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Female Perception Of Male Quality Influences Male Success In The Red Flour Beetle

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Abstract

The red flour beetle *Tribolium castaneum* is a pest species that is found primarily in food grains. Sexual selection, both male-male competition and female choice, are a strong evolutionary force in this species and affect the morphology and diversity of the beetle populations based on its environment. Previous work shows that female perception of male quality indicated by a male's copulatory courtship and not the male quality itself affects male fertilization success, thereby signaling the importance of post-copulatory female choice. However, it is not clear if females can manipulate paternity based on other male cues that are used for pre-copulatory mate choice. Here we tested if females can manipulate paternity based on a pheromone cue by artificially altering male pheromone cue but not male quality itself. We predicted that male beetles that are determined to be attractive based on the enhanced pheromone cue will have a higher fertilization success. Our results supported this prediction. Thus, our data adds to the evidence for female ability to control post-copulatory processes in this species.

Keywords: copulation, female control, red flour beetle, post-copulation mechanisms

1. Introduction

Mating preference is defined as "the sensory and behavioral properties that influence the propensity of individuals to mate with certain phenotypes"[7]. The red flour beetle, *Tribolium castaneum*, is known for its ability to reproduce rapidly making it a prime candidate for sexual selection studies[12] including mating preference [6,11]. In this species, female mating preference may operate before, during or after copulation and may be based on different male cues [5,6,11]. For example, prior to copulation, females may use male pheromone (4-8 dimethyl decanal) as a criterion for mate choice [6,11]. During copulation, females pay attention to stridulation by males [3,4,6,11]. In both these cases, males that are more attractive to females have an overall higher share of paternity [2, 3,6,11].

Though it is known that pheromone attractiveness influences male share of paternity, it remains unclear whether female manipulation after copulation or male quality itself (good genes) is responsible for higher paternity success of preferred males [11]. Previous studies investigating post-copulatory female choice based on pheromone cue and copulatory courtship used an experimental design which measured male success in sperm precedence studies with rival males [3,4,9].

Here we test whether male or female controlled processes affect post-copulatory outcomes of mating by artificially altering male pheromone cue in the absence of multiple mating by females. This experimental design better enables us to separate the various post-copulatory processes (cryptic female choice versus sperm competition). We predict that if female control is critical for deciding on male paternity success, then males with artificially enhanced pheromone cue will have higher paternity success than untreated males. On the other hand, if male quality itself affects paternity success, then manipulation of pheromone cue will not influence paternity success of males.

2. Methods and Materials

2.1 General protocols

Adult *T. castaneum* were purchased from Carolina Biological Supply (Burlington, NC, USA). 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 [8,9,10]. Males and females were separated as pupae to ensure virginity and kept in separate vials. Thus, all beetles used in the study were virgin, young adults.

2.2 Pheromone source

The pheromone 4,8 dimethyldecanal is an aggregation pheromone and a sexual attractant in this species [6,11]. A synthetic version of this pheromone was acquired from Trece Inc. (Adair, OK, USA).

2.3 Marking male beetles

Adult males were marked with a black Sharpie on the thorax[8, 10]. The mark is identifiable during mating trials to distinguish between the male and the female.

2.4 Beetle pheromone exposure

Forty 1-dram vials were prepared with a label number and a long film of filter paper. Twenty of the pieces were dabbed with the pheromone 4-8 dimethyldecanal using a capillary tube then dried so the beetles would not stick to the oil. Males assigned to either a pheromone vial or a control vial at random and placed there for 30 minutes before the mating trial. The observer was not aware of which vials contained pheromone to avoid bias during the observations.

2.5. Mating behavior trials

Behavioral observations were conducted in 35 mm Petrie dishes lined with filter paper and flour medium in ambient light and temperature conditions [10]. In mating behavior observations, trials were 15 min long between a virgin male and a virgin female. The following behaviors were recorded: mating frequency and duration and contacts between beetles[10].

2.6 Egg and larvae production

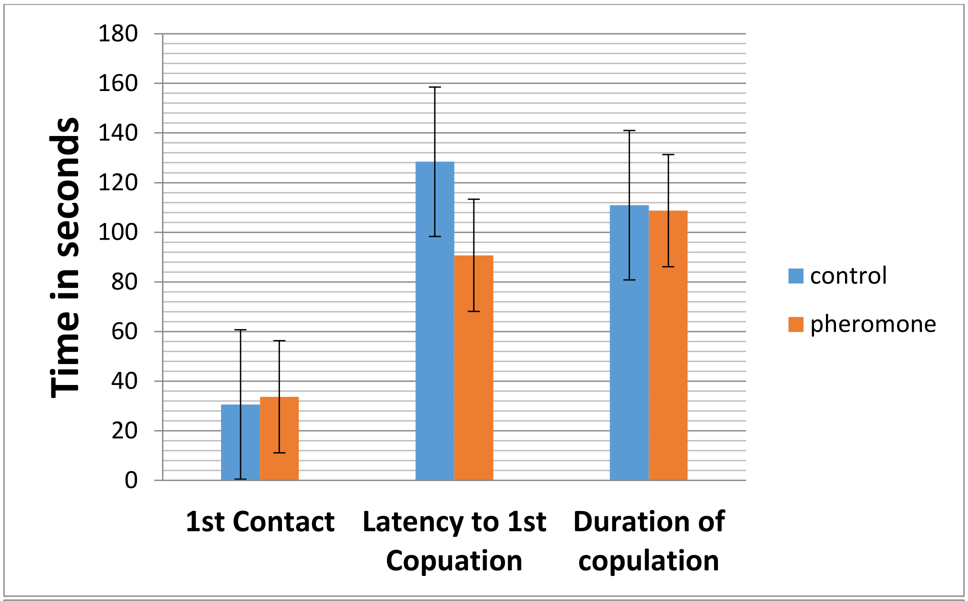
At the conclusion of the mating trial the female was removed from the petri dish and placed in a vial with double sifted medium [10] then placed in the incubator for a period of three days. After that time, the total number of eggs and larvae in each vial were counted. Because females were virgins at the start of the mating trial, therefore the offspring were sired by the experimental males. Thus, this provided an accurate measure of males' reproductive success post-copulation.

2.7 Statistical analysis

A t-test was performed on the data to test for a significant difference in mating behaviors (mating frequency and duration, contacts between beetles) and offspring production of the males manipulated with pheromone versus the control group.

3. Results

Mating behavior trials: Mating behaviors of males manipulated with pheromone were not different from control males. The time of first contact after initiation of trial (t-test, $df = 38$, $P = 0.72$, Figure 1 A) was not different between the two types of males suggesting that both males were as likely to locate or be located by females. The latency to copulation or the time since the start of the trial until the first copulation was not different between the two males (t-test, $df = 38$, P = 0.29, Figure 1 A) suggesting that pheromone treated and control males were similar in their ability to acquire a mating. The duration of copulation, a possible indicator of male investment in a copulation as well as of female quiescence [2] was also not different between the two types of males (t-test, df = 38, P = 0.93, Figure 1 A). Similarly, we did not find a significant difference in copulation frequency, an indicator of male investment as well as female quiescence [2], between pheromone treated and control males (Figure 1B, t-test, df = 38, P = 0.9).



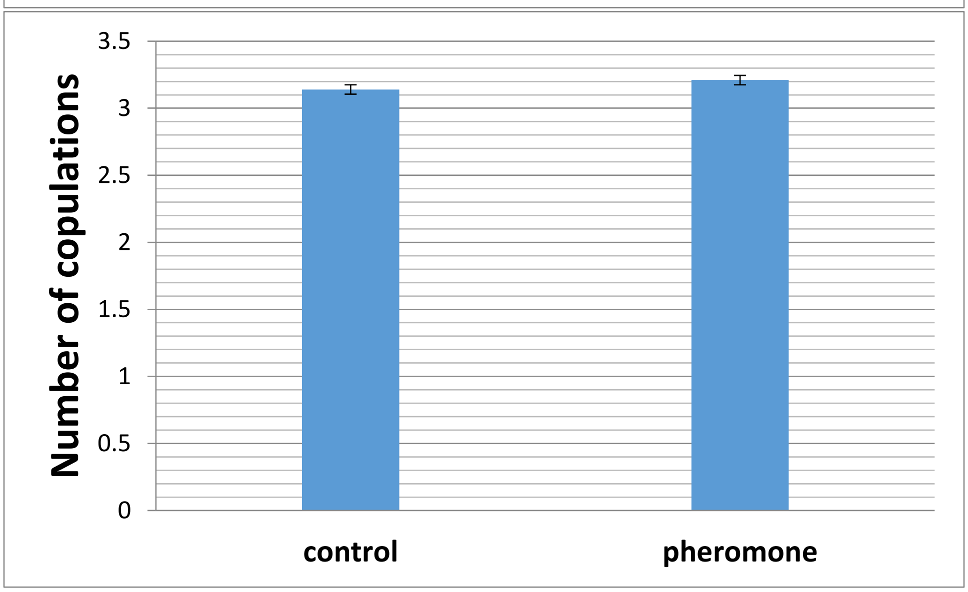


Figure 1: Mating behavior of beetles. Males were either treated with pheromone or left untreated (control). Means and SE are shown.

A. Time to first contact, Latency to first copulation and Duration of copulation. These three measures of mating behavior were not significantly different between pheromone treated and control males.

B. Copulation frequency. The number of copulations did not differ between pheromone treated and control males.

Egg and larvae production: Offspring production (eggs and larvae) of females mated with pheromone enhanced males was significantly higher than females mated to untreated (control) males (Figure 2, t-test, df = 38, P = 0.02).

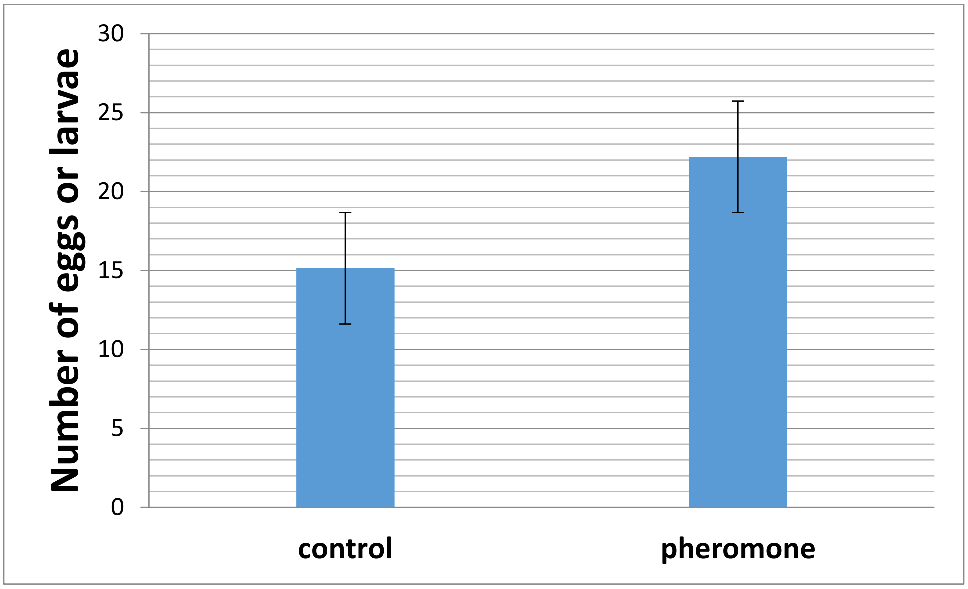


Figure 2: Number of eggs and larvae produced by females mated to control (untreated) males and pheromone treated males was significantly different. Means and SE are shown.

4. Discussion

The overall aim of this study was to test if females had the ability to control post-copulatory processes based on their perception of male quality via the male pheromone cue. To test this, we manipulated male pheromone cue by exposing some males to a synthetic version of the sexual attractant found in this species and examined post-copulatory success of males after mating behavior observations. We predicted that manipulating male pheromone cue in this manner will result in altering female perception of male quality and therefore post-copulatory success of males.

We found that male mating behavior was not different between treated and untreated males. Because mating behavior is strongly controlled by males [10], it is not surprising that we found no differences between behaviors under the two conditions. However, we did find a significant difference between egg and larvae production by females mated to pheromone treated males compared to partners of untreated males. This is strong evidence for female manipulation of paternity based on their perception of higher quality of pheromone treated males. Females are possibly manipulating sperm storage and usage after copulation [1,2,6,11]. Previous studies in this beetle have indicated that male reproductive success depends on female perception of male quality and not the male quality itself [3, 4]. Our study adds to the existing evidence for female control of post-copulatory processes in the red flour beetles [3,4,6,11].

This study suggests that female choice based on indicators of male quality is adaptive because females can manipulate egg production based on their perception of male quality. Alternatively, another possibility is that the pheromone itself is a stimulant, which provokes egg production independent of female control. Future studies will explicitly test these two alternative hypotheses. Another shortcoming of this study was that we did not have sufficient time to allow all the eggs to hatch to larvae (giving a better estimate of fertilized and viable offspring). Future studies will carefully examine the viability of the eggs after the initial 3 days.

5. Acknowledgements

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