*. *Oikos* 71:321
- Fox CW, Dublin L, Pollitt SJ (2003) Gender differences in lifespan and mortality rates in two seed beetle species. *Funct Ecol* 17:619–626
- Fox CW, Hickman DL, Raleigh EL, Mousseau TA (1995) Paternal investment in a seed beetle (Coleoptera: Bruchidae): influence of male size, age, and mating history. *Ann Entomol Soc Am* 88:100–103
- Fox CW, Moya-Laraño J (2009) Diet affects female mating behaviour in a seed-feeding beetle. *Physiol Entomol* 34:370–378
- Fox J, Weisberg S (2018) An R companion to applied regression. Sage publications
- Harano T (2012) Water availability affects female remating in the seed beetle, *Callosobruchus chinensis*. *Ethology* 118:925–931
- Iglesias-Carrasco M, Bilgin G, Jennions MD, Head ML (2018) The fitness cost to females of exposure to males does not depend on water availability in seed beetles. *Anim Behav* 142:8
- Johnstone RA, Keller L (2000) How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. *Am Nat* 156:368–377
- Jönsson KI (1997) Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos* 78:57
- Kibar H, Kibar B (2019) Changes in some nutritional, bioactive and morpho-physiological properties of common bean depending on cold storage and seed moisture contents. *J Stored Prod Res* 84:101531
- McNamara KB, Sloan NS, Kershaw SE, Van Lieshout E, Simmons LW (2020) Males evolve to be more harmful under increased sexual conflict intensity in a seed beetle. *Behav Ecol* 31:591–597

- Messina FJ, Fry JD (2003) Environment-dependent reversal of a life history trade-off in the seed beetle *Callosobruchus maculatus*. *J Evol Biol* 16:501–509
- Moya-Laraño J, Fox CW (2006) Ejaculate size, second male size, and moderate polyandry increase female fecundity in a seed beetle. *Behav Ecol* 17:940–946
- Nestel D, Papadopoulos NT, Pascacio-Villafán C, Righini N, Altuzar-Molina AR, Aluja M (2016) Resource allocation and compensation during development in holometabolous insects. *J Insect Physiol* 95:78–88
- Ofuya TI (1995) Multiple mating and its consequences in males of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J Stored Prod Res* 31:71–75
- Paukku S, Kotiaho JS (2005) Cost of reproduction in *Callosobruchus maculatus*: effects of mating on male longevity and the effect of male mating status on female longevity. *J Insect Physiol* 51:1220–1226
- Poças GM, Crosbie AE, Mirth CK (2020) When does diet matter? The roles of larval and adult nutrition in regulating adult size traits in *Drosophila melanogaster*. *Journal of Insect Physiology*, 104051
- R-Development-Core-Team (2013) R: A language and environment for statistical computing
- Reinhardt K, Naylor R, Siva-Jothy MT (2011) Male mating rate is constrained by seminal fluid availability in bedbugs, *Cimex lectularius*. *PLoS ONE* 6, e22082
- Ryne C, Nilsson PA, Siva-Jothy MT (2004) Dietary glycerol and adult access to water: effects on fecundity and longevity in the almond moth. *J Insect Physiol* 50:429–434
- Sanghvi K, Zajitschek F, Iglesias-Carrasco M, Head ML (2021) Sex- and trait-specific silver-spoon effects of developmental environments, on ageing. *Evol Ecol* 35:367–385
- Savalli U, Fox C (1999) The effect of male size, age, and mating behavior on sexual selection in the seed beetle *Callosobruchus maculatus*. *Ethol Ecol Evol* 11:49–60
- Savalli UM, Fox CW (1998) Genetic variation in paternal investment in a seed beetle. *Anim Behav* 56:953–961
- Searle SR, Speed FM, Milliken GA (1980) Population marginal means in the linear model: an alternative to least squares means. *Am Stat* 34:216–221
- Simpson SJ, Raubenheimer D (2012) The nature of nutrition: a unifying framework from animal adaptation to human obesity. Princeton university press
- Takashi Yamane JG, Johanna Liljestrand R, Göran, Arnqvist (2015) Male seminal fluid substances affect sperm competition success and female reproductive behavior in a seed beetle. *PLoS ONE* 10
- Therneau TM, Grambsch PM (2000) The cox model, Modeling survival data: extending the Cox model. Springer, pp 39–77
- Ursprung C, den Hollander M, Gwynne DT (2009) Female seed beetles, *Callosobruchus maculatus*, remate for male-supplied water rather than ejaculate nutrition. *Behav Ecol Sociobiol* 63:781–788
- Van Lieshout E, McNamara KB, Simmons LW (2014) Why do female *Callosobruchus maculatus* kick their mates? *PLoS ONE* 9, e95747
- Vincent A, Head ML, Iglesias-Carrasco M (2020) Sexual conflict and the environment: teasing apart effects arising via males and females. *Anim Behav* 162:57–66
- Vrtilek M, Chuard PJ, Iglesias-Carrasco M, Zhang Z, Jennions MD, Head ML (2021) The role of maternal effects on offspring performance in familiar and novel environments. *Heredity*, pp 1–14
- Wickham H (2016) *Ggplot2: elegant graphics for data analysis*. Springer International Publishing
- Wilson CJ, Buzatto BA, Robinson SP, Tomkins JL (2014) Sociosexual environment influences patterns of ejaculate transfer and female kicking in *Callosobruchus maculatus*. 94:37–43
- Wilson CJ, Tomkins JL (2014) Countering counteradaptations: males hijack control of female kicking behavior. *Behav Ecol* 25:470–476
- Zhang Z, Head ML (2021) Does developmental environment affect sexual conflict? An experimental test in the seed beetle. *Behav Ecol* 33:147–155