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Does Male Mate Choice vary with Environment?

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Abstract

Both male and female red flour beetle, *Tribolium castaneum*, have a high mating rate. Male beetles demonstrate clear pre-copulatory mate choice. Males prefer mature females over immature females, virgin females over previously mated females, and younger females over older females. We hypothesize that male choice is affected by the environment and test if male mate preference varies when males' environment are changed. In the experiment, male environment was manipulated by changing food availability and keeping males in starvation or optimal nutrition environments. Choosiness can be inferred by how quickly males mate, how long they investigate the female, and the frequency and duration of copulations. We predicted that the males that are malnourished will be less choosy than the males that are nourished. To determine the results for male choosiness and reproducing capabilities, this experiment examined their behavior and the outcome of the mating as measured by offspring production. Our results suggest that male mating behavior and mate choice is affected by nutritional environment.

Keywords: *Tribolium castaneum*, mate choice, environment

1. Introduction

Animal behaviors are shaped by the environment they are in. For example, *Tribolium castaneum*, the red flour beetle, when exposed to environments that differed with respect to population density (low or high), food availability (deficient or optimal), and competition from heterospecifics (present or absent), as immature beetles were found to have differences in their development time, fecundity, and cannibalism rates[1].

Red flour beetles are commonly used to research mating behaviors, including mating behavior characteristics (frequency, duration, etc.), outcomes (fecundity, offspring quality etc.), and mate choice (criteria) [2]. For example, male red flour beetles demonstrate clear pre-copulatory mate choice[2]. Males prefer mature females over callow (immature) females, virgin females over previously mated females, and younger females over older females[3, 4, 5]. This research focuses on the interaction between environment and male mate choice behavior. Sexual selection theory predicts that male choosiness would be higher when variance in mate quality is high, but would be constrained by factors such as cost of mate assessment, mate search etc.[6]. There is very little research on male mate choice in red flour beetles especially in varying environments. Most research that has been conducted focuses on female red flour beetles. We predict that male mate choice will be influenced by the environment they experienced. Specifically we expect that males that experienced sub-optimal conditions would be less choosy and invest less energy in mating activities because of the costs of mate assessment. We also expect to find that males that experience sub-optimal environments would likely transfer less sperm or would be less preferred by their mating partners and therefore would produce fewer offspring than males that experienced optimal environments.

T. castaneum typically thrive in warm temperatures and yeast-rich food environments[7]. They are also found in dense aggregations[7]. In this study, the nutritional environment was manipulated into ‘starvation’ and ‘optimal nutrition’. Two experiments were performed to assess effects of environment on male mate choice. For the first experiment, we assessed differences in mating behaviors of starved and control males when exposed to a single virgin female by

comparing: 1) time it took to make the first touch, 2) the number of contacts, 3) the number of chases, 4) the number of copulations, and 5) number of attempts. After that, female egg production was measured to determine if there was any difference in sperm production and /or post-copulatory mate choice by females. In the second experiment, male mate choice was tested by assessing the interactions of a male when exposed to a high quality (virgin) and a low quality (previously mated) female.

2. Methodology

2.1 Beetle Source and Maintenance:

Carolina strain beetles were acquired from Carolina Biological Company. Beetles were raised in standard flour and yeast medium (95% flour, 5% yeast) in a dark incubator kept at 29ºC at 70% RH[8]. Beetles were sexed as pupae and males and females were kept in groups of 5 till approximately 7 days post emergence.

2.1.1 starvation

Male beetles approximately 7 days post emergence were assigned to two groups at random. Half the males were assigned to starvation group and kept in empty glass vials. Males were starved for 3 to 7 days[9]. The other half were assigned to control group and kept in glass vials with flour and yeast medium.

2.2 Experiment 1: Copulatory Behavior And Post-Copulatory Success Of Starved And Control Males

The aim of this experiment was to test if males' environment effected their mating behavior. Thus, we observed mating behavior of males from environment with no food (starved males) and males from optimal food environment (control males). Male behavior was observed for 30 min when exposed to a virgin female beetle in a 35 mm diameter plastic petri dish lined with filter paper and flour[8]. Because males and females were sexed as pupae, all beetles were virgins. Males were marked with a black sharpie to distinguish them from females. Marking them in this way does not alter mating behavior[10]. Thirty starved and 30 control males were used in this experiment and individually exposed to a virgin female. Females were assigned at random to mate with either control or starved males. Each beetle was used only in a single trial. The following behaviors were noted: 1) time to 1st touch, 2) number of copulations, 3) number of mating attempts, 4) number of chases, and 5) number of touches between beetles. To test if the males' environment effected their post-copulatory success in the form of egg production, a subset of the females were allowed to lay eggs for 2 days. Eggs were counted on the third day.

2.3 Experiment 2: Mate Choice Of Starved And Control Males

The aim of this experiment was to test if males' environment effected their mate choice. To this end, males from different food environments (starved or control) were exposed simultaneously to a virgin and a mated female. Mated females were previously paired with virgin males and separated 24 hours before the experiment. Experimental males were marked black and females marked with green. Individual trials were randomly assigned to have either the virgin female or the mated female to be marked with a green sharpie. In half the trials, virgin females were marked, and in the other half the mated females were marked. We noted which female was approached first by the male and which female was copulated with first.

2.4. Statistical Tests

Copulatory behavioral traits including 1) time to 1st touch, 2) number of copulations, 3) number of attempts, 4) number of chases and 5) number of touches for control and starved males were compared using t-tests. The frequency of single, multiple or no copulations among starved and control males was compared with a chi-square test.

3. Results

3.1 Experiment 1: Copulatory Behavior And Post-Copulatory Success Of Starved And Control Males

We predicted that starved males would show less mating vigor as indicated by courtship effort and success rate than control males. Starved males did not differ significantly with respect to time to first touch (t-test, df = 58, P = 0.13, Figure 1a), number of copulations (t-test, df = 58, P = 0.25, Figure 1b), number of attempts (t-test, df = 58, P = 0.31, Figure 1c) or number of touches (t-test, df = 58, P = 0.57, Figure 1e). The only aspect of mating behavior that was different between the two groups was the frequency of chases, which was significantly lower in starved males compared to chase frequency of control males (t-test, df = 58, P = 0.005, Figure 1d).

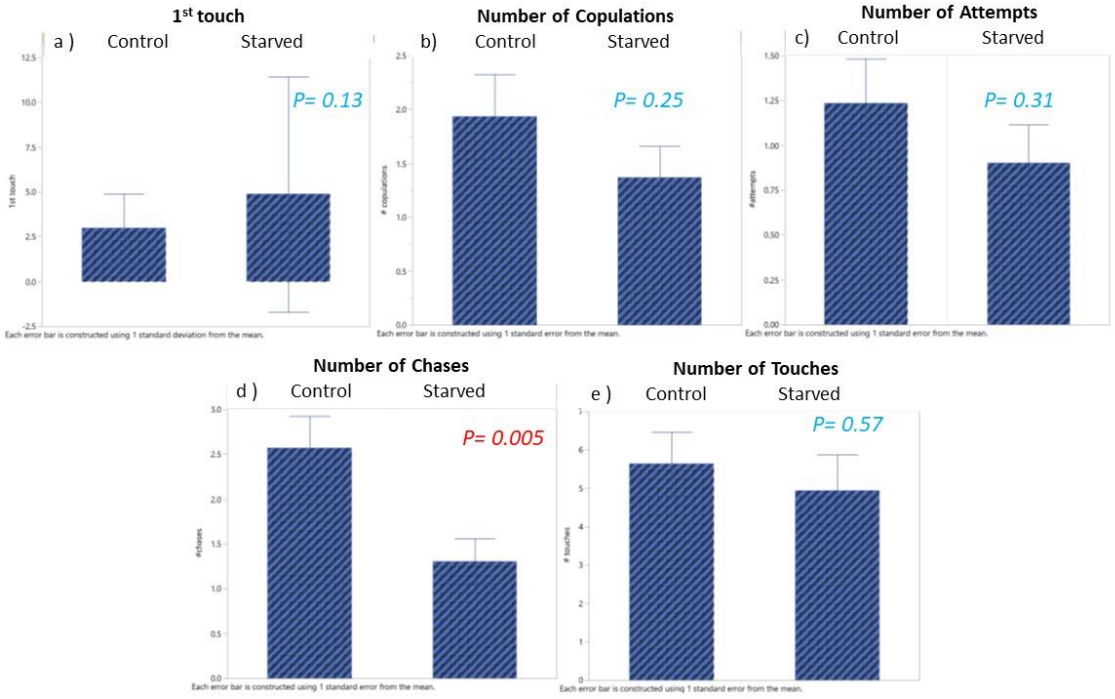
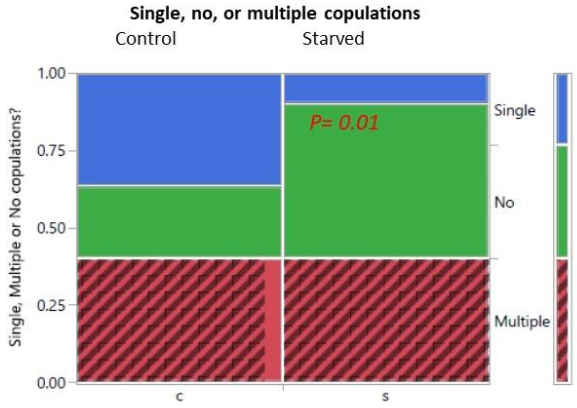


Figure 1: Effect of starvation on male mating behavior. Virgin males (starved or control) were exposed to a virgin female in a plastic petri dish lined with filter paper and flour for 30 min. Behaviors noted were: a) time to 1st touch, b) number of copulations, c) number of mating attempts, d) number of chases, and e) number of touches between beetles (N= 30). Means and Std. Errors are shown. Results of t- tests are shown.

The starvation treatment effected whether males copulated never, singly or multiply in the 30 min observation period (contingency chi-square test, Chi- square = 7.83, df = 2, N = 60, P = 0.01). Specifically starved males were more likely to be unsuccessful at mating than control males (Figure 2).



Single, no, or multiple copulations for Control and Starved Males

	Control	Starved
Single mating	11	3
No mating	7	11
Multiple matings	12	12

Figure 2: Effect of starvation on male mating frequency. Virgin males (starved or control) were exposed to a virgin female in a plastic petri dish lined with filter paper and flour for 30 min. Males were classified into three groups based on whether they mated a single time, multiple times or never in the 30 min period (see table above). Results of chi square test are shown with the figure.

We expected that starved males would differ from control males in post-copulatory success and that starved males would produce significantly fewer eggs than control males either due to lower sperm production or due to female choice against them. The egg production of partners of starved and control males was compared with a t-test and did not differ significantly (t-test, t = 1.19, df = 25, P = 0.29, Figure 3).

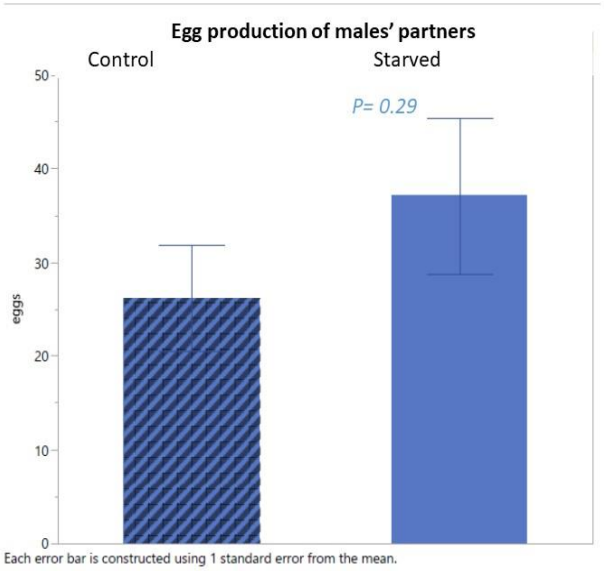


Figure 3: Males' partner's egg-production. Results of t- tests are shown.

3.2 Experiment 2: Mate Choice Of Starved And Control Males

We expected that starved males would differ from control males in mate choice and that starved males are more likely to mate with less preferred (mated) females. We found that when exposed to virgin and mated females, males approached virgin females at frequencies no different from the frequencies at which they approached mated females (Chi-square test, chi- square = 0.345, N = 12, df =1, P = 0.7; Figure 4 a). However, starved males mated with only virgin females and control males only with mated females (Chi-square test, chi- square = 9.56, N = 7, df =1, P = 0.04; Figure 4 b).

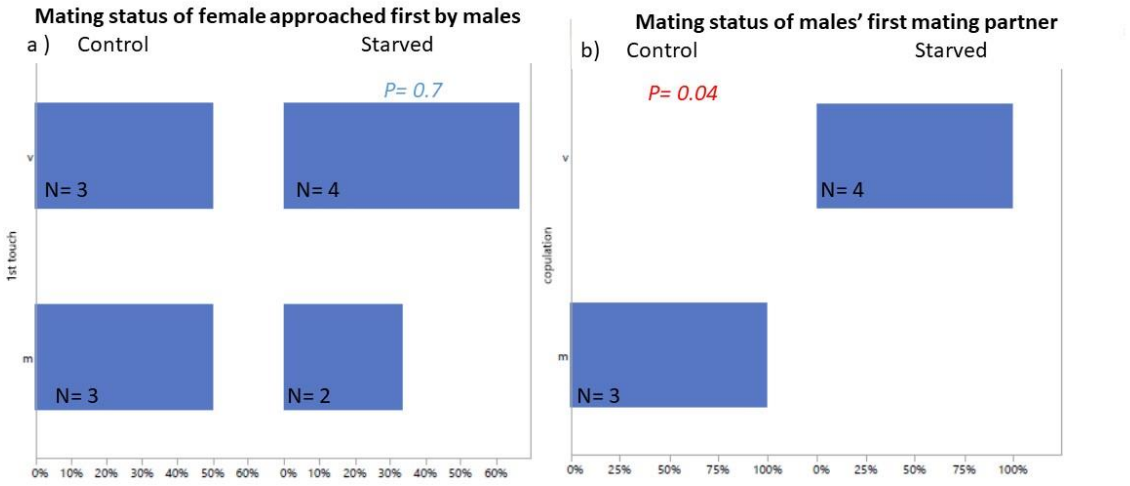


Figure 4: Male preference for virgin and mated females in a simultaneous choice experiment was inferred from the female males approached first (a) and copulated with first (b) (N = 12). Sample sizes of each group are indicated in the bars. Results of chi-square tests are shown.

4. Discussion

Beetle environment influenced male mating behavior as well as mate choice. The first experiment indicated that starvation affected certain aspects of male mating behavior. Specifically, starved males had lower chase frequency (Figure 1). Similarly, starvation was more likely to result in a lack of successful copulation (Figure 2). Though male flour beetles are known to mate with up to seven females in 15 minutes[11], 15 out of the 30 males in starvation condition, compared to 7 out of 30 control males, did not copulate even once in the 30 min observation period (Figure 2). This suggests that males adjust their effort at copulation when subjected to starvation. This result is consistent with the "silver-spoon" hypothesis, which suggests that animals developing under a nutritionally optimal conditions will outperform animals that do not grow in nutritionally optimal conditions[12].

Based on the mate choice experiment (Figure 4), we concluded there was a difference between starved and control males in their preferred mates. Previous studies suggest that red flour beetle males consistently approach virgin females first and copulate more frequently with virgin females[4]. However, once contact was made with a female, males were as likely to copulated with a virgin females as a previously mated female[4]. Male preference for virgin females resulted in higher reproductive success of the males [3, 4] who sired more offspring due to lack of a rival in sperm competition.

Our results from mate choice experiment were somewhat different from previous studies because starved and control males did not preferentially approach virgin females first (Figure 4a). However, starved males only mated with virgin

females and control males only mated with previously mated females. This pattern is contrary to what we expected. One explanation for this may be that females exert strong mate choice[5] and previously mated females are more discerning about their second partners and do not mate with a poor quality male. Red flour beetles exhibit last male sperm precedence[5] therefore, females might avoid poor quality last male. Our future study will explore this by testing if mated females choose control males when simultaneously exposed to a starved and a control male. Red four beetle populations differ in mating behavior traits[2], therefore, it's possible that beetle populations vary in male mate choice criteria. Previous work in our lab has shown that males consistently mate more frequently with mated females, in the present study too, control male beetles consistently approached the mated females for copulations whereas the starved males mated only with virgin females. Though the small sample size makes these data inconclusive, we will continue to test if starvation affects male mate choice and explore the differences in criteria for mate choice among different populations of this species.

Our results indicate that behavioral differences did not result in differences in egg production (Figure 3). This suggests that male sperm production and sperm transfer was unlikely to be different from starvation. The lack of difference in egg production suggests that females did not discriminate against starved males.

Prior work on effect of nutritional environment on male red flour beetles revealed that when fed different nutritional diets, males did not show significant variation in olfactory attractiveness to females, mating or insemination rates, and sperm defense (P_1) or offense (P_2)[13]. This study however showed a significant difference in starved and control males with respect to likelihood of mating (Figure 2).

Another multi-generational study in this beetle, that examined male mating and insemination success across three nutritional environments found significant phenotypic plasticity among male genotypes[14]. The results from present study are consistent with the finding that nutritional environment shapes reproductive traits[13,14] as well as behaviors such as response to pheromone[15] in red flour beetles. Other insects such as fruit-flies also show influence of nutritional environment on male mating behavior[16,17].

Overall, these data support the hypothesis that environments indeed have an effect on male mating behavior and potentially choosiness. Future work will include mate choice assays with larger sample sizes

5. Acknowledgements

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