Temperature stressed males are less attractive to female red flour beetles

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Abstract

Male red flour beetles (*Tribolium castaneum*) are shown to attract females through an aggression pheromone that may be altered due to varying degrees of stress (Wade, 1984). If these females do follow certain pheromone cues, then we would predict that they have a preference when given a choice between stressed and non-stressed males. This was tested by putting two sets of temperature stressed and non-stressed males in an arena with females and noting each interaction through a series of trials. By taking note of each mating, aggression, and simple encounter, we were able to find that females do prefer a non-stressed male. While stress can suppress multiple mechanisms in beetles, this sexual preference study provides insights into the reproductive processes of this pest and other invertebrates.

Purpose

In order to better understand how to control food stored product pests, such as the red flour beetle, it is critical to understand the factors that affect reproduction.

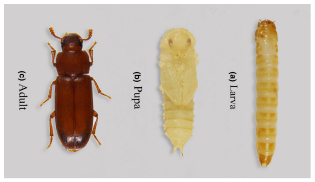
Questions, Hypotheses, and Predictions

Question: When given the option of males that are stressed by temperature or not stressed, which will the female *Tribolium castaneum* choose to mate with?

Hypothesis: I hypothesize that the female red flour beetle will choose to mate with non-stressed males.

Study System

The red flour beetle (*Tribolium castaneum*) is in the family Tenebrionidae and class Insecta. These beetles are considered as stored product pests for eating the remnants of grains or cereals and live up to 3 years. For mating rituals, the males stimulate the female elytra with their tarsi which has been suggested to have evolved from cryptic female choice, where females can choose which sperm from which partner will win (Fedina, 2007). Previous studies have shown that stressed males (by starvation) transferred less sperm than non-stressed, and that female pre-mating discrimination has been based on olfactory cues such as their natural pheromone attractants (Edvardson, 2000). This helps us suggest that the females tested do choose the non-stressed individuals over the stressed, due to possible pheromone cues or physical stimulation success.

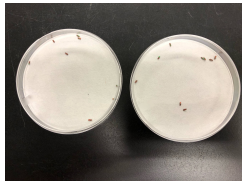


Khan, I., Prakash, A. and Agashe, D. (2016), Divergent immune priming responses across your beetle life stages and populatons. Ecology and Evoluton, 00: 1–9. doi: 10.1002/ece3.2532.

Methods and Experimental Design

30 females and 20 males were sexed and separated during the pupa stage. The females were then placed into a large secure container with unlimited food and space, while the males were divided into two separate containers. 10 males were marked with orange on their backs and placed into a medium sized container with food and then placed into a fridge at (4°C) for 24 hours. The remaining 10 males were marked with green and secured in a large jar with unlimited food and space at room temperature (22°C) for the same amount of time.

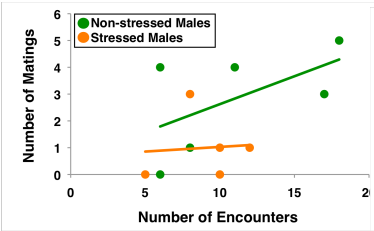
After a full cycle of 24 hours to properly stress the individuals, two groups consisting of three stressed and non-stressed males were placed in an arena with three unstressed females for 20 minutes of monitored interactions. Each group ran through three trials, each trial using the same males but an exchange of females. Each association between males and males or males and females was recorded, as well as mating interactions and how long they lasted.



Results

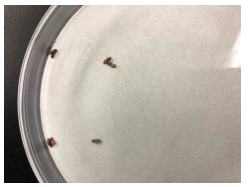
We found that, by providing females the choice between stressed and non-stressed males in a secured arena for 20 minutes, females preferred the non-stressed males when accepting a mate partner by a significant amount.

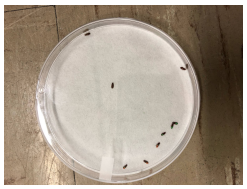
When noting interactions during the study, we also considered which male-on-male aggressive interactions occurred and how long each copulation (roughly) took place and then tallied their interactions. The non-stressed individuals seemed to initiate the aggressive communication onto the stressed males much more often than stressed on non-stressed. These interactions were to show dominance by "false-mating". When roughly timing each true mating interaction, we found that they lasted between 1 minute and 5 minute in all trials.



Conclusions

We found that females mated more frequently with non-stressed males, despite encountering both types of males equally. Moreover, non-stressed were more aggressive. Through this experiment and other sources, we find that females make a conscious choice of which male to mate with.





Future Directions

The original plan was to stress the males using food and space availability, however time restraints and survival limited this approach. In the future, I would like to re-run this experiment using food stress and then compare the results to see if females respond similarly to different kinds of stress. I would also like to use more sets of males since there seemed to be a decrease in energy through the three trials. The more males to test, the more results I could compare to get a concrete answer to the question. If I could expand more on this same project, I would like to test if there is a pheromone difference in attraction or if it is purely physical, as well as time the extent of each mating interaction more efficiently.

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