Aug 6th, 9:30 AM - 12:00 PM

Manipulation of foraging rate in honey bees in a natural setting

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Abstract
The accumulation of reactive oxygen species (ROS) and free radicals within tissues creates oxidative stress, causing damage and eventual aging in an organism. Intense activity can increase the level of oxidative stress that occurs within an organism. In honey bees, this activity occurs during foraging flights. To measure levels of oxidative stress resulting from low and high foraging activity, we set up three colonies: a colony with a pollen trap to cause increased foraging, a normal colony (control), and a colony with an artificial waterfall to limit foraging. We counted flights of marked foragers at each colony for 5 to 6 hours per day. As predicted, more pollen foraging flights occurred in the pollen trap colony. There was a steady rate of foraging activity in the normal colony, and low rates of foraging in the waterfall colony. Foragers tagged for age and with matching foraging rate data from each colony were collected in liquid nitrogen and frozen at -80° C. Future studies will measure expression of genes involved in oxidative stress in the brains and flight muscles of these bees utilizing western blots (to measure protein expression) and quantitative real-time PCR (to measure RNA expression).

Manipulation of foraging rate in honey bees in a natural setting

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Abstract

The accumulation of reactive oxygen species (ROS) and free radicals within tissues creates oxidative stress, causing damage and eventual aging in an organism. Intense activity can increase the level of oxidative stress that occurs within an organism. In honey bees, this activity occurs during foraging flights. To measure levels of oxidative stress resulting from low and high foraging activity, we set up three colonies: a colony with a pollen trap to cause increased foraging, a normal colony (control), and a colony with an artificial waterfall to limit foraging. We counted flights of marked foragers at each colony for 5 to 6 hours per day. As predicted, more pollen foraging flights occurred in the pollen trap colony. There was a steady rate of foraging activity in the normal colony, and low rates of foraging in the waterfall colony. Foragers tagged for age and with matching foraging rate data from each colony were collected in liquid nitrogen and frozen at -80° C. Future studies will measure expression of genes involved in oxidative stress in the brains and flight muscles of these bees utilizing western blots (to measure protein expression) and quantitative real-time PCR (to measure RNA expression).

Introduction

In a natural setting, the activity level of an adult worker honey bee (*Apis mellifera*) is age-related, a type of behavior known as temporal polyethism. During the first 2-3 weeks of the adult life, worker bees have a lower level of activity, performing tasks within the hive such as nursing or maintaining the hive. Nurse bees do not fly often, leaving the hive only to eliminate waste. The worker bees' level of activity increases when they are about 3 weeks old, when they begin to go on frequent foraging flights. Foraging flights are over 8 kilometers per day, primarily to gather pollen or nectar (Winston, 1987). During aerobic metabolism, especially during intense activity such as flying, free radicals, (also known as reactive oxygen species (ROS)) are produced (Yan and Sohal, 2000). High levels of ROS have been shown to contribute to aging in bees. A study by Williams et al. in 2008 concluded that higher oxidative stress due to an increase in flight intensity leads to aging and senescence in bees, lending credit to this theory. This study aims to manipulate the natural foraging activity of experimental bee colonies to collect samples representing various levels of foraging effort. In future studies, these samples will be analyzed for molecular signatures of oxidative stress.

References

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Winston, M.L. 1987. The Biology of the Honey Bee. Harvard University Press: Cambridge MA.

Yan, L.J., Sohal, R. S. 2000. Prevention of flight activity prolongs the life span of the housefly, *Musca domestica*, and attenuates the age-associated oxidative damage to specific mitochondrial proteins. Free Radic. Biol. Med. 29, 1143-1150.

Acknowledgements

The authors thank MME and AA for their instruction and guidance through this experiment. Also, much appreciation to Marina Pozzoli, Georgina Mancinelli, Azucena Benito, and David Vardukyan for their assistance. This research was performed though the Summer Undergraduate Research Program at UNLV. This project was funded by NSF grant (IOS-0725030) to MME.

Methods

Three small colonies controlled for number of bees, queen presence, and amounts of pollen and nectar were set with (1) a pollen trap, (2) no changes, and (3) an artificial waterfall simulating rain. The pollen trap was hypothesized to induce more foraging flights, the colony without changes was a control for normal flight, and the waterfall colony was hypothesized to prevent flight - in order to manipulate high, medium, and low levels of activity, respectively. The frequency of bee flight was recorded daily for 5 weeks. Foraging bees were collected and frozen using liquid nitrogen. These frozen bees are being stored at -80°C for future analysis.

- **Group selection**

Three groups of age-matched bees were selected from three different experimental colonies at the emerging period. Selected bees were marked by individually numbered tags according to their hive (Fig. 1) and then released and tracked.

- **Setting manipulation**

A pollen trap (Fig. 2) was set to manipulate a high level of bee activity (frequency of flights per day). Bees in this colony were able to forage from sunrise to sunset, and were hypothesized to increase recruitment of foragers and foraging activity in response to the trap. In the medium control group (Fig. 3), bees were allowed to forage naturally without manipulation from sunrise to sunset. A waterfall device (Fig. 4) mimicking rainfall was set for the low control group. Bees in this setting were able to forage for only about 4 hours per day compared to the normal 12-14 hours per day in the natural setting colony or pollen trap colony.

- **Observation and collection**

The tagged bees' activities were observed and their frequency of flight was recorded for about 5 weeks. Foraging bees from the three colonies were collected, frozen in liquid nitrogen, and stored at -80°C.

Fig. 1: Various tagged bees

Fig. 2: Pollen trap colony

Fig. 3: Control colony

Fig. 4: Waterfall colony

Results and Discussion

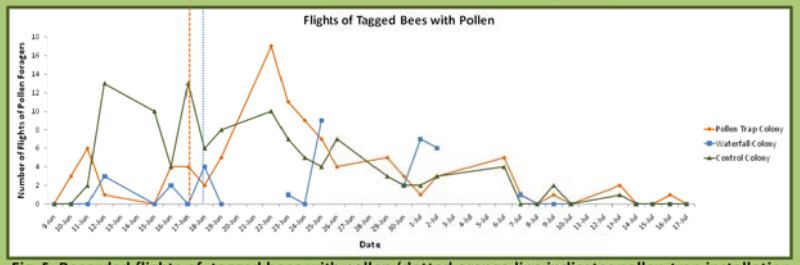

Fig. 5: Recorded flights of tagged bees with pollen (dotted orange line indicates pollen trap installation on 6/17 and dotted blue line indicates waterfall installation on 6/18)

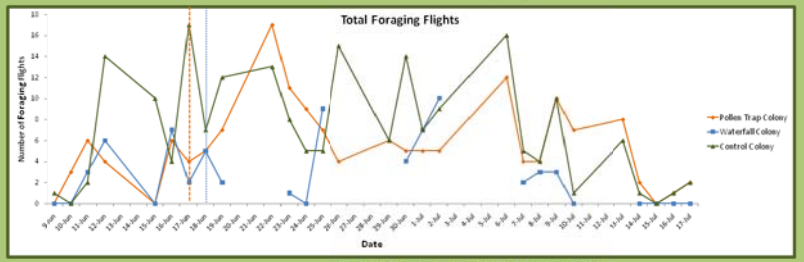

Fig. 6: Foraging flights of tagged bees (pollen and nectar foragers combined)

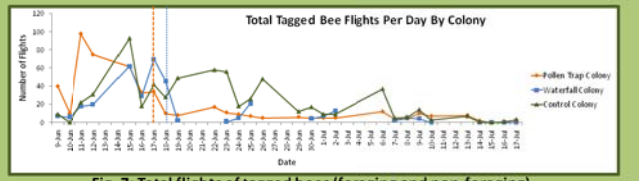

Fig. 7: Total flights of tagged bees (foraging and non-foraging)

Fig. 8: Flights of bees tagged on 6/2/09

Fig. 9: Flights of bees tagged on 6/5/09

- As Fig. 5 shows, the pollen trap did induce more pollen foraging after its installation. When compared to the two other colonies, the pollen trap colony conducted more pollen foraging flights during the experimental period.
- Fig. 6, which shows total foraging records for the three colonies, demonstrates that the pollen trap slightly reduced and the waterfall greatly reduced the number of foraging flights of their respective colony, an expected outcome, for both the pollen trap and waterfall reduce the entrance to the colony in some manner. The number of foraging flights for the control colony, in comparison, demonstrated a relatively steady pattern.
- In Fig.7-9, flight peaks for all three colonies occur somewhat simultaneously throughout the graphs, showing a natural pattern of foraging highs and lows due to pollen/nectar availability, a result of the experiment being conducted in a natural setting.
- In Fig. 8 and Fig. 9, the flights of the oldest tagged bees and the youngest tagged bees are shown, and similar patterns of increasing and decreasing flights during their lifespan can be observed. When comparing Fig. 8 to Fig. 9, it is seen that as the flights of the older bees decrease, the flights of the younger bees increase, indicating increasing recruitment of younger foragers as older foragers naturally die.

Future Studies

- Future research will analyze the bees tagged and collected in this experiment at the molecular level, through analysis of brain and thorax tissue.
- Protein expression will be measured through the use of western blots.
- Quantitative real-time PCR (Polymerase Chain Reaction) will be applied to measure RNA expression in the samples.
- The results of these two analyses and their relation to collected flight data will provide insight into the role gene expression plays in oxidative stress.