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Correlation Between Pre-Copulatory And Cryptic Male Choice In The Red Flour Beetle

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Abstract

Male mate choice is a universal phenomenon yet many aspects of male mate choice evade a full understanding. An insect model system, the promiscuous red flour beetle (*Tribolium castaneum)* was used to explore different components of male mate choice. The aim of this pilot study, is to establish female attributes that are used as mate choice indicators by males. Next it examines the correlation between pre-copulatory and cryptic male choice behaviors. The study tested whether pre-copulatory and cryptic mate choice by males is correlated. It does so by first establishing female attributes that are preferred by males (mating status, nutrition status, and age) and next testing for evidence of cryptic male choice. In no-choice tests, males preferred virgin (unmated) females to mated females and young females to old females. Further, males invest more towards preferred 'young' females in terms of time spent in copula. There was no evidence for cryptic female choice for virgin females. Thus, female age is a good indicator of female quality and forms the basis for pre-copulatory and cryptic male choice.

Keywords: *Tribolium castaneum*, pre-copulatory male mate choice, cryptic male choice, red flour beetles

1. Introduction

Male mate choice is 'differential male sexual response to reproductively mature conspecific females' and is ubiquitous in the animal kingdom[2]. Male choice is manifested as pre-copulatory preference for certain females or cryptic choice[2]. Here we test the hypothesis that male pre-copulatory choice (as indicated by acceptance or rejection of a mate and copulation frequency) and cryptic choice (as indicated by copulation duration, mate guarding duration) are correlated.

Assessing pre-copulatory male choice is possible by examining 1) acceptance of rejection of females, 2) variation in intensity of frequency of courtship and /or copulation attempts and 3) intensity of male-male competition for females[2]. On the other hand, cryptic male choice may be assessed by examining the resources invested by males such as 1) nuptial gift, 2) ejaculate, 3) duration of copulation, 4) duration of mate guarding and 4) paternal care/investment[2].

Male *Tribolium castaneum,* (red flour beetles) is an ideal system for study of sexual selection[8]. Males display a preference for virgin females and mature females as opposed to mated females or immature females respectively[3]. Males also prefer novel partners rather than ones they have mated with before[1]. Because the correlation between pre-copulatory male choice and cryptic male choice has not been examined in this system[7], here we investigate the correlation between pre- copulatory male choice and cryptic male choice.

2. Methods and Materials

2.1 Study Species And General Protocols

Beetles were acquired from Carolina Biological Supply Company. Beetles were raised in whole wheat flour and Brewers' yeast mix (95:5 w/w) at 29° C and 70% RH in a dark incubator following previously established standard protocol[4, 6]. Males and females were separated as pupae to ensure virginity and kept in separate vials. Beetles were marked with a sharpie in behavioral observations so that the observer could tell them apart[5]. Behavioral observations were conducted in 35 mm Petrie dishes lined with filter paper and flour medium in ambient light and temperature conditions[6]. In mating behavior observations, the following behaviors were recorded: mating frequency and duration, contacts between beetles, attempts, and chases[6]. We tested for the effect of three female attributes mating status, nutritional status, and age on male choice.

2.2 Experiment 1: Effect Of Female Mating Status On Male Mate Choice

First, to test if female mating status influenced male mate choice, we observed males in the presence of Mated and unmated virgin Control females. In the first step, individual virgin females were mated with virgin males for 24 h and separated from each other. Next, we placed the experimental male with an Control female or a Mated female into a petri dish and observed the latency to touch i.e. the minute after trial began when beetles first contacted each other and copulation frequency over a 30 min period (N = 40).

Next, to determine if males invest more in preferred females we compared male mate guarding for preferred and non-preferred mates. To test if male mate preference influenced male mate guarding in the presence of a rival, we placed two males with one marked female per petri dish (mated or control) and recorded the number of copulations and the duration over 30 min (N = 10).

2.3 Experiment 2: Effect Of Female Nutritional Status On Male Mate Choice

To determine if nutritional status of females influences male mate choice, we observed male behavior with Starved and unstarved Control females. First, females were starved for 7-10 d. Next, a Starved or Control female was placed with one male in a petri dish and observed for 30 min (N= 40).

2.4 Experiment 3: Effect Of Female Age On Male Mate Choice

To determine if female age influences male mate choice, we observed male behavior with Young and Old females. Young females in this experiment were less than 4 weeks old and Old females were 7 months old. One young or old female was placed with a male in a petri dish and observed for 30 min (N= 40).

To determine if males invest more in preferred females we placed one Young and Old female with one male in a Petri dish and observed behavior for 30 min. The observer was unaware of which female being marked in the second experiment (N = 12).

3. Results

3.1 Experiment 1: Effect of female mating status on male mating behavior

We expected that mating status would affect male mate choice because males of this species are known to prefer virgin females. Results of the mate choice trials supported this prediction and males mated more frequently with unmated (control) females (Figure 1 A; t-test, $P < 0.05$). This was because males were less likely to mate with previously mated females (Figure 1 B, Chi-square test, $P < 0.05$), though there was no difference in initial interest in the mated females as indicated by the latency to contact, (Figure 1 C; t-test, $P = 0.17$)

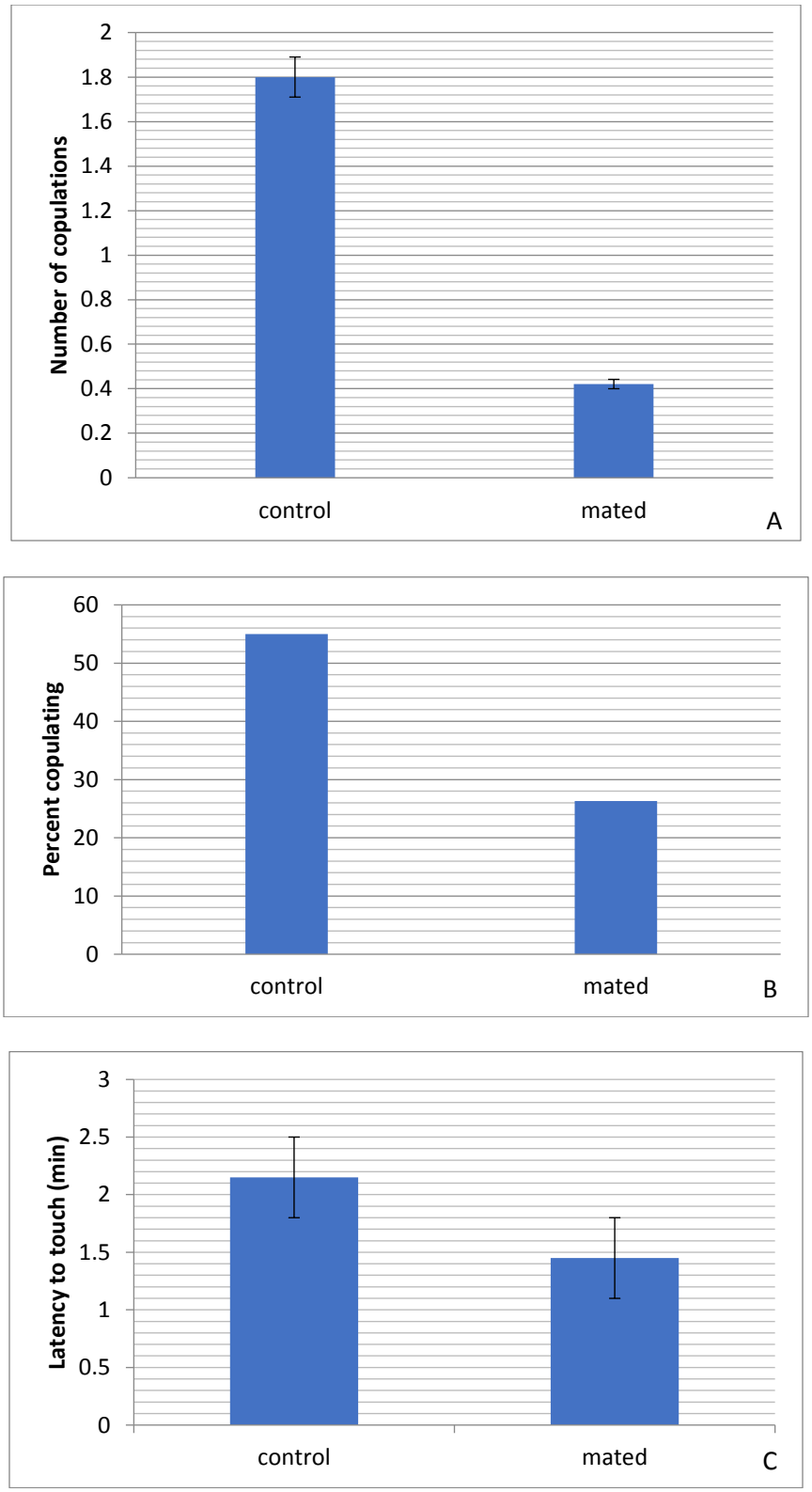


Figure 1: Effect of female mating status on male behavior. A. Average copulations observed between males and females (SE are shown). B Percent of trials in which mating was observed (N= 40). C. Latency to touch (SE are shown).

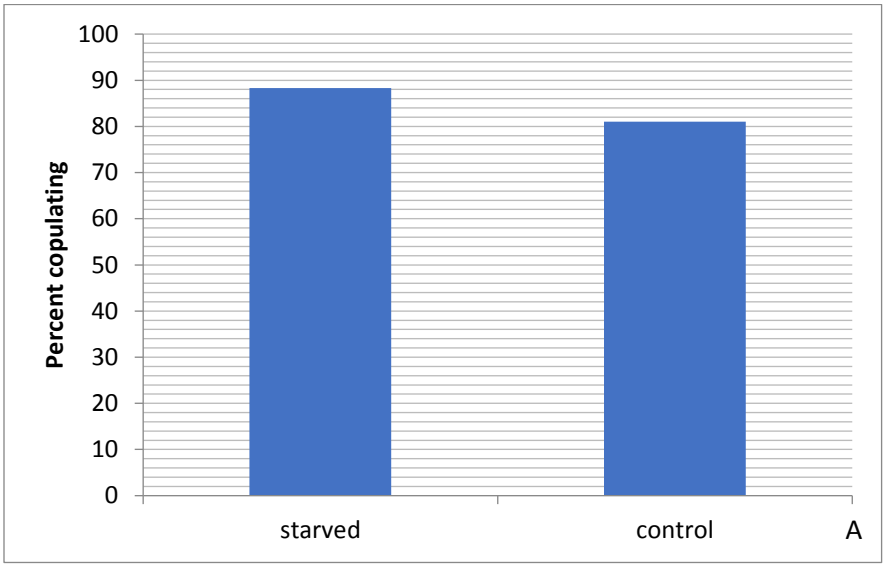
Male investment in copulation efforts towards mated and unmated females in the presence of a rival was not significantly different. Specifically, copulation frequency, average copulation duration and total time spent in copula did not differ between mated and control females (Table 1, t-test, $P > 0.05$)

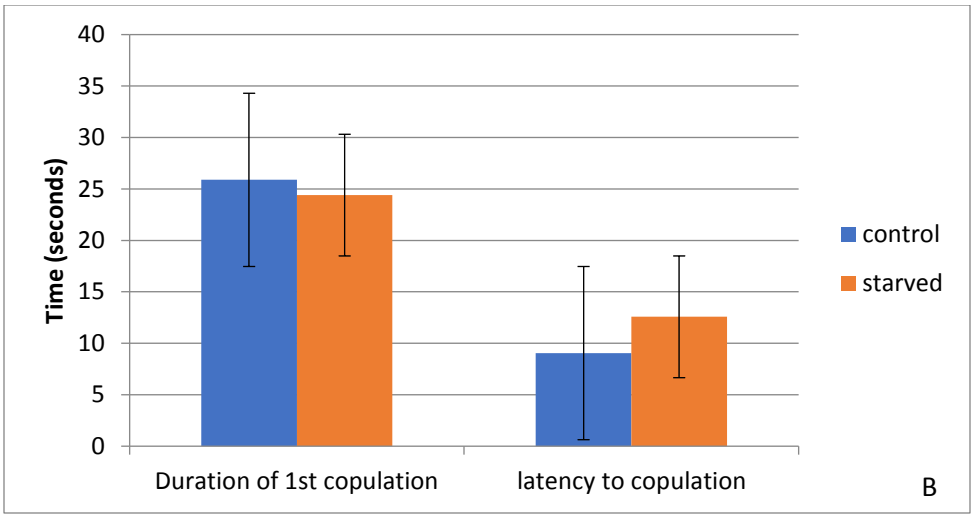
Table 1: Effect of female mating status on male mating behavior in the presence of a rival
(Means and SD are shown)

	control	**mated**
Number of copulations	2.5 (1.3)	2.4 (2.1)
Total time in copula (s)	108.8 (70.3)	174 (112.8)
Average copulation duration	47.8 (29.2)	89.8 (65.6)

3.2 Experiment 2: Effect Of Female Nutrition Status On Male Mating Behavior

We expected that female nutritional status would affect male mating behavior because starvation would presumably have a negative effect on female capacity for egg production. However our results did not support this prediction (Figure 2). Overall, there was no difference in the percent of trials in which males copulated with a Straved and Control females (Figure 2A, Chi-square test, $P > 0.05$). Similarly, there was no difference in latency to first copulation or duration of first copulation bteween Starved and Control females (Figure 2 B, t-tests, $P > 0.05$).





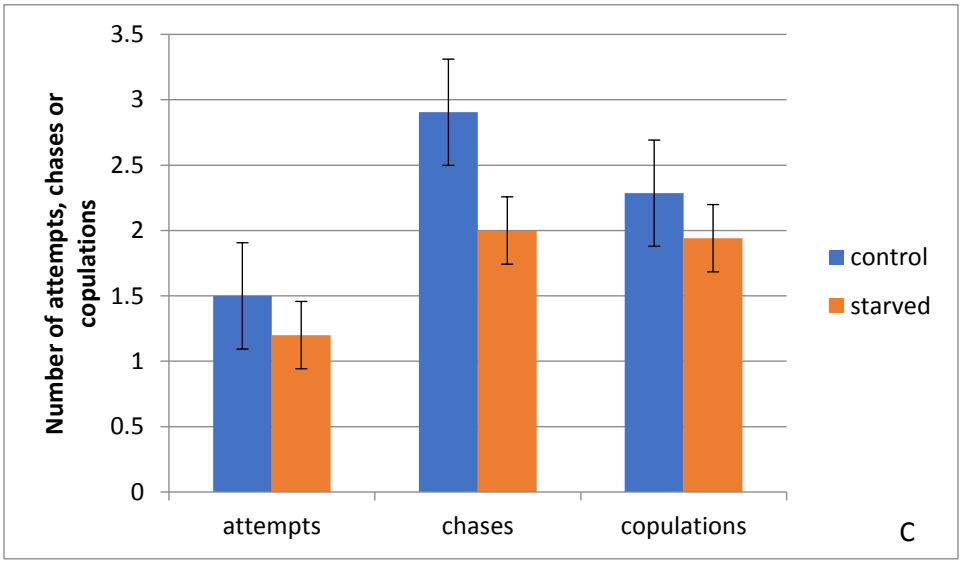
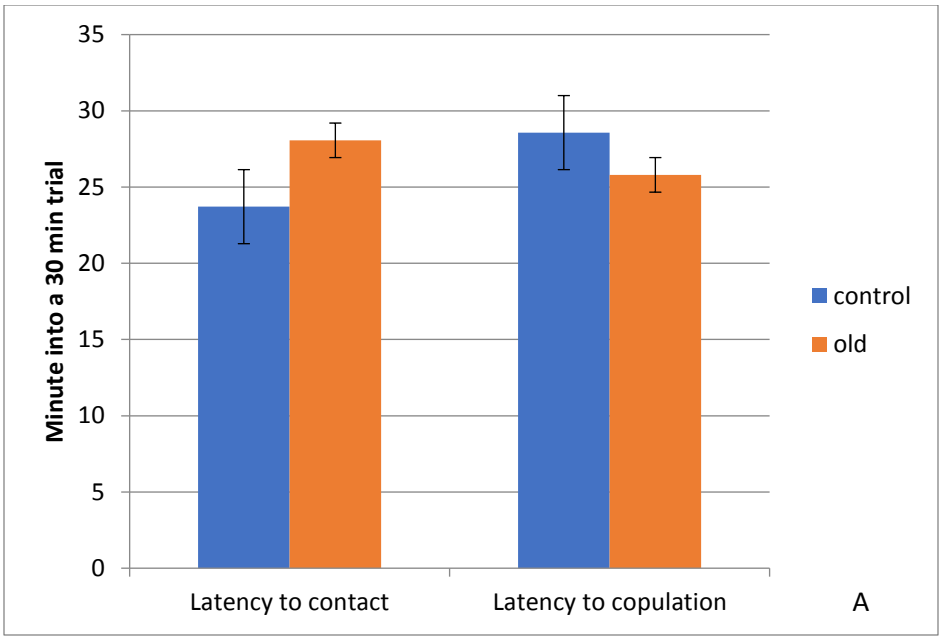


Figure 2: Results of tests with a single male and female (either Control or Starved). A. Percent of trials in which mating was observed. B. Duration of first copulation and Latency to copulate (time after start of trial when copulation occurred). C. Frequency of attempts, chases and copulations. Means and SE are shown. No significant difference was found in any variable (t-test, $P > 0.05$).

3.3 Experiment 3: Effect of female age on male mating behavior

We expected males to discriminate against older females because older females are less fecund[6]. Our data support this prediction and males showed preference for younger females (Figure 3). The percent of trials in which copulation was observed was not different (93% for both Old and Young).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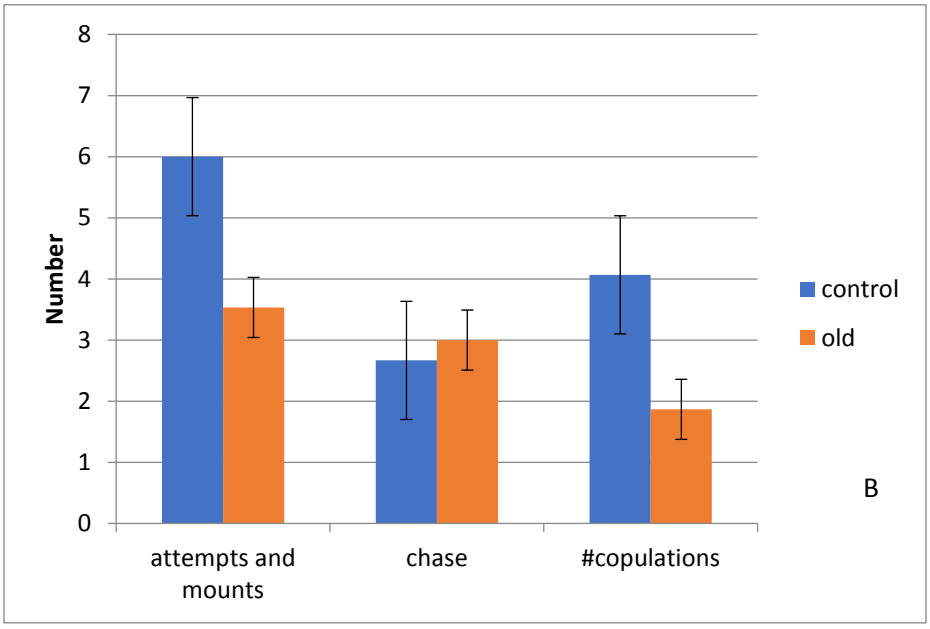
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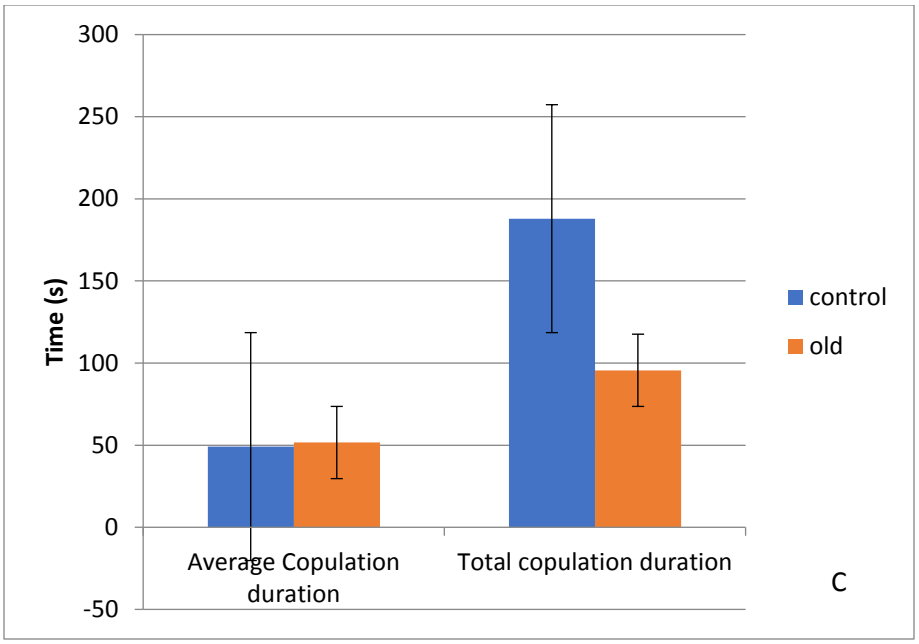

Figure 3: Results of tests with a single male and female (either Control or Old).A. Latency to contact between beetles and Latency to copulate (t-tests, $P < 0.05$ for both). B. Number of attempts (t-test, $P > 0.05$), chases (t-test, $P > 0.05$) and copulations (t-test, $P < 0.05$). C. Average copulation duration (t-test, $P > 0.05$) and total time in copula (t-test, $P < 0.05$). Means and SE are shown.

In simultaneous choice test, males did not invest more copulatory effort into Young females compared to Old females. Male investment in copulatory efforts as indicated by attempts, chases, copulations did not differ significantly toward Old and Young (control females) (Table 2, paired t-tests, $P > 0.05$). Similarly, neither latency to contact, nor latency to copulation was different toward the two females (Table 3, Paired t-tests, $P > 0.05$).

Table 2: Male mating behavior when offered the choice between both old and young (control) females. (Means and SD are shown. Paired t-test, $P > 0.05$.)

	control	**old**
Attempts	3.8 (3.12)	3 (3.3)
Chases	1.0 (1.5)	1.8 (1.9)
Copulations	0.75 (0.6)	0.91 (0.6)
Latency to contact	17.3 (9.1)	18.7 (8.9)
Latency to copulation	17.3 (9.1)	22.5 (9.5)

4. Discussion

Our hypothesis for this research was that pre-copulatory male preference as indicated by acceptance or rejection of a mate and copulation frequency correlates with cryptic male choice as indicated by copulation duration, mate guarding duration. We expected that males exhibit clear pre-copulatory mate choice favoring females with traits associated with higher fecundity (virgin females, well-fed females, and young females) and that mate guarding and/or duration of copulation will be longer with preferred females.

Pre-copulatory male choice: We tested for male choice with regard to three female attributes, mating status, nutritional status (condition) and age. As expected males preferred unmated females to mated females. Males rejected mated females more frequently (Figure 1B) and copulated more frequently with unmated (control) females (Figure 1

A). However, there was no significant difference in the latency to contact towards mated and unmated females (Figure 1 C). Males did not appear to discriminate on the basis of female condition (nutritional status) (Figure 2 A and C). Younger females were preferred by males compared to older females as indicated by copulation frequency (Figure 3B), but not rejection rates (7% for each type of female). Similarly, males seemed more 'eager' to mate with young females as suggested by the shorter latency to copulation and contact towards young females compared to old females (Figure 3A).

Cryptic male choice: In the first experiment, pre-copulatory male choice for unmated females suggested that this was a useful indicator of female quality in this system. Therefore, we followed up by conducting a second experiment for cryptic choice. Male cryptic choice was assessed by measuring total male investment towards mated and control (unmated) females by two rival males exposed to a single female in a petrie dish. Because cryptic male choice may be inferred from difference in male-male competition, we expected to see higher copulation frequency and duration in copula with the preferred virgin females compared to the mated females. However, we found no evidence of cryptic male choice in relation to female mating status (Table 1).

In the second experiment testing for preference based on female nutritional status, we detected no sign of cryptic male choice as assessed by duration of copulation (Figure 2). The lack of pre-copulatory choice, combined with the lack of evidence for cryptic male choice based on nutritional status of females lead us to conclude that this is not a significant indicator of female quality in this species. Therefore, we did not conduct additional experiments testing for cryptic male choice.

In the third experiment, we detected pre-copulatory preference for young females as well as indication of higher copulation duration invested in young (control) females compared to older females (Figure 3). Therefore, we followed up the initial experiment with a second one in which, males were exposed to an Old and a Young (control) female simultaneously, but found no indication of male preference in this experiment (Table 2).

Correlation between pre-copulatory and cryptic male choice: Out of the three female attributes tested, only two, age and mating status were associated with male preference. Out of these two only the latter, female age, appeared to invoke cryptic male choice as seen by longer time spent in copula. Thus, we found partial evidence for correlation between pre-copulatory and cryptic male choice.

Summary: In this pilot study, our aim was to identify useful indicators of female quality that male red flour beetles use as a criterion for mate choice. In this objective we were successful in identifying female age as a key indicator of mate quality. Multiple aspects of male behaviors before, during and after copulation were associated positively with this female trait in a no- choice test (Figure 3), however when we tested this in a simultaneous choice test we found no mate choice on the basis of female age (Table 2). In future, we will expand on our exploration of correlation between pre-copulatory and cryptic male choice by repeating the simultaneous choice experiments with larger sample size, testing for mate guarding in the presence of a rival and examining post-copulatory outcome of male mating with old and young females.

5. Acknowledgements

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