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Short Title: Condition Dependence in *T. molitor*

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Abstract

Traits under strong directional selection are predicted to be condition dependent, and thus increase in development when an organism acquires more resources. This prediction has been tested for a variety of traits, particularly those under precopulatory sexual selection. However, few studies compare the condition dependence of a variety of phenotypic traits, potentially subject to different selective forces. Here we examine the condition dependence of several important life history traits, including those under both pre- and postcopulatory sexual selection, in the mealworm beetle, *Tenebrio molitor*. We manipulated condition by randomly assigning larvae to one of: high-, medium- or low-quality diets. For males reared on the three diets we measured: a) adult body size and lifespan, b) pheromone attractiveness, c) weight of their ejaculate transferred to females, and d) fecundity of their female mates. Males raised on a high-diet were larger and lived longer than males raised on medium- and low-diets. Although females were significantly attracted to male pheromones, there were no significant differences amongst condition treatments in male attractiveness, nor ejaculate transfer. Furthermore, mates' fecundity was also not affected by male condition. We discuss these results considering previous work on trade-offs and condition dependence of life history traits.

Key words: condition dependence, sexual selection, direct benefits, pheromones, lifespan, *Tenebrio molitor*

Significance Statement

Theory suggests that traits that are under strong selection should be sensitive to the amount of resources an organism has available (i.e. dependent on condition), such that when those resources are plentiful then these traits should increase disproportionately to other traits that are not so important to relative fitness. Much research has been done on the condition dependence of precopulatory traits under strong sexual selection, but not as much on postcopulatory traits. Our research focused on measuring the effect of resources on both pre- and postcopulatory traits in male mealworm beetles as well as their effect on their mates' relative fitness. Interestingly, although we found that important life history traits such as body size and adult lifespan were condition dependent, precopulatory traits such as pheromone attractiveness and postcopulatory traits such as ejaculate transfer were not. Our study highlights the complicated nature of tradeoffs in resource allocation faced by individuals under sexual selection.

Introduction

Sexual selection, or competition for mates, is known to be very strong and is thought to be responsible for some of the most elaborated traits seen in nature (Darwin 1871, Andersson 1994). These so-called secondary sexual traits are predicted to be costly to produce and can thus act as indicators of mate quality (Zahavi 1975; Grafen 1990). Theory suggests that traits under strong sexual selection should be sensitive to the amount of resources an organism has available (i.e. dependent on condition, Rowe and Houle 1996), such that when those resources are plentiful then these sexual traits should increase disproportionately to other traits that are not so important to relative fitness (Cotton et al. 2004). A wide variety of secondary sexual traits are indeed found to be condition dependent across animals, from lateral body colouration in ambush bugs (Punzalan et al. 2008), to calling song in field crickets (Hunt et al. 2004), to male weaponry in beetles (House et al. 2016) (reviewed in Cotton et al. 2004).

In addition to acting on ornaments and weapons like those listed above that function in precopulatory competition for mates, sexual selection operates to select traits that are advantageous after copulation (Parker 1970). For example, sperm competition is thought to select for greater numbers of sperm in male ejaculates, which increases the likelihood of paternity in polyandrous species (reviewed in Birkhead and Møller 1998; Simmons 2001; Wedell et al. 2002; Arnqvist and Rowe 2005). Furthermore, male ejaculates also contain substances in addition to sperm (Eberhard and Cordero 1995), many of which are known to affect female fitness (e.g. Chapman et al. 1995). Female *Prochyliza xanthostoma* flies who ingest the ejaculate of their mate both lay more eggs and lay them sooner than females who are prevented from doing so (Bonduriansky et al. 2005). And the ejaculates of preferred male

Gryllus lineaticeps field crickets have been shown to increase female fecundity (Wagner et al. 2001) and lifespan (Wagner et al. 2001; Wagner and Harper 2003). Male ejaculates are often costly, and so both sperm production and non sperm ejaculate components are predicted to be condition dependent. Condition dependence of male ejaculates has been demonstrated in several taxa. For example, male Trinidadian guppies (*Poecilia reticulata*) in good condition had higher sperm loads than males in poor condition (Pitcher and Evans 2001). And, in the two-spotted ladybird beetle (*Adalia bipunctata*), males in high condition transferred larger ejaculates, but containing fewer sperm than ejaculates transferred by males in low condition (Perry and Rowe 2010). Comparisons among multiple traits in the degree of condition dependency is very useful in making inferences about the importance of those traits to relative fitness (Cotton et al. 2004). Our study examined the condition dependence of a range of phenotypic traits, ranging from those known or likely to be under sexual selection (precopulatory and postcopulatory) to those not known to be sexually selected, in the yellow mealworm beetle, *Tenebrio molitor*.

T. molitor beetles are small (13-17mm long, Rutowski 1982) darkling beetles (Coleoptera, Tenebrionidae) that are pests of stored grain products (Dunkel 1992). Little is known about the natural mating system of *T. molitor*, although females are known to be polyandrous (Drnevich 2003). Males transfer a spermatophore to females which releases sperm 5-10 minutes after copulation has been completed (Gadzama and Happ 1974). Females can store sperm from multiple males for hours and last male (i.e. a female's most recent mate) sperm precedence has been demonstrated (Drnevich et al. 2001a; 2003; Siva-Jothy et al. 1996). Females derive direct benefits from the male ejaculate in the form of increased fecundity (Drnevich et al. 2001b;

Worden and Parker 2001).In response to the risk of sperm competition, male *T. molitor* increase sperm content in the ejaculate when in the presence of a rival male (Gage and Baker 1991). It is also apparent that males compete for mates pre-copulation as well. The presence of a sex pheromone in both sexes of *T. molitor* has been well documented (Valentine 1931; Tschinkel et al. 1967; Happ 1969) and has been shown to communicate both reproductive status (Carazo et al. 2004), immunocompetence (Rantala et al. 2002) and parasite load (Worden et al. 2000). Rantala et al. (2003) have shown that the male pheromone is condition dependent, and females mated to males with preferred pheromones gained direct benefits in the form of increased lifespan (Vainikka et al. 2006).

In this study, we investigated the condition dependence of important life history traits (body size and lifespan) not known to be sexually selected, and both precopulatory (male mating behaviour and pheromone attractiveness) and postcopulatory (mass of ejaculate transfer) sexually selected traits in *T. molitor*. We manipulated the quality of food resources available to larval *T. molitor* and measured the effects on these traits. We predicted that males reared on higher quality diets would: a) mature larger and live longer, b) have more attractive pheromones (e.g. Rantala et al. 2003), and transfer a larger volume of ejaculate (e.g. Perry and Rowe 2010) to females while mating, than males reared on lower quality diets.

Materials and Methods

Study Animals

All *T. molitor* used in this experiment were obtained as larvae from Ward's Natural Science. We housed these larvae in an environmental chamber set at 70% relative humidity, 25° C and a 12hr: 12hr light: dark cycle. This colony of larvae was fed a diet of whole wheat flour (Robin Hood® brand) mixed with Brewer's Yeast (Bulk Barn®) in a 9:1 ratio. Female pupae were sexed (Bhattacharya et al. 1970) and removed from the colony. These females were kept on an identical standard diet and housed as a group to ensure virginity. Upon reaching adulthood, the date was recorded, and virgin female adults were housed separately in plastic containers (AMAC brand, 3.02 cm length x 3.02 cm width x 6.67 cm height). These females would be used in the mating trials with males whose diets we manipulated.

From the initial group of *T. molitor* larvae, we haphazardly selected a total of 300 larvae of varying sizes and unknown instar number to be subjected to condition manipulation (see below). These focal individuals were weighed to the nearest 0.1 mg using an electronic balance (Mettler AE 50) and randomly assigned to one of three diet treatments (see below). We housed these larvae individually in plastic containers (see above) with an excess of food, which was changed every 40 days to avoid degradation from the buildup of waste products. At pupation, the day was noted, weight measured as above, and sex determined by the morphology of the eighth abdominal segment (Bhattacharya et al. 1970). We then placed the pupae back into their individual container with food, a small piece of paper towel as a foothold for newly emerging adults, and a plastic microfuge tube cap (Fisher Scientific, 1.5 mL flat top Micro centrifuge tube) filled with moistened cotton to provide a moist environment for molting adults. During the

course of this experiment, mites were noticed twice in sporadic instances in the colony and in individual containers across all treatments. The effects of these mites are unknown, but because they were present on all treatments any effects should not bias our results. To reduce mite numbers, the food of the colony and individuals was replaced whenever mites were found. To measure the effects of condition manipulation (see below) on adult survival, all individually-housed beetles were checked every two days and the date on which each was found dead was used to calculate lifespan (date of death – date of adult emergence).

Condition Manipulation

The 300 experimental larvae were each assigned randomly to one of three diet manipulation treatments (as recommended by Cotton et al. 2004) differing in percentage of digestible food: high (99%), medium (75%) and low (50%). The digestible portion was the same food given to the colony (9:1 ratio of whole wheat flour and brewer's yeast) and the indigestible component was cellulose powder (KEYCEL 200CT, Canada Colours and Chemicals Ltd.). All individuals were provided their assigned diet ad libitum for their entire lives.

Measuring Male Attractiveness

We placed individual adult focal males on filter paper (42.5 mm diameter) 6-10 days post-eclosion, within a small Petri-dish (Falcon, 50 mm diameter x 9 mm height). This method has been used in many different systems to elicit behavioural responses to pheromonal cues laid down by focal individuals on the filter paper (e.g. Otte and Cade 1976; Tachon et al. 1999; Worden and Parker 2001; Rantala et al. 2002; 2003). A filter paper was also placed in a

158 container of the focal male's diet as a control for experimental diet cues that may be present on
159 focal males. After 24 hours, the male pheromone-scented and control filter papers were
160 removed, cut in half, and placed in a large glass Petri dish (Pyrex, 100 mm diameter x 15 mm
161 height) on opposite sides of the dish with straight edges facing inwards. We then placed a virgin
162 female (six to eight days post-eclosion, collected as pupae from the larger colony) in the Petri-
163 dish under a small plastic cap (35 mm diameter, 10 mm height) to restrict her movement (assay
164 modified from Rantala et al. 2002; 2003; Vainikka 2006; see also Worden and Parker 2001).
165 After a ten-minute acclimatization period, the cap was removed and during the next ten
166 minutes we recorded the filter paper that the female's mouthparts touched first and the time
167 the female spent on each filter. Time on a filter paper was recorded as long as the female's
168 mouthparts were in contact with the filter paper. An attractiveness score was calculated by
169 subtracting the time spent on the control filter paper from the time spent on the male
170 pheromone filter paper. Females used in the attractiveness trials were then placed back into
171 the stock colony and not used again.
172
173 ***Measuring Mating Behaviour and Ejaculate Transfer***
174 We paired each focal male with an unmanipulated virgin female (six to eight days post-
175 adult eclosion) and weighed both to the nearest 0.01 mg using an electronic balance (Mettler
176 AE 240). The focal male and the virgin female were then placed into a glass Petri-dish, each
177 under a small plastic cap (similar as those used in attractiveness trials) for a five-minute
178 acclimatization period after which the caps were removed and male and female allowed to
179 mate. During each mating trial we recorded: i) latency to first contact, ii) total number of

contacts, iii) latency to copulate and iv) copulation duration. Contacts were recorded if antennae from one individual came into contact anywhere on the other beetle's body. Copulation duration was recorded starting when the male inserted his aedeagus and ending when he removed it from the female. If mating did not occur after ten minutes, the male was recorded as having not mated (this occurred in 4 trials).

Within approximately one minute of the end of copulation, noted by the removal of the male aedeagus from the female, we weighed both individuals to the nearest 0.01 mg to determine the change in weight during mating.

Fecundity Consequences

After mating, we housed females individually in containers (3.02 cm x 3.02 cm x 6.67 cm, AMAC Plastic Products) with stock food (9:1 whole wheat flour: brewer's yeast). Each female was given a small piece of paper towel, to provide a substrate for gripping. Four days after mating, the period during which singly mated females lay most of their eggs (Drnevich et al. 2001b, Worden and Parker 2001), the female food was sifted and the number of eggs was counted.

Statistical Analyses

We assessed deviations of our data from the assumptions of parametric statistics using the Kolmogorov-Smirnov test and boxplots. Where these assumptions of normality and homoscedasticity were violated and it was not possible to correct using transformations, we used nonparametric and randomization tests. All statistical tests were performed with SPSS

(version 23) for windows at an alpha of 0.05. For logistical reasons related to maintaining individuals on their diet treatments, blinded methods were not used in data collection. However, in practice the treatment group of each individual was not apparent to the observer because identification labels were not visible during behaviour assays and the order in which individuals were observed was randomized.

Results

Larval Survival and Body Size

When initially assigned to the different diets, there were no significant differences in larval weights across treatment groups (mean±SE: low = 87.5±2.5 mg, medium = 86.0±2.3 mg, high = 88.5±2.1 mg, ANOVA: $F_{2, 297} = 0.289$, $p = 0.749$). Diet treatment significantly affected larval survival to adulthood (Chi-square = 18.487, df = 2, $p < 0.001$). Larvae raised on the low-quality diet had lower survival to adulthood (64%) than those raised on high- (89%) and medium- (80%) quality diets (low vs high, Chi-square = 17.383, df = 1, $p < 0.001$; low vs medium, Chi-square = 6.348, df = 1, $p = 0.012$), but high-and medium-quality diets did not differ in larval survival (Chi-square = 3.092, df = 1, $p = 0.079$). The sex ratio at adulthood differed among diet treatments (20 males: 44 females, 41 males: 39 females, and 48 males: 41 females in the low-, medium-and high-quality diets respectively; Chi-square = 8.671, df = 2, N = 233, $p = 0.013$). Given that it is impossible to sex larval *T. molitor* we cannot know the sex ratio in each treatment at the start of the experiment, however, the treatment differences in sex ratio at adulthood suggests that the low-quality diet was harder on males than females.

The diets did not affect male and female pupation weight differently (GLM: Sex * Diet, $F_{2, 227} = 1.525$, $p = 0.220$), therefore the Sex * Diet interaction term was removed from the model. In this reduced model, Diet significantly affected pupation weight (GLM: $F_{2, 229} = 16.090$, $p < 0.001$, Fig. 1), and there was no main effect of Sex on pupation weight (GLM: $F_{2, 229} = 0.095$, $p = 0.758$, Fig. 1). Individuals reared on the high-quality diet were significantly heavier than those raised on either the medium-or low-quality diets (Tukey HSD: high vs medium, $p = 0.046$; high vs low, $p < 0.001$) and medium-diet beetles were heavier than low-diet beetles (Tukey HSD: $p = 0.002$, Fig. 1).

Male Attractiveness and Mating Behaviour

A further 14 males had to be excluded from analysis because they either died before they were scheduled to be mated or we failed to collect at least one response variable, leaving 16, 38 and 41 in the low, medium-and high-quality diet treatments.

We tested whether females were significantly attracted to the male pheromone filter paper over the control filter paper. To do this we calculated 95% confidence limits for each of the mean attractiveness scores using 10000 bootstrap samples (i.e. the 2.5 and 97.5 percentiles of these 10000 bootstrapped means represent the upper and lower confidence limits). Since the attractiveness score is calculated as the difference between the time spent on the male pheromone filter paper and the time spent on the control paper, if the confidence limits excluded zero (no preference) then females significantly preferred either the male pheromones (positive values) or the control (negative values). This analysis showed that female *T. molitor* preferred the male pheromone filter paper over the control filter paper for all three treatments

(Low: Attractiveness Score = 36.6s, 95% CI = 19.8-53.2s; Medium: Attractiveness Score = 60.4s, 95% CI = 45.9-75.9s; High: Attractiveness Score = 46.0s, 95% CI = 29.6-62.7s).

Kolmogorov-Smirnov tests of normality for male attractiveness, time to first contact, number of copulation attempts, latency to copulate, and copulation duration revealed statistically significant departures from normality that were not corrected by transformation. We therefore conducted separate Kruskal-Wallis tests for each dependent variable and adjusted the experiment-wise Type-I error rate with the sequential Bonferroni method (Holm 1979). Although low-diet males had the lowest attractiveness score, longest latency to copulate and shortest copulation duration, there were no statistically significant differences among the treatments in any of the dependent variables, and measured effect sizes were small (Table 1).

Ejaculate Transfer

Diet treatment had no statistically significant effect on either female or male weight change during mating (Multivariate GLM: Pillai's Trace = 0.092, $F_{4, 184} = 2.212$, $p = 0.069$). And although females tended to lose less weight during mating as diet quality of their mates increased, and males tended to lose more weight during mating as their diet quality increased, male and female weight change was not negatively correlated as predicted if our measure of weight accurately reflected the transfer of ejaculate from male to female during copulation (Spearman's rho: all $r > -0.058$, all $p > 0.261$; correlations calculated both across and within diet treatments).

Adult Lifespan

Two additional females were removed from the analysis due to missing survivorship data, leaving sample sizes of 16M:43F, 38M:38F and 41M:41F on the low, medium- and high-quality diets respectively. High-diet males survived longer than low- and medium-diet males (mean adult male lifespan±SE: low = 53.1±3.5 days, medium = 58.8±2.4 days, high = 68.5±2.8 days; Kaplan-Meier Survival Analysis: high vs low: Log Rank = 13.64, $p < 0.001$; high vs medium: Log Rank = 8.53, $p = 0.003$), although medium- and low-diet males did not differ in their survivorship (medium vs low: Log Rank = 2.20, $p = 0.138$). Females reared on high-and medium-quality diets lived significantly longer than females reared on the low-quality diet (mean adult female lifespan±SE: low = 47.5±2.3 days, medium = 66.6±3.0 days, high = 70.7±3.1 days; Kaplan-Meier Survival Analysis: high vs low: Log Rank = 28.36, $p < 0.001$; medium vs low: Log Rank = 19.15, $p < 0.001$), but did not differ from each other (high vs medium: Log Rank = 0.89, $p = 0.345$).

Fecundity of Female Mates

After controlling for both female and male size, diet treatment in males had no statistically significant effect on the fecundity of females mated to them (GLM: $F_{2, 90} = 2.455$, $p = 0.092$). Heavier females did have higher fecundity ($F_{1, 90} = 5.006$, $p = 0.028$), but male size did not affect their mates' fecundity ($F_{1, 90} = 0.278$, $p = 0.599$).

There were no statistically significant correlations between mated female fecundity and any measure of male attractiveness for either all treatments pooled (all |Spearman rho| < 0.126, $p > 0.224$) or within each treatment (all |Spearman rho| < 0.271, $p > 0.100$).

Discussion

We manipulated condition (the pool of resources available to allocate towards competing life history traits, Rowe and Houle 1996) by raising larval *T. molitor* on low-, medium- and high-quality diets. Diet quality significantly affected larval survival, adult body size and adult survival, with low-condition individuals surviving less well, attaining smaller body sizes and living shorter lives than individuals in medium- and high-condition, confirming the condition dependence of these important life history traits. Although male *T. molitor* pheromones from all treatments were attractive to females, we did not detect an effect of condition on measures of male attractiveness, including the attractiveness of his pheromones (Table 1). Finally, we were unable to detect any effect of our condition manipulation on either the ejaculate transferred by males or on their mates' fitness (survival and fecundity).

Body size is an important determinant of male mating success in many mating systems (reviewed in Andersson 1994), although little is known about its importance to male *T. molitor* beetles. We found that body size in both male and female mealworm beetles was condition dependent (Fig. 1), which would be predicted if this trait is under strong sexual selection in males (Cotton et al. 2004) and probably fecundity selection in females. Larger body size is often correlated with greater success in direct male-male physical combat (e.g. Hack 1997, Hooper et al. 2016) and larger body size is thought to be advantageous in buffering the energetic demands of mating displays, or endurance rivalry (Andersson 1994). Most studies on the mating system of *T. molitor* have focused on the attractiveness of male pheromonal cues (see below) with no

311 published work on the extent of direct male-male competition, whether that be through direct
312 physical combat, scramble competition or displays of endurance, despite the fact that *T. molitor*
313 is a common model organism used in both teaching (e.g. Rutowski 1982) and research. Here we
314 call for more research on the basic natural history of *T. molitor* mating, as this would both
315 advance our understanding of the ecology of mating systems and potentially prove useful in
316 controlling pests of stored products.
317 Lifespan is most often positively correlated with male secondary sexual traits (Jennions et
318 al. 2001), despite Darwin's original suggestion that the most vigorously displaying (and
319 therefore most successful) males would suffer mortality costs (Darwin 1871). The relationship
320 between condition and lifespan is therefore of interest, with some studies finding positive
321 relationships between condition and male lifespan (e.g. Kotiaho 2000, Pike et al. 2007, Judge et
322 al. 2008) and some finding negative relationships (e.g. Hunt et al. 2004, Hooper et al. 2016). In
323 our study, we found that as experimentally manipulated condition increased, so too did male
324 lifespan. These results suggest that a longer adult life is under strong sexual selection in *T.*
325 *molitor*, as it is in other species such as field crickets (e.g. Zuk 1987) and anurans (e.g. Murphy
326 1994). However, without more detailed knowledge of the breeding phenology of *T. molitor*, our
327 results are difficult to interpret.
328 Previously, researchers have shown that, when subject to a severe starvation regime as
329 an adult, the attractiveness of male *T. molitor* pheromones declined with reduced adult
330 condition (Rantala et al. 2003). In contrast, our condition manipulation involved diet quality
331 rather than quantity, occurred during the larval period before adult body size is fixed, and had
332 no detectable effect on male pheromones, which were consistently attractive to females across

condition treatments. These contrasting results suggest that male pheromones indicate current condition rather than past condition. In our experiment, when faced with fewer larval resources, male *T. molitor* may have traded off adult body size and survival in favour of maintaining their pheromone attractiveness. Similar findings occurred in studies into the effects of adult and juvenile condition on sexually selected traits in the field cricket *Gryllus campestris* (Holzer et al. 2003, Scheuber et al. 2003a,b, 2004). Adult condition affected chirp rate, a component of male calling effort known to be preferred by females (Scheuber et al. 2004), but when condition was manipulated during the juvenile stage, male *G. campestris* traded off size (as indicated by song pitch for chirp rate (Scheuber et al. 2003). Alternatively, pheromones may be relatively cheap to produce and our manipulation of diet quality was insufficient to affect their production. Elucidating the patterns of both the acquisition of resources and their allocation to different traits is an important area of study (van Noordwijk and de Jong 1986). Our study illustrates one interesting pattern, and future studies should investigate the influence of other resources (e.g. water) as well as the effects of varying quantity at different life stages.

As recommended by a review of the condition dependence literature (Cotton et al. 2004), we varied condition over three treatment levels. However, our low-condition treatment may have exerted selection on several of the adult life history parameters because larval survival on the low-quality diet was significantly lower than on either the high- or medium-quality diets. Furthermore, the low-quality diet appeared to affect males more severely than females. Given that acquisition is likely affected by a large proportion of an individual's genome (Rowe and Houle 1996), survival of larvae in the low-quality diet may have been nonrandom with respect to male genetic quality. This may explain why there were no effects of diet on either

attractiveness, ejaculate transfer or mate fecundity (assuming their increased importance to fitness relative to body size and lifespan). Our results argue for caution in studies seeking to manipulate condition, as treatment effects attributed to differences in condition could be confounded with genetic differences resulting from selection.

Male ejaculate traits are known to be condition dependent (e.g. Perry and Rowe 2010, Kahri and Cox 2015), however, we were unable to detect an effect of condition on ejaculate transfer. This may have been because our measure of ejaculate transfer was not accurate because of a lack of ambient humidity control. If the chief beneficial ingredient in male ejaculates is water (e.g. Droge-Young et al. 2016), then random fluctuations in room humidity may have introduced noise into our measurement of treatment differences. Alternatively, the effect of our manipulation of condition may not have been apparent in a single mating, but manifest as an effect on male mating rate. For example, although males in all diet treatments may have been able to transfer ejaculates in their first mating, as diet quality decreased, males may have had to take longer reproductive time outs to recoup resources lost during earlier matings (e.g. Kaldun and Otti 2016). Furthermore, males in our study may not have invested maximally in ejaculate transfer because they perceived the risk of sperm competition to be low (e.g. Gage and Baker 1991). Future studies should manipulate ambient humidity as well as measure male mating rate in experiments involving both virgin and nonvirgin females to fully address these questions.

Previous studies suggest female *T. molitor* gain direct benefits from mating (Drnevich et al. 2001b, Worden and Parker 2001) and ejaculate-derived mating benefits to females are widespread (reviewed in Vahed 2007, Gwynne 2008). We were unable to detect any such direct

benefits, measured either as female mate lifespan or fecundity. Dietary restriction of female *T. molitor* did not significantly affect the lifetime fecundity benefit of mating multiply (Worden and Parker 2001), although these experiments were not conducted under conditions of controlled humidity. Hydration is increasingly being recognized as an important material benefit of female multiple mating, especially for species that inhabit very dry environments such as desert-dwelling crickets (e.g. Ivy et al. 1999) and beetle pests of stored grain products (e.g. Ursprung et al. 2009, Droge-Young et al. 2016). *T. molitor* is also a stored product pest (Dunkel 1992) and if the main benefit to females of multiple mating is hydration, then our lack of ambient humidity control and manipulation could explain the lack of significant effect seen in our study. Clearly manipulating water availability and more control over ambient humidity in future experiments would address this question. More detailed examination of the condition dependence of both sperm and non-sperm (e.g. water content) components of male *T. molitor* ejaculates would help clarify these alternative hypotheses.

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Data Availability Statement

The dataset generated during and/or analysed during the current study are available in the Open Science Framework repository, https://osf.io/3zm57/.

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535

Tables

Table 1. Summary of mating behaviour differences (median [interquartile range]) among the males reared on low- (N = 16), medium- (N = 38) and high-quality (N = 41) diets. Effect sizes for each Kruskal-Wallis test are given by ε^2.

Variable	Low	Medium	High	χ^2	p	ε^2
Attractiveness Score (s)	29.5 (7.5-70.3)	46.5 (24.0-97.3)	44.0 (16.0-66.5)	2.184	0.336	0.023
Time to First Contact (s)	48.0 (20.5-97.8)	48.0 (19.8-105.0)	52.0 (23.0-119.5)	0.124	0.940	0.001
Copulation Attempts (#)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-1.5)	3.972	0.137	0.042
Latency to Copulate (s)	72.5 (31.5-115.8)	61.0 (39.5-164.3)	46.0 (23.0-77.5)	6.151	0.046*	0.065
Copulation Duration (s)	96.0 (87.3-119.0)	107.0 (93.0-147.3)	109.0 (95.5-130.0)	3.511	0.173	0.037

* not significantly different after sequential Bonferroni correction (Holm 1979)

544 **Figure Legends**

545

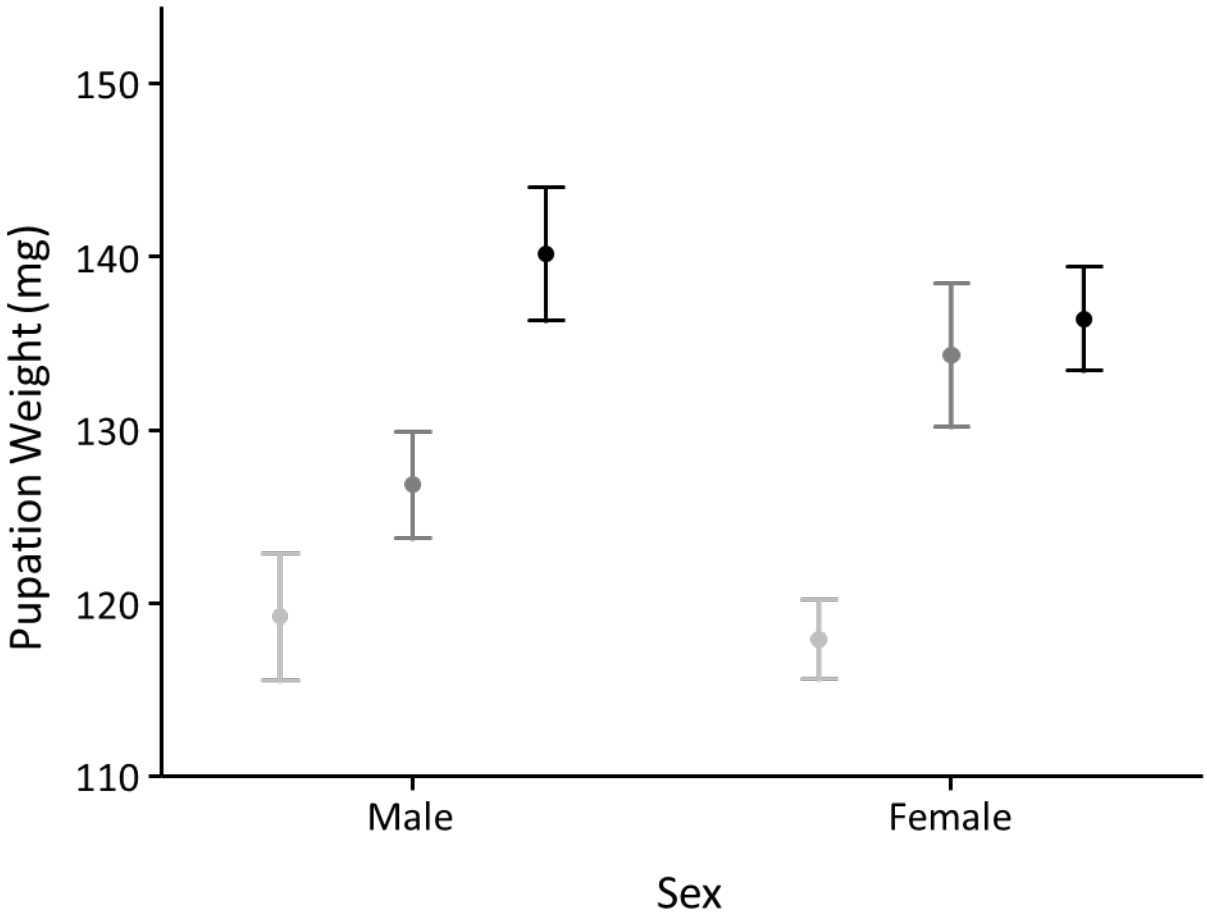
546 Figure 1. Mean (±SE) weight at pupation of individuals raised on low- (light grey), medium-

547 (dark grey) and high- (black) quality diets.

548

549 **Figures**

550



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552 McConnell and Judge, Fig. 1