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Investigation of gene and protein expression based on Honey Bee (Apis mellifera) aging, flight experience, and behavior

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Honeybees undergo a process of adult behavioral development, spending their first 2-3 weeks working inside the constant environment of the hive. At about 3 weeks of age workers leave the hive as foragers who gather pollen and nectar. Previous research found that bees show an enormous decline in immunity as a result of their transition from regular hive jobs to more difficult foraging activities. Foragers can be forced to go back into hive-tasks, thus becoming “reverted nurses” which may also allow a reversal of immunosenescence. Understanding how this happens could prove to be useful because if there is flexibility in honeybee immunity it could lead us to a better understanding of the human immune response since the honeybee has a very similar genome to that of humans. I plan to use protein and gene expression analysis, along with other measurements to understand how forager bees change back into nurses and how this effects their immune response and their process of senescence and aging.

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Objectives

-Determine differences in gene and protein expression between age-matched bees of differing behavioral types (precocious foragers and typical-age nurses – 7-9 days old, typical-age foragers and over-aged nurses – 19-22 days old, and reverted nurses – 24-26 days old)

- Measure global gene expression of these groups using microarray and quantitative real-time PCR technology to identify genes involved in aging

- Measure expression of different proteins from the genes identified using western blotting

Introduction

Honey bee society is made up of three different castes: a reproductive queen, male drones, and sterile female workers. Adult bees mature through a process known as temporal polyethism (age-based behavioral development) which causes the bees to change their tasks in the hive based on their age. One- to two-week old bees nurse young larvae; two- to three-week old bees build the hive comb, take care of the queen, guard the hive, and take out dead bees. After the third week, bees spend most of the day flying while foraging for pollen, nectar, and water outside the nest (Fig. 1). Workers can switch jobs based on the current needs of the colony. If the older foragers are lost or die the younger bees mature sooner, becoming precocious foragers to provide necessary nutrients for the colony. Similarly, if the younger nurse bees are removed from the hive or their numbers decline, the foragers revert to nursing again to feed the larvae (Winston, 1991). Although the phenomenon of these "reverted" nurses is well known, the physiological and genomic changes that occur during this process are not. Each of these bee types shift by age, behavioral group, and flight experience (Table 1). Due to the flexibility of this model system, these variables can be manipulated and controlled to study changes in gene and protein expression during these shifts in life history.

Flight is metabolically expensive in honey bees (reviewed in Roberts and Elekonich, 2005). Older bees are less able to mitigate oxidative stress caused by flight leading to senescence and death (Williams et al., 2008). We intend to study this process further by determining the expression of key genes and proteins in bees differing by age, behavior, and flight experience.

Honey bees serve as important biomedical models. Many of these honey bee genes are highly conserved and share high sequence similarity with human orthologs. Understanding their function and expression will help us to better understand our own genes, just as studying aging in honey bees will help us to better understand aging in humans.

Bee type	Behavior	Age	Flight Experience
Precocious forager (PF)	Out-of-hive	7-9 days old	~1 week
Typical-age nurse (TN)	In-hive	7-9 days old	<1 day
Typical-age forager (TF)	Out-of-hive	19-22 days old	~1 week
Overage nurse (ON)	In-hive	19-22 days old	<1 day
Reverted nurse (RN)	In-hive	24-26 days old	~1-2 weeks

Table 1. Honey bee sampling groups

Figure 1. Honey bee behavioral development

Materials and Methods

Colony Manipulation:
- We made 8 normal colonies using single drone-inseminated (SDI) queens to obtain brood of two genotypes (V8H x CD and MH x MH, 4 queens of each).

- Newly emerged bees from SDI colonies were each tagged with different-colored enamel paint (Fig. 2) on their thorax to keep track of their age and genotype and introduced into a small nucleus hive to create a "single cohort" colony (SCC).

Collections:
- We collected five different groups: precocious foragers, typical-age nurses, typical-age foragers, overage nurses, and reverted nurses (Table 1).

- Foragers were identified by the paint mark on their thoraces as they returned to the colony entrance and collected directly into liquid nitrogen. Bees observed feeding larvae were identified as nurses and simultaneously collected from each SCC. Reverted nurses were first observed as foragers, then trapped in an empty hive as they returned. Larvae and a queen were introduced into this nurse-free environment to cause foragers to revert to nurse-like behaviors. Three days later the reverted bees were collected.

- All samples were stored at -80°C to preserve RNA and proteins until processing.

Sample Processing:
- Following partial lyophilization, brains will be dissected from head capsules and flight muscle dissected from thoraces on dry ice and stored at -80°C.

- RNA will be extracted from brains and flight muscle using the PicoPure kit (Arcturus).

Microarray:
- RNA samples will be quantified by Nanodrop, used to make double-stranded cDNA, and then aRNA using the MessageAmp II aRNA kit (Ambion).

- aRNA will be labeled using the Kreatech ULS-Cy 3/5 aRNA labeling kit (Kreatech) and hybridized to DGRC oligonucleotide microarrays (Univ. of Illinois, Urbana-Champaign) after aRNA fragmentation (Ambion reagents).

- Labeled arrays will be scanned by GenePix 4000B software (Molecular Devices) and analyzed for significant differences in global gene expression.

Quantitative Real-Time Polymerase Chain Reaction:
- To confirm the microarray results, expression levels of selected genes will be measured with qRT-PCR using SybrGreen labeling with an iCycler (BioRad).

Western Blots:
-Brains will be dissected as before. Brains and thoraces (flight muscles) will placed into JLB lysis buffer and homogenized. Total protein will be measured with the Bradford assay. Each sample will be diluted to 2µg/16µl with JLB, Laemmli buffer and BME. Sample and control proteins will be separated on a 10% SDS-PAGE gel.

- Following transfer (Fig. 3), membranes will be immunoprobed with an antibody specific for the protein. After incubation with a HRP-conjugated secondary antibody, proteins will be visualized with ECL (Amersham) for quantification on a Typhoon Phosphoimager (Amersham).

Figure 2. Paint-marked workers at colony entrance

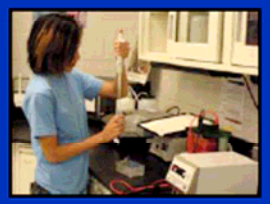

Figure 3. transfer procedure for western blot process

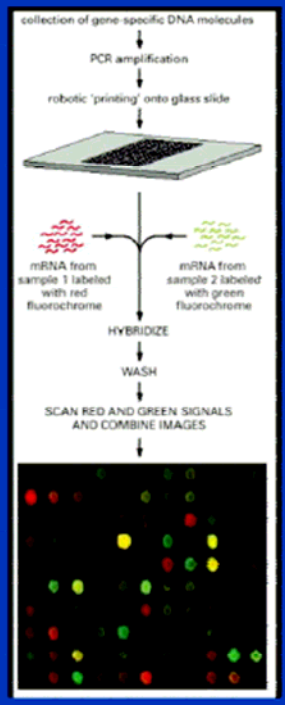

Figure 4. Microarray process

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Future Directions

•These experiments are ongoing – in the next few weeks we will be dissecting collected bees and running the first arrays.

• Expression of genes of interest will be verified with quantitative real-time PCR followed by measures of proteins to further explore function and regulation of these molecules.