Analysis of Genetic Polymorphisms in Aging Genes of *A. mellifera*

Theo Kuchar* and Andrew D. Ammons
Goshen College, 1700 S. Main St., Goshen, IN 46526

***email: tkuchar@goshen.edu**

Abstract

The eusocial society of honey bees allows for an age-based division of labor among the sterile female workers. Through the development of the worker honey bees, this temporal polyethism, which allows for the distribution of labor, is affected by many different genes. Six genes were studied and tested in worker honey bees at different developmental periods from different hives; *jhamt*, *trxr-1*, *nAChRa5*, *stnB*, *rtet*, and *MRPL20*. Using designed primers for each specific gene, the samples were amplified using PCR, and then separated based on size through gel electrophoresis. Amongst the samples of foragers, nurses, one-day olds, pupae, and larvae, no differences in gene structure were noticed for the samples of *jhamt*, *trxr-1*, *nAChRa5*, *rtet*, and *MRPL20*. The *stnB* gene, however, was expressed differently in the larvae, as compared to the pupae and adult bees. Further research showed that the *stnB* gene develops into its adult form as the honey bee develops into its pupal phase.

Introduction

The most widely accepted theory of aging is the free radical theory of aging. This theory proposes that the respiratory use of oxygen creates wastes at the cellular level, called reactive oxygen species (ROS), which, over time, degrade the cell's proteins, lipids, and DNA. This happens, however, when the antioxidant levels and defense systems of the cell are overwhelmed by too many ROS, or when the antioxidant levels and defense systems are too weak to function against ROS (Metcalfe, 2010.)

This experiment used the European honey bee (*Apis mellifera*) as a model organism to examine the influence of genetic polymorphisms in aging genes on honey bee lifespan and aging. *A. mellifera* was chosen for this experiment because its aging process is behaviorally influenced, and therefore can be manipulated (Caron, 1999). The DNA structure of larvae, pupae, newly emerged, nurse, and forager bees were compared to one another. Forager bees have the highest mass-specific metabolic rate measured for any metazoan, and is 10-100 times greater than that of nurse bees (Vance, et. al., 2009.) Because of this, it was predicted that the forager bees would be exposed to more ROS, and thus have differences/polymorphisms in aging genes, as compared to nurses. Six candidate genes were chosen. These genes play crucial roles in growth and development (*jhamt*), reduction of oxidative stress (*trxr-1*), the function of the nervous system (*nAChRa5* & *stnB*), the immune system (*rtet*), and DNA translation (*MRPL20*).

Materials and Methods

Sample Collection

The samples of *A. mellifera* used for this project were collected from the bee hives on the facilities of Goshen College in Northern Indiana.

Genetics

DNA of the collected samples of foragers, nurses, day old bees, pupae, and larvae were obtained using the protocol for a typical phenol/chloroform DNA extraction (Ammons and Hunt, 2008.) The DNA was then amplified through polymerase chain reaction (PCR), and separated based on size through gel electrophoresis.

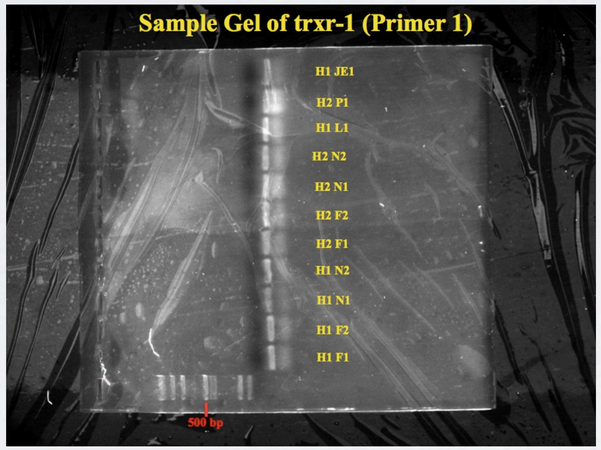

Fig. 1 Sample gel of *trxr-1* (primer 1), H-hive, F-forager, N-nurse, L-larva, P-pupa, JE-1day old

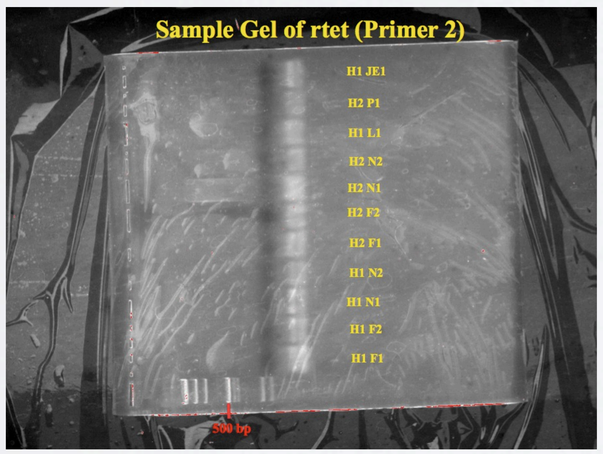

Fig. 2 Sample gel of *rtet* (primer 2), H-hive, F-forager, N-nurse, L-larva, P-pupa, JE-1day old

Results

Two sets of primers were designed for each of the six tested genes; *jhamt*, *trxr-1*, *nAChRa5*, *stnB*, *rtet*, and *MRPL20*. All of the designed primers successfully isolated their specific target gene. After testing the primers, each gene was put through PCR with samples of *A. mellifera* in different developmental stages from different hives. The sample gels of *jhamt*, *trxr-1* (Fig. 1), *nAChRa5*, *rtet* (Fig. 2), and *MRPL20* showed no DNA polymorphisms amongst any of the bee samples. A possible size polymorphism was noticed for the larval sample in the *stnB* sample gel (Fig. 3) With further investigation, the *stnB* gene in larvae and pre-pupae is shorter and consists of only one band, whereas that of pupae and adult bees is larger in size and contains two bands (Fig. 4)

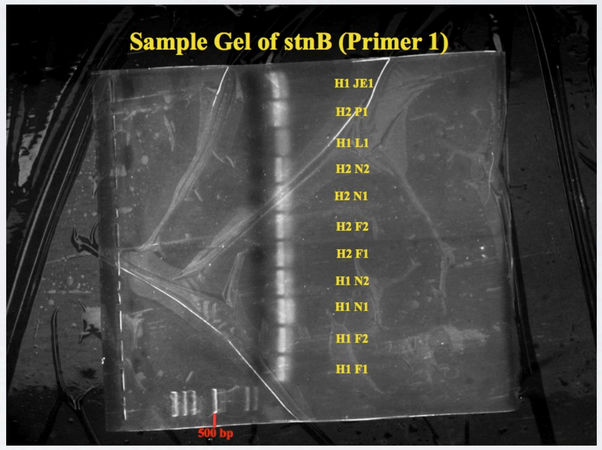

Fig. 3 Sample gel of *stnB* (primer 1), H-hive, F-forager, N-nurse, L-larva, P-pupa, JE-1day old

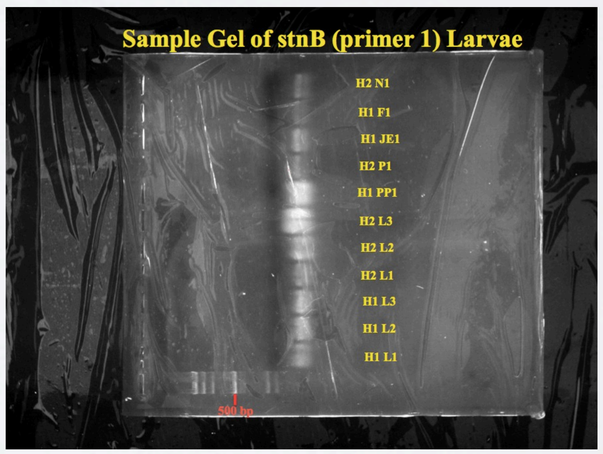

Fig. 4 Sample gel of stnB larvae (primer 1), H-hive, F-forager, N-nurse, L-larva, PP-pre pupa, P-pupa, JE-1day old

Discussion

When testing the designed primers, each one produced identical polymorphisms among three different forager bees from the same hive. Once the primers were known to be successful in isolating the genes, a sample agarose gel with bees in different developmental stages was run for each gene. As shown by Fig. 1 and Fig. 2, no differences were observed in the gels. As a result, *A. mellifera* does not seem to exhibit differences among the *jhamt*, *trxr-1*, *nAChRa5*, *rtet*, and *MRPL20* genes in structure. This does not mean that these genes do not differ in the honey bee throughout different developmental stages; further research at the RNA and protein level could indicate differing expression of genes. This future research could include enzyme digests and quantitative real-time PCR (qRT-PCR) of these genes.

After completing the sample gel of *stnB*, a size polymorphism was noticed for the larval sample, as compared to the pupa and adult samples. Further research confirmed the difference in the *stnB* gene of the larvae as compared to the pupae and adult bees. As shown by Fig. 4, the samples of the larvae and pre-pupae are shorter in length and consist of a fuzzy, spread out single band, whereas the pupa and adult bees express a clear double band. Amongst several possible explanations, this difference in gene structure may indicate that the nervous system of the larva is not fully developed, unlike that of a pupa or adult worker bee. The difference in structure in the *stnB* gene could indicate clear signs of aging development, with regard to synaptic plasticity, in the bee nervous system. Even though the *stnB* gene is not widely understood in the honey bee, research on this gene in *Drosophila melanogaster*, the fruit fly, suggests that it encodes for endocytotic proteins related to signal transduction in the nervous system. In order to make conclusions about the difference in the *stnB* gene between larvae and adult bees, however, further research must be done.

References

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