

The Effects of Aging and Behavioral Development on Gene Expression in Bumble Bees, *Bombus huntii*

Introduction

Senescence, also known as aging, is the decline in physiological function, reproductive success, and survival due to age (Finch, 1990). While the effects of aging on the whole organism are well documented, it is only in recent years that we have begun to explore the cellular level mechanisms underlying aging. One theory of aging is that the body produces and accumulates reactive oxygen species (ROS), called free radicals, which react with other molecules in the body causing damage to the body.

The accumulation of ROS, also known as oxidative stress, can be caused by many daily activities such as eating and exercising. In addition, it appears that reducing ROS can increase lifespan. The first such study was done in 1930 and found that reducing caloric intake in mice (which lowers the amount of superoxide radicals) increased the mice's longevity (Karp, 2010). Free radicals can attack the cell's proteins, lipids, carbohydrates and DNA which causes damage to the cell's membrane and DNA and can eventually cause cell death (Singh, *et al.*, 2004). As an organism gets older, its body is more susceptible to damage from the free radicals as it becomes less efficient at producing antioxidants which could alter its gene expression. Thus, studies suggest that accumulation of free radicals can lead to neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Yuan, *et al.*, 2010).

To protect the body from free radicals, antioxidants are present to prevent or retard excess oxidation reactions in the body. In addition, cells contain several enzymes which destroy free radicals thereby minimizing damage. Two well characterized examples are the enzyme superoxide dismutase (SOD), which destroy the superoxide radical, a type of radical formed when oxygen has an extra electron (Karp, 2010), and catalase (CAT), an enzyme that facilitates the decomposition of H_2O_2 to H_2O and O_2 so that no superoxide radicals form. When the equilibrium between antioxidants and ROS is disturbed, ROS can start to attack the nucleic acids which cause changes in the DNA (Harman, 1988).

I intend to study the cellular basis of aging in the bumble bee, *Bombus huntii*, to see if this animal can serve as a good model for studies of the genetic cellular basis of aging. If so, I will also see if I can identify novel genes that are related to cellular aging and relate those genes to specific tissues. Bumblebees offer several advantages over mammalian models. First, because

they have short life spans, it will be possible to study the cumulative effects of oxidative stress from birth to death in the short summer period. Second, they can easily be observed while living a normal life (as opposed to caged lab animals), allowing real data of their behavior to be obtained. Moreover, because different individuals within a bumblebee colony specialize for different behavioral tasks, such as foraging and in-hive brood care, I will be able to analyze how differences in life history (flying vs. not, reproducing vs. not) are correlated to changes in gene expression and age.

In the proposed research, I hypothesize that older bumble bees will show higher levels of key oxidative species than newly emerged bumble bees. I also predict that bumble bees of the same age, but of different behaviors will differ in the amount of enzymes such that bumble bees that have higher physiological stress will have higher expression levels of genes encoding the free radical inactivating enzymes than less stressed bumble bees. I will look for these differences using micro array analysis of gene expression.

To explain my study of the oxidative stress in bumble bees, I will first review their life history. Bumblebees are social insects and live communally in hives with many workers. The workers specializing as nurses take care of new larvae and honey in the hive, whereas foragers must leave the nest to find and collect pollen. Foragers must expend more metabolic energy as they have to fly and navigate. In a study on honey bees, increased flight and cognition in these foragers increases the oxidative stress put on the flight and brain tissue, respectively, leading to more ROS's (Williams *et al.*, 2008). The same study suggested that the different tasks in honey bees lead to differences in senescence, as foragers expend more energy in flight and cognition, which produce higher levels of free radicals, and reduced the foragers' longevity. Therefore, by comparing age-matched bumble bee nurses and foragers using microarray, possible differences could be seen in gene expression depending the bee's life style.

The extent of oxidative stress differs from tissue to tissue. For my study, I aim to focus on tissue types with relatively high levels of oxidative stress, such as the brain, locomotive, and reproductive tissues. The brain is a metabolically expensive organ. Thus, it is expected that older bees that participated in more mentally challenging tasks, such as older foragers, will be more susceptible to damage from free radicals, as antioxidant production decreases with age. The second tissue, the locomotive muscle tissue, has also been found to be affected by ROS in a study on mice that participated in strenuous exercise (Ji, 1999). Flight muscles in bees, a type of

locomotive tissue, are one of the most energetically expensive organs to control and maintain (Schippers *et al.*, 2009). Therefore, it is expected that the flight muscle of older foragers, who have been flying most of their lives, will have more damage compared to the nurses. The third tissue is the reproductive tissue as it also receives much damage from ROS. As a result, the brain, flight, and reproductive tissue of *B. huntii* will be examined.

Microarray analysis will be used to determine differential gene expression in the nurses and foragers. The gene expression of the bees is predicted to vary with nurses having the lowest amount of genes that code for antioxidative enzymes, while the bees that forage the most will have the highest amount of genes. Other differences in gene expression will be analyzed to determine if age and behavioral lifestyles affect any other genes. Such differences appear likely as not all aging appears to be directly related to the accumulation of ROS.

Many recent studies were conducted on honey bees to see how senescence is affected by ROS, SOD, and CAT (Williams *et al.*, 2008; Seehuus, 2006). These studies agree that antioxidants can help slow down aging by counteracting ROS. In my proposed study, I will (1) use microarray analyses to determine if there are any differences in gene expression between the various levels foraging and whether any specific affected gene can be identified; and (2) determine how age plays a role in the gene expression of *B. huntii* with respect to behavioral lifestyles .

Methods/Timeline-

In order to determine how age and behavioral development affects gene expression, behavioral studies will be performed on *Bombus huntii*. Queen bumble bees of *Bombus huntii* will be collected in the wild in late April as they emerge from hibernation. I will then initiate several colonies and mark each new batch of worker bees with different color paint to keep track of their age. Once the colony is stabilized and the bees have differentiated into their roles, I will observe the hives for 6 hours (3 hours on each of 2 successive days) to determine which bees leave the nest (these are the foragers) using audio tape, I will also simultaneously video tape the hive so that I can gain a complete record of the activities undertaken by the in hive workers. After this, I will distinguish the nurses from the foragers using numbered tags.

Once I have described the workers and their tasks, I will freeze the bees in liquid nitrogen, which will help preserve them, and ship them with to UNLV over dry ice to perform further genetic analyses. Following the bees, I will be assisted by Professor Michelle Elekonich,

who has been researching this topic on honey bees, in examining the brains, flight, and reproductive tissue of the nurses and foraging bees using microarray to determine the differences in differential gene expression which will take about a week. The data from the microarray will be used to better understand how physiological stress from reproduction (vs. no reproduction), flight (vs. no flight), and cognitively demanding tasks (vs. no cognitively demanding tasks) affect gene expression in nurses and foragers.

References

- Belles, M., Alonso, V., Linares, V., Albina, M.L., Sirvent, J.J., Domingo, J.L., Sanchez, D.J.. 2010. Behavioral effects and oxidative status in brain regions of adult rats exposed to BDE-99. *Toxicology Letters*.
- Finch, C.E. 1990. *Longevity, Senescence, and the Genome*. Chicago: The University of Chicago Press.
- Harman, D. 1988. Free Radicals in Aging. *Molecular and Cellular Biochemistry*. 84:155-161.
- Ji, L.L. 1999. Antioxidants and Oxidative Stress in Exercise. *Proceedings of the Society for Experimental Biology and Medicine*. 222:282-292.
- Karp, G. 2010. *Cell and Molecular Biology Concepts and Experiments*, sixth ed. John Wiley & Sons Inc., NJ, p.34.
- Remolina, S.C., Hafez, D.M., Robinson, G.E., Hughes, K.A. 2007. Senescence in Worker Honey Bees, *Apis mellifera*. *Journal of Insect Physiology*. 53(10): 1027-1033.
- Schippers, M.P., Dukas, R., Smith, R.W., Wang, J., Smolen, K., McClelland, G.B. 2006. Lifetime Performance in Foraging Honey-bees: Behaviour and physiology. *Journal of Experimental Biology*. 209: 3828-3836.
- Seehuus S.C., Krekling T., Amdam, G.V. 2006. Cellular Senescence in Honey Bee Brain is Largely Dependent of Chronological Age. *Experimental Gerontology*. 41(11): 1117-1125.
- Shippers, M.P., Dukas, R., McClelland, G.B. 2010. Lifetime- and Caste-Specific Changes in Flight Metabolic Rate and Muscle Biochemistry of Honeybees, *Apis mellifera*. *Journal of Comparative Physiology B*. 180:45-55.
- Singh, R.P., Sharad, S., Kapur, S. 2004. Free Radicals and Oxidative Stress in Neurodegenerative Diseases: Relevance of Dietary Antioxidants. *Journal, Indian Academy of Clinical Medicine*. 5(3):218-25.

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Williams, J.B., Roberts, S.P., Elekonich, M.M. 2008. Age and Natural Metabolically-Intensive Behavior Affect Oxidative Stress and Antioxidant Mechanisms. *Experimental Gerontology*. 43(6): 538-49.

Yuan, H., Zhang, Z.W., Liang, L.W., Shen, Q., Wang, X.D., Ren, S.M., Ma, H.J., Jiao, S.J., Liu, P. 2010. Treatment Strategies for Parkinson's Disease. *Neuroscience Bulletin*. 26(1): 66-76.