

The Effect of Chronological Age and Foraging Experience on Mental Flexibility in Bumble Bees

A Major Qualifying Project Report
submitted to the Faculty of
WORCESTER POLYTECHNIC INSTITUTE
in partial fulfillment of the requirements for the
Degree of Bachelor of Science
by

Erin Beaulieu

Caitlin Heindl

Report Submitted to:
Professor Robert J. Gegear

April 25, 2013

The views and opinions expressed herein are those of the authors and do not necessarily reflect the positions or opinions of Worcester Polytechnic Institute.

This report is the product of an educational program and is intended to serve as partial documentation for evaluation of academic achievement. The report should not be construed as a working document by the reader. WPI routinely publishes these reports on its web site without editorial or peer review.

Table of Contents

Table of Figures	2
Table of Tables	3
Abstract	4
Introduction.....	5
Mental Flexibility and Task Switching	5
Bees as Foragers and Flower Constancy.....	6
Bees as Pollinators	7
Bee Population Declines	7
Importance of Pollinators and Impact of Declining Populations	8
Influence of Aging	9
Experiment	10
Methods	11
Bees.....	11
Flowers and arrays	12
Training.....	13
Testing.....	13
Variables	16
Percent Task Repetition	16
Switch Cost	16
Results.....	17
Task Repetitions.....	17
Switch Costs.....	18
Discussion	20
Acknowledgements.....	22
References.....	23

Table of Figures

Figure 1: The (a) bumble bee colony was kept in a (b) cardboard box containing the hive which was attached to (c) a large mesh enclosure with experimental array; also where bees were allowed to fly and forage daily	11
Figure 2: Bumble bee with chronological age marking (blue & green) on thorax and foraging age marking (white) on abdomen	12
Figure 3: a) Flower made of an Eppendorf tube with foam corolla b) Flower inserted into foam board ...	13
Figure 4: Experimental Procedure Schematic – a) pre-training arrays b) training array c) 2 task array d) 1 task yellow array e) 1 task geranium array	14
Figure 5: Proportion of Task Repetitions over 120 visits for (a) young chronological and foraging age, (b) old chronological and young foraging age, (c) old chronological and foraging age. Data points with differing letters are significantly different	17
Figure 6: Proportion of Task Repetitions for Last Block of 40 Visits comparing (a) bees with young and old chronological ages, while controlling for foraging age, (b) bees with young and old foraging ages, while controlling for chronological age	18
Figure 7: Switch Costs over 120 visits for (a) young chronological and foraging age, (b) old chronological and young foraging age, (c) old chronological and foraging age. Data points with differing letters are significantly different (statistically, $p \leq 0.05$) from each other.....	18
Figure 8: Switch Costs for Last Block of 40 Visits comparing (a) bees with young and old chronological ages, while controlling for foraging age, (b) bees with young and old foraging ages, while controlling for chronological age	19

Table of Tables

Table 1: Classification of Tested Foragers based on Chronological and Foraging Ages	12
Table 2: Division of Tested Bees among Age Groups and Number of Tasks	15

Abstract

Mental flexibility is the ability of the brain to rapidly and effectively shift from one mental operation to another, or task switch. Additional time is needed to complete the extra mental processing involved with performing a task switch; this is known as a switch cost. This process is used by foraging bumble bees when they are faced with many different flower types and must decide when to switch between types. Evaluating the cost of switching between flower types can be used to measure a bee's mental flexibility. It is possible that age or foraging experience could decrease mental flexibility, as seen in other organisms, resulting in a higher switch cost. The purpose of this study was to determine if a bumble bee's age or foraging experience impacts its mental flexibility, therefore altering its ability to forage proficiently. A novel behavioral assay was used to measure a bee's foraging efficiency. It was found that higher levels of foraging experience led to decreased mental flexibility; this was independent of the bee's chronological age, which had no impact on mental flexibility. These results indicate that increased experience correlates with cognitive decline in bumble bees. This effect could have ecological implications in that it could compound with environmental stressors, helping to explain critical bumble bee population declines.

Introduction

Mental Flexibility and Task Switching

Mental flexibility is the ability of the brain to rapidly and effectively shift between two mental operations, or task switch (Wecker *et al.*, 2005). In humans, task switching involves the use of several areas of the brain (Dove *et al.*, 2000) and necessitates a “task-set reconfiguration.” This process requires the subject to change the stimulus in focus, determine or retrieve from memory the new goal and the method of reaching that goal, and responding in a way that allows it to meet the goal (Monsell, 2003). Furthermore, the brain has to inhibit the processes working on the previous task (Monsell, 2003), and, if both tasks are new to the subject, overcome any memory interference (learning one task shortly after another can block and potentially erase the memory of the first task; this effect wanes as the subject better learns both tasks – Dukas, 1995; Chittka *et al.* 1999).

Additional time is needed to complete the extra mental processing involved with performing a task switch, and studies have shown that there is a cost to voluntarily switching between tasks, with reaction times increasing when a task changes as compared to when a task repeats. The difference between these two reaction times is known as the switch cost (Arrington, 2004; Dove *et al.*, 2000). In our study, we look at this switch cost in foraging bumble bees. Bumble bees are faced with many different flower types when foraging and they must decide to switch between the flower types (perform a task switch) or forage on a single flower type. It does not benefit the bee to switch between flower types if the switch cost is high; the bees are then wasting more time deciding which flower to visit, decreasing their overall foraging efficiency. In this case, bees should favor a more constant foraging behavior, where they forage from a single flower type. If the cost of switching is low, however, it is advantageous to forage from multiple flower types, because there is not a significant time cost to choosing to visit a different flower type.

This trend has been observed in bees in several studies. Bees that switch between two foraging tasks (such as foraging based on odor and foraging based on color) forage less efficiently than bees that complete only one foraging task. When the flowers were spaced farther apart, the bees had more time to process the two potential tasks and choose to switch between them. As a result, the switch cost decreased and the bees switched between flower tasks more often (Leigher and Rinaldo, 2012). Bees also demonstrated decreased foraging rates when switching between flowers with numerous varying traits. This is likely a result of the fact that distinguishing between dissimilar flowers requires increased use of time and activated memory to store and process the various traits (a higher switch cost exists; Gegear & Laverty, 2005). Because the switch cost is higher, the bees’ efficiency, as expressed by foraging rate, decreases. As there is a documented cost to switching between tasks, a constant foraging behavior in bumble bees has been shown to be the result of an economic foraging decision, based, at least in part, on the bees’ working memory limitations (Gegear & Thompson, 2004; Leigher & Rinaldo, 2012; Gegear & Laverty, 2005).

Bees as Foragers and Flower Constancy

In each bumble bee colony there is one dominant queen bee whose main responsibility is laying eggs to maintain the colony's population. The rest of the colony consists of female workers (with the exception of late summer and early fall when new queens and male bees are produced to prepare for winter) that either maintain the hive and new eggs or leave to forage for food (bumblebee, n.d.). Foragers need to bring back enough food to ensure that the colony survives and that new eggs can be laid and supported. It is therefore predicted that bumble bees adopt optimal foraging methods to maximize the ratio of energy gained per energy used. This requires the bee to obtain the maximum reward while expending the minimum amount of energy (Wells & Wells, 1983).

In spite of this many pollinating insects, including bumble bees, have displayed a seemingly suboptimal foraging technique, a specialization phenomenon known as "flower constancy." When demonstrating this behavior, the pollinator will visit only a single flower species, despite the fact that flowers of equal or greater reward are also available (Waser, 1986; Chittka *et al.*, 1999; Gegear & Lavery, 2001). This foraging method has clear benefits for the flowers, as they have decreased risk of losing their pollen to or having their stigmas (pollen receiving part of flowering plants) obstructed by pollen from heterospecific (of a different species) flowers (Waser, 1986; Gegear & Lavery, 2001). However, the benefits of flower constancy for the pollinators have not been as evident, as it can result in less efficient foraging when flowers of equal or great reward and within a shorter traveling distance are bypassed (Wells & Wells, 1983).

Several hypotheses have been made that link flower constancy in bees to cognitive limitations. One such hypothesis is the "interference hypothesis". This hypothesis argues that, because of cognitive limitations on the short term or working memory of bees, bees may forget how to handle a specific flower type if they learn how to handle a novel flower type. This occurs because the memory of handling the new flower type interferes with and potentially replaces the memory of how to handle the old flower type (Waser, 1986; Lewis, 1986; Goulson, 2000; Gruter & Ratnieks, 2011). This would favor constancy to minimize the loss of handling memories and the cost of relearning techniques (Gegear & Lavery, 2001; Chittka *et al.* 1999). Another hypothesis, known as the "learning investment hypothesis," reasons that, in order to learn how to manipulate and recognize a new flower type, a bee has to use time and energy in a less efficient way than if it foraged from a known flower type (Goulson, 2000; Chittka *et al.*, 1999; Waser, 1986; Gruter & Ratnieks, 2011). Constancy is a way to avoid these periods of lower efficiency. The "search image hypothesis" is yet another possible explanation for flower constancy. This idea postulates that a bee selectively chooses a flower to search for, which makes it more attentive to the cues of that flower and better able to discriminate that flower type from the background. If bees cannot mentally process search images of more than one flower type (cannot recognize multiple rewarding flower types when scanning the foraging environment), then they

are more likely to be constant because they are actively searching for only one flower type (Goulson, 2000; Gruter & Ratnieks, 2011).

There are also several floral variables that can affect the extent to which bumble bees exhibit flower constancy. For example, bees have been shown to be less constant when the distance between flowers increases (increased traveling time and cost) and more constant when the flowers are spaced more closely together (Leigher & Rinaldo, 2012; Gegear & Thompson, 2004; Chittka *et al.*, 1999). Another factor influencing constancy is the similarity between the available flower types. When available flower types differ in more than one trait (color, size, odor, shape, etc.), bees are more likely to show constancy than if the flowers only differ in one trait, such as color (Gegear & Lavery, 2005; Chittka *et al.*, 1999). The reward amount of visiting each flower has also been shown to impact constancy; when a known flower species provides a high reward, bees are less likely to switch between flowers and incur the switch cost. When obtaining less reward from a flower species than normal, bees show a higher likelihood of switching (Chittka *et al.*, 1999). Many of these impacts are also dependent on the bee's knowledge of the reward status of the available flowers. If bees are trained to two rewarding flower types and have learned that they both offer an equally high reward, they are more likely to switch between the two and visit closer flowers than if they have learned that the two species offer relatively low rewards (Gegear & Thompson, 2004). In other words, it is worth incurring the switch cost if rewards are high in both flower types but not if rewards are low in both flower types. On the other hand, if bees are uncertain about the reward status of novel flowers and are acquiring an acceptable reward by visiting one known flower type consistently, they have less need to learn about other potential food sources. There is no advantage to expending time and energy sampling other flowers and taking the risk of not collecting enough food when the bees are already collecting the amount of food they need. This could easily change if the bees' constant flower choice no longer provides an adequate amount of food (Chittka *et al.*, 1999; Gruter & Ratnieks, 2011).

Bees as Pollinators

While bees are foraging, they are simultaneously acting as animal pollinators. Pollination is the reproductive process by which male gametes (pollen) are transferred to the female reproductive organ (ovule) within the flower. Typically, animal pollination involves individuals passively picking up pollen from one flower and transferring it to another flower as they are searching for food (pollen and nectar rewards contained in the flower; Delaplane and Mayer, 2000). Social bees are largely responsible for the pollination of about 130 agricultural plants in the United States, and nearly 400 agricultural plants worldwide. Different bee species vary in which plants they pollinate, their foraging habits, and their adaptability (Committee, 2007).

Bee Population Declines

Concerns have been raised over noted bee population declines in recent decades in various locations worldwide, including in the United States (Committee, 2007). While no one cause is to blame, the combinations of causes have negative effects and may result in damages to ecological communities as well as an increased difficulty to achieve pollination in the agricultural industry.

This may in turn lead to increased pricing on all food or products produced from such pollinated plants. It may also indirectly affect other economic sectors, such as livestock, which may be fed using foods produced by plants dependent on pollination (Committee, 2007).

Causes known to negatively affect bee colony health include pathogens, parasites, pesticides, transgenic crops, invasive species and climate change (Committee, 2007). While their effects range in severity, they collectively create big issues for bee populations. Pathogens, such as *Pacnibacillus larvae*, infect colonies and can be fatal. While treatment is available for some of the most common pathogens, they spread easily and cause fatalities if left untreated (Committee, 2007). Another cause affecting entire colonies is parasitic infection. Parasitic mites became a growing issue starting in the 1980's, with states seeing the biggest losses attributed to parasites, ranging from 30-80%, in 1995-1996 (Delaplane and Mayer, 2000).

The effects of pesticides differ in severity based on the type of pesticide and the affected species of bee, but they range from death to the weakening of the individuals' navigational and foraging ability (when sub-lethal doses are used - Committee, 2007). While the usage of pesticides has received negative attention, most colony losses occur from accidental and careless application or failure to follow provided recommendations (Committee, 2007). Similar to pesticides, certain transgenic crops have been attributed to having negative effects on pollinator species for their production of pesticidal proteins intended to target specific pest species (Committee, 2007). Invasive species are another cause of population declines; Africanized honey bee colonies specifically affect colonies of European honey bees. Their colonies grow faster, are able to nest in a wider variety of locations, and reproduce more often, giving them an advantage over European species. The small hive beetle, which has a diet of pollen and honey, is seen as a potential threat to colonies. Native to South Africa, it was first found in the United States in 1998, and its brooding habits cause damages to colonies and bee keeping equipment (Committee, 2007). Climate change can also affect pollinators. With changing temperatures, precipitation, carbon dioxide concentrations, ozone, and UV light levels, plant growth and flowering is altered, in turn affecting the species that forage on them (Committee, 2007). Habitat loss, caused by agriculture, grazing, urbanization and fragmentation of natural habitat into areas too small to support diverse communities, is also a primary cause of bumble bee population declines in Europe (Goulson, 2008).

Importance of Pollinators and Impact of Declining Populations

Approximately 85% of all flowering plants (approximately 300,000 different species; Ollerton *et al.*, 2011) rely on animals for pollination. As a result, pollinators play a vital role in the communities they inhabit. Reductions in pollinator populations lead to reduced plant fertility and negatively affect overall plant diversity (Moller *et al.*, 2012). Lack of pollination could lead, in the most extreme circumstances, to ecosystem collapse. For example, in certain tropical communities, figs are consumed by 80% of vertebrates populating the area. Losing their pollinators would cause enormous reductions in fig production resulting in detrimental effects to the populations relying on figs as a food source (Allen-Wardel *et al.*, 1998). In one case taking

place in New Brunswick, use of pesticides killed a large number of the bee populations in one area, and a reduction in blueberry production resulted (Allen-Wardel *et al.*, 1998). This in turn affected a range of organisms from birds and insects to bears and humans. The reduction of pollinator species in ecological communities results in harmful outcomes to those plant species they visit. This leads to further damages to all those species utilizing the plants, whether it be as a food source, a source of shade, a nesting habitat, or as protection.

From an agricultural standpoint, the tremendous importance of animal pollinators has been recognized for years. Indeed, 1/3 of the food that humans consume is the direct or indirect result of a pollination event (Committee, 2007). For example, crops such as apples, almonds, avocados, blueberries and cranberries, are produced exclusively via animal pollination (Delaplane and Mayer, 2000). Moreover, crop plants that do not receive adequate pollination may produce small, misshapen fruit in lower yields (Delaplane and Mayer, 2000). From an economic view, crops in this condition have a lesser value; therefore the presence of pollinators is highly beneficial. While many different pollinators exist, the most human-managed and semi-domesticated are bees. Animal pollination is estimated to be valued in the billions but varies from setting to setting based on the crop dependence on animal pollination (Committee, 2007). Therefore, declining pollinator populations would have devastating effects on the agricultural community.

Influence of Aging

As mentioned above, there are already several environmental stressors (pathogens, pesticides, parasites, etc.) thought to be negatively impacting bee population declines; this may be the result of these stressors altering the bees' mental flexibility. It is possible that age or experience could be magnifying these impairments, thus amplifying the effect on bee populations. Chronological age has been shown to negatively affect cognitive performance for numerous animals throughout the animal kingdom, such as rats (Gage *et al.*, 1984), rabbits, mice (Engle & Barnes, 2012), and humans (Christensen *et al.*, 1997; Wecker *et al.*, 2005). It was predicted that this trend would carry over to bumble bees, meaning that bumble bees with a higher chronological age would show a decrease in mental flexibility. This would result in higher switch costs and therefore less efficient foraging.

Honey bees, however, have been found to perform equally well at chronologically old and young ages (Behrends & Scheiner, 2010), causing researchers to investigate the effect of social role (forager or nest bee) on the honey bees' mental flexibility. This role can change with changes in colony demography, with bees becoming foragers at chronological ages ranging from 5-200 days (Behrends *et al.*, 2007). While honey bees of differing chronological ages did not demonstrate a decrease in ability to learn or discriminate odors, differences in cognitive performance were found in those bees with more foraging experience. When learning new odors, honey bee foragers that had been foraging for 15 or more days demonstrated slower acquisition rates (Behrends *et al.*, 2007). It was also observed that bees with higher foraging ages learned odors more accurately and showed the ability to discriminate between odors more easily. Bees with

both young and old foraging ages eventually reached the same level of performance, the only difference found was in learning pace (Behrends *et al.*, 2007). Based on these results, it was predicted that bumble bees would show a decrease in mental flexibility as they gain foraging experience, again resulting in higher switch costs and overall less efficient foraging.

Experiment

In this study, a series of experiments was designed to compare the mental flexibility, in terms of flower constancy and switch costs, of bumble bees of varying chronological ages and amounts of foraging experience (as determined by the number of days foraged, or foraging age). This was accomplished by measuring and comparing the bees' performance on arrays that allowed for a single foraging task, either color- or odor-based, and arrays that allowed for two simultaneous foraging tasks, both color- and odor- based, with both tasks being of equal reward. The bees were split into three groups, chronologically young with a low foraging age, chronologically old with a low foraging age, and chronologically old with a high foraging age. It tested two predictions. The first prediction was that bumble bees with increased chronological age would show declines in mental flexibility. The second prediction was that, like in honey bees (Behrends *et al.*, 2007), cognitive aging in bumble bees is affected by the role the bee has in the colony, with cognitive decline beginning in bees after they start foraging. Therefore, an increased foraging age will also result in diminished mental flexibility. A decrease in mental flexibility results in less efficient task switching. The bees with increased chronological and foraging ages will therefore be less efficient foragers and demonstrate a higher cost for switching between tasks. The results of this study could be used to determine when in a bee's lifetime it is able to forage optimally by making economic foraging decisions. Less efficient foraging decisions made as a result of decreased mental flexibility may have negative consequences on colony survival and reproduction because the efficiency of the foragers is an integral part of the wellbeing of the colony (Wells & Wells, 1983). This knowledge could be useful in learning how to increase the overall fitness of a bumble bee colony and the bumble bee population in general.

Methods

Bees

This experiment used colonies of the species of bumble bees *Bombus impatiens*. The colonies were obtained from Biobest Biological Systems in Leamington, Canada. The colony (Figure 1a) was kept in a 30x22.5x19 cm cardboard box containing a hive (Figure 1b) attached with a wire mesh tube approximately 2.5 cm in diameter to a larger mesh enclosure (Figure 1c). This space, where the bumble bees foraged, was 1.83x1.83x1.83 m. The enclosure contained a table upon which the constructed feeding and testing arrays were set. It was lit with three fluorescent 32 watt bulbs and one ultra violet 32 watt bulb, all 1.22 m long.

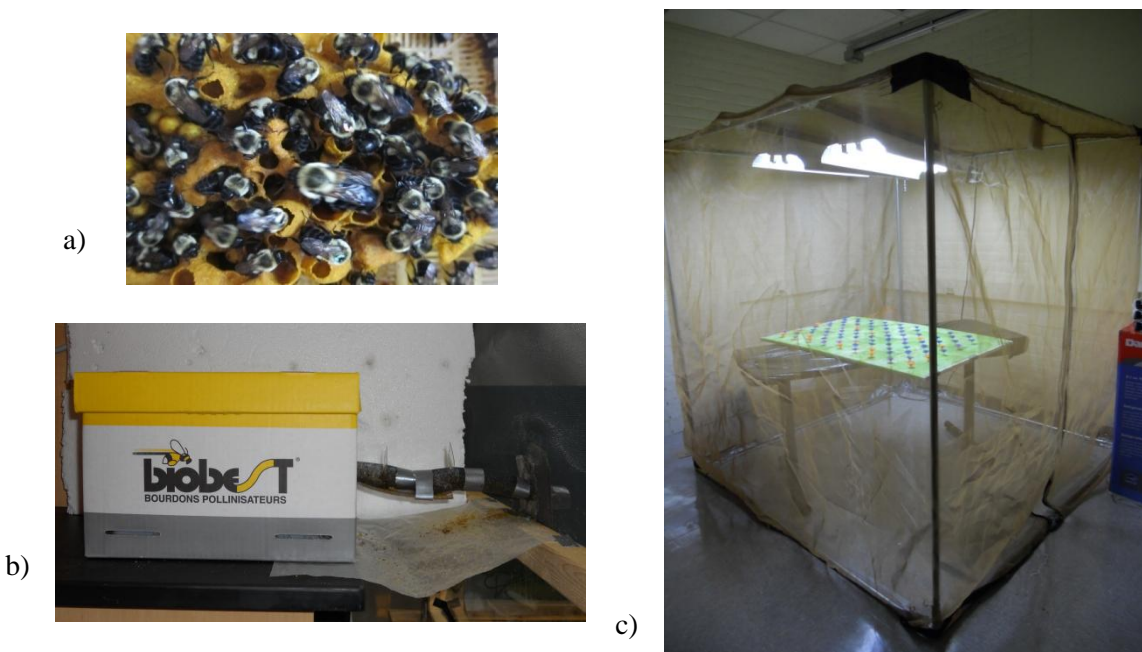


Figure 1: The (a) bumble bee colony was kept in a (b) cardboard box containing the hive which was attached to (c) a large mesh enclosure with experimental array; also where bees were allowed to fly and forage daily

The bees were fed a 30% sucrose solution twice a day and given one pollen log daily. Upon arrival, every bee in the colony was marked with white non-toxic acrylic paint on the thorax above the wings. To mark the bees, all lights in the room except a red heating lamp were shut off and forceps were used to grasp the bee by its hind leg. The bee was then chilled in a refrigerator until unconscious. Every day the colony was observed for newly hatched bees, and newborn bees were marked with a different color non-toxic acrylic paint on the thorax to track chronological ages. The feeding array was observed daily while the bees foraged, and any bees seen foraging on the array for the first time were captured using clear plastic vials or a butterfly net. They were then marked with thin line(s) of non-toxic acrylic paint of varying colors on the abdomen to track foraging age. An example of a marked forager can be seen below in Figure 2.

Bees were classified into three groups based on their chronological and foraging ages, as shown below in Table 1. Each test bee was tested only once and then removed from the colony.



Figure 2: Bumble bee with chronological age marking (blue & green) on thorax and foraging age marking (white) on abdomen

Table 1: Classification of Tested Foragers based on Chronological and Foraging Ages

Group	Chronological Age	Foraging Age
Young Chronological Age & Young Foraging Age	≤ 19 days	≤ 6 days
Old Chronological Age & Young Foraging Age	≥ 20 days	≤ 6 days
Old Chronological Age & Old Foraging Age	≥ 20 days	≥ 7 days

Flowers and arrays

Flowers were constructed using 1.5 mL Eppendorf centrifuge tubes with the cap removed. Flower tubes were clear, blue, orange, purple, or yellow. To imitate the flower-like appearance of a corolla, circular foam cut-outs were secured around the mouth of the tube to create a 3.0 cm diameter; foam color was matched to tube color. Figure 3 below depicts a constructed flower and a flower inserted in a foam board with a bee showing relative size. Yellow, orange, and purple flowers were unscented. White feeding flowers were scented with apple and blue flowers were scented with clove, mint, or geranium. Odors were prepared by mixing 4 μ L scented oil with 196 μ L pentane. Odors were dispensed in 5.0 μ L amounts on the foam corolla of designated flowers. Arrays were constructed from 3.5 cm thick foam boards covered with patterned green construction paper.

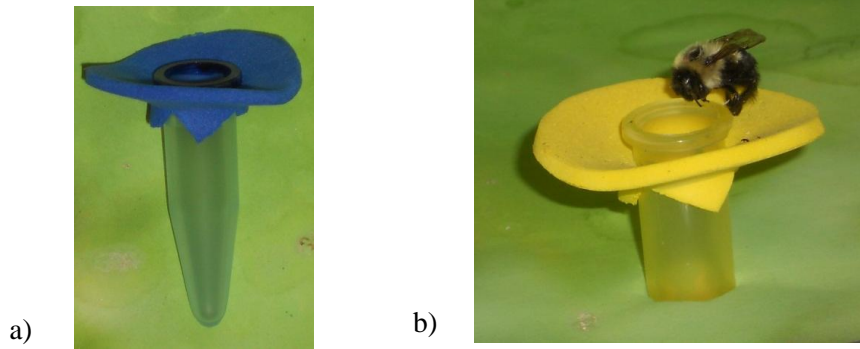


Figure 3: a) Flower made of an Eppendorf tube with foam corolla b) Flower inserted into foam board

Training

Before being tested, bees went through pre-training and training procedures. The pre-training step taught foragers to recognize the rewarding flower tasks: yellow unscented (color task) and blue geranium-scented (odor task). Two pre-training arrays (Figure 4a) each contained six of either the yellow unscented or geranium-scented blue flowers, filled approximately halfway with sucrose solution. Each forager was observed foraging on an array until it completed three trips returning to the colony. The array was then switched and the process was repeated for the array with the other reward task. This was done either immediately before training and testing or one day prior.

After pre-training, bees were trained using an array (Figure 4b) containing three of each type of reward flower in an alternating pattern. This training step taught the bees to switch between the two flower tasks in a single foraging run and trained them to the reward volume that would be used during testing, 2.0 μL of sucrose. All but one of each type was hidden by a cover made of construction paper. During the initial visit, the bee selected which reward type to visit; following this initial visit, the bee was only exposed to one flower at once in an alternating pattern, with the remaining flowers covered. After visiting all six flowers, the training array was removed and the bee was immediately tested.

Testing

On the test array, flowers were evenly spaced apart in nine staggered rows of ten. The flowers had 12.0 cm between them both horizontally and vertically and 8.5 cm between them diagonally. During testing, there were always 32 rewarding flowers and 58 non-rewarding flowers. Reward flowers were yellow unscented and blue scented with geranium and contained 2.0 μL 30% sucrose solution. Non-reward flowers contained 2.0 μL water. Sucrose and water were dispensed at the bottom tip of the tube to ensure consistency and to prevent delays in foraging. Figure 4c below shows the arrangement of flower colors and odors on a two task array. Blue flowers labeled (1) were scented with clove, blue flowers labeled (2) were scented with mint, and blue flowers labeled (3) were scented with geranium. One task arrays omitted one of the reward flower types; these arrays were used to account for any differences in handling times between yellow and geranium flowers. The results from the one task arrays were also compared to those of the two task array to determine the time cost of switching between two tasks. One task

geranium arrays replaced all the yellow flowers with geranium-scented blue flowers (Figure 4d) and one task yellow arrays replaced all the geranium-scented blue flowers with yellow flowers (Figure 4e).

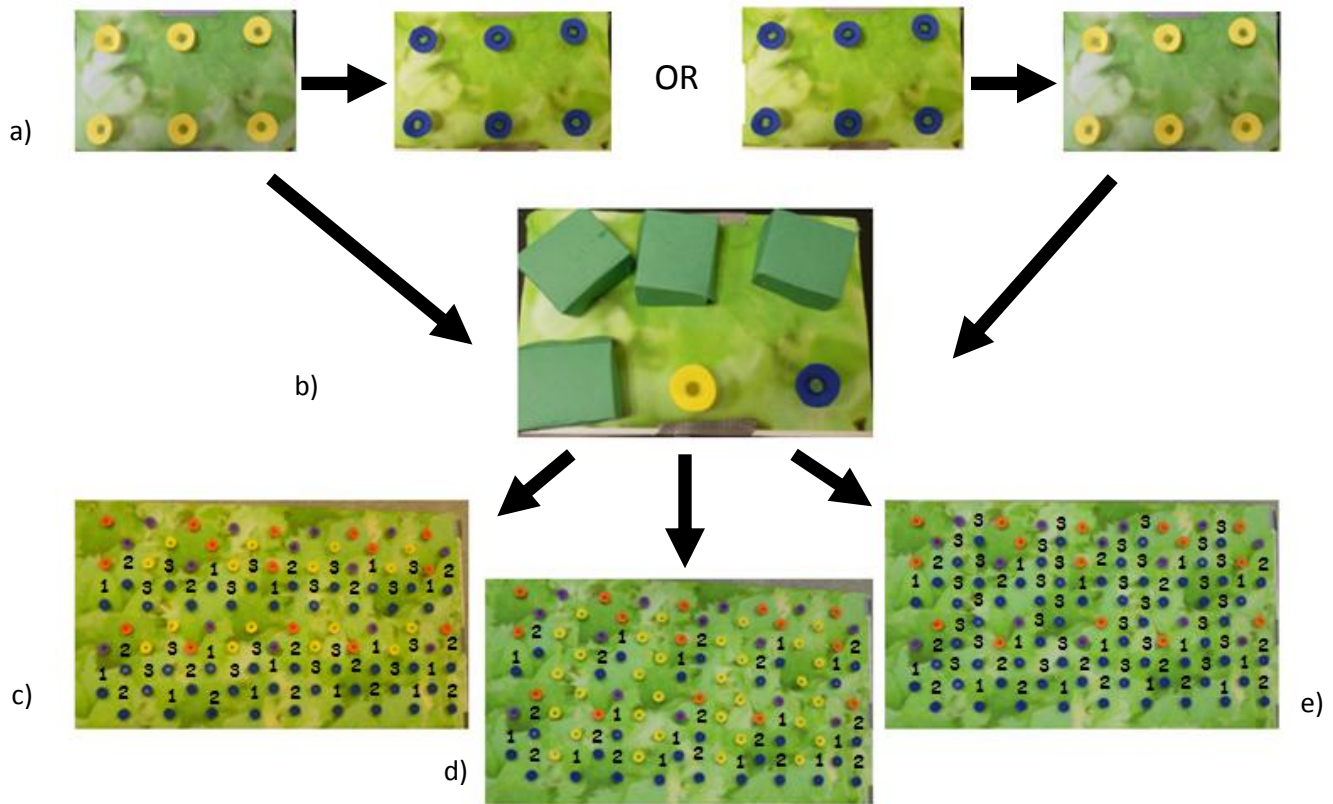


Figure 4: Experimental Procedure Schematic – a) pre-training arrays b) training array c) 2 task array d) 1 task yellow array e) 1 task geranium array

Every experimental run was video-taped for later analysis. Only one bee was exposed to the testing array at a time; the rest of the bees were removed from the mesh enclosure and the wire tube was blocked off. Upon removing the training array, each bee was then exposed to the testing array. It was noted which type of flower the bee visited and whether the visit was considered ‘full’ or ‘halfway.’ A full visit consisted of the bee completely entering the flower and consuming the sucrose reward while a halfway visit consisted of the bee partially entering the flower but not consuming the sucrose reward. To distinguish between the scents of the blue flowers on the video, the number of the scent was announced aloud. After a bee completed a full visit to a reward flower, the flower was refilled with 2.0 μ L 30% sucrose solution. Testing concluded after the bee made a total of 120 full visits to rewarding flowers. If a two task array was being tested and the bee showed specialization (foraged exclusively from only one reward type), all the flowers of the preferred type were removed and foraging continued until the bee made 20 visits to the other reward type. Data was collected and analyzed for 77 bees, divided among the groups as described in Table 2.

Table 2: Division of Tested Bees among Age Groups and Number of Tasks

Group	Number of Bees Tested on Two Task Array	Number of Bees Tested on One Task Array
Young Chronological Age & Young Foraging Age	18	15
Old Chronological Age & Young Foraging Age	14	12
Old Chronological Age & Old Foraging Age	8	10

Variables

Percent Task Repetition

A task repetition is defined as when a forager moved from one rewarding flower task (either yellow or geranium scent) to the same flower task over consecutive visits. For each bee tested on the two task array, we determined the proportion of task repetitions over the 120 recorded visits (these 120 visits were blocked into 3 groups of 40 visits). Values ranged from 0, indicating the bee switched tasks every visit, to 1, indicating that a bee performed the same task every visit. A value of 0.5 would indicate random task selection given the distribution of tasks on the array.

Switch Cost

A switch cost is defined as the increase in decision time associated with switching between different foraging tasks. Decision time is the amount of time from when the forager left a flower to when it landed on the next flower. We calculated switch cost by comparing decision times on the two task array with the mean decision time on the one task arrays. To determine switch costs for each bee tested on the two task array, the average decision time for bees on the one task arrays was subtracted from each individual's decision time on the two task array. We determined the switch cost for the 3 blocks of 40 visits (composing the 120 visit test). A value of 0 indicated that the bees showed no difference in decision time between the one and two task arrays (i.e. they did not experience a switch cost).

Results

Task Repetitions

All three test groups performed significantly more task repetitions on the two task array than expected by random task selection (random task reps =0.5; young chronological and foraging ages $t_{17} = 5.77$, $p < 0.0001$; old chronological age and young foraging age $t_{13}=6.31$, $p < 0.0001$; old chronological and foraging ages $t_7=3.74$, $p = 0.01$), suggesting that there is some cost associated with task switching. However, test groups exhibited very different patterns in the frequency of task repetitions over the 120 flower visits (Figure 4). A repeated measures ANOVA was performed; it showed that there was a difference in the number of task repetitions completed by the bees with young chronological and foraging ages ($F_{2, 17} = 5.79$, $p = 0.02$). Tukey's pair-wise comparison was used to determine that there was a significant increase in task repetitions between the first block of 40 visits and the third block of 40 visits (Figure 4a). The group with old chronological age and young foraging age showed no significant change in the number of task repetitions they performed throughout the test (Figure 4b; ANOVA: $F_{2, 13} = 2.59$, $p = 0.11$). A repeated measures ANOVA showed that there was a difference in the number of task repetitions completed by the bees with old chronological and foraging ages ($F_{2, 7} = 3.94$, $p = 0.08$). Tukey's pair-wise comparison was used to determine that there was a significant decrease in repetitions between the first block of 40 visits and the second block of 40 visits (the third block of 40 was not significantly different from either of the first two blocks as a result of an outlier; Figure 4c).

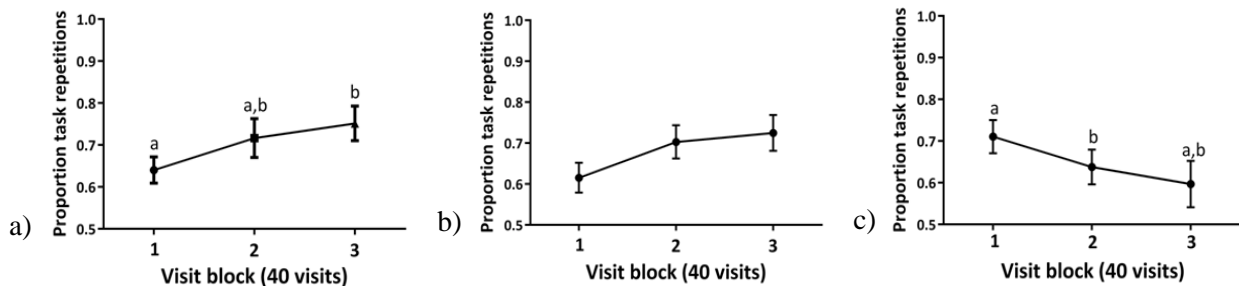


Figure 5: Proportion of Task Repetitions over 120 visits for (a) young chronological and foraging age, (b) old chronological and young foraging age, (c) old chronological and foraging age. Data points with differing letters are significantly different

The two variables, chronological age and foraging age, were then examined independently of each other using an unpaired T test in the last block of 40 visits (used as an indication of bees' final foraging behavior; Figure 5). When controlling for foraging age, there was no difference in percent task repetitions between chronologically young and old bees (Figure 5a; $t_{29} = 0.44$, $p = 0.66$). When controlling for chronological age, there was a near significant difference ($t_{19} = 1.81$, $p = 0.08$) in percent task repetitions between bees with young and old foraging ages (Figure 5b). Bees with a young foraging age repeated tasks more often than bees with an old foraging age.

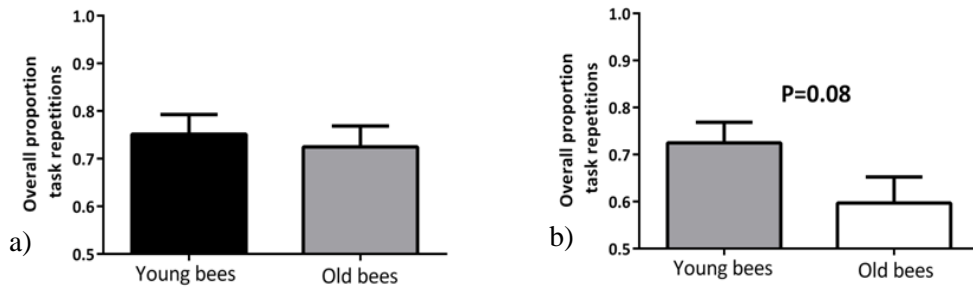


Figure 6: Proportion of Task Repetitions for Last Block of 40 Visits comparing (a) bees with young and old chronological ages, while controlling for foraging age, (b) bees with young and old foraging ages, while controlling for chronological age

Switch Costs

All three groups showed an increase in decision time when switching between tasks compared to repeating the same task, indicating that bees exhibit a robust switch cost. However, there was some difference in the temporal dynamics of switch costs among the groups tested (Figure 6). A repeated measures ANOVA was performed. It showed that the group with young chronological and foraging ages exhibited a difference in their switch cost over the course of the test ($F_{2, 17} = 6.16, p = 0.01$). Tukey's pair-wise comparison showed that there was a significant decrease in decision time between the first block of 40 visits and the last two blocks of 40 visits (Figure 6a). The group with old chronological age and young foraging age showed no change in their switch cost throughout the test (Figure 6b; ANOVA: $F_{2, 13} = 1.06, p = 0.36$). The group with old chronological and foraging ages also showed no change in their switch cost throughout the test (Figure 6c; ANOVA: $F_{2, 7} = 0.46, p = 0.56$).

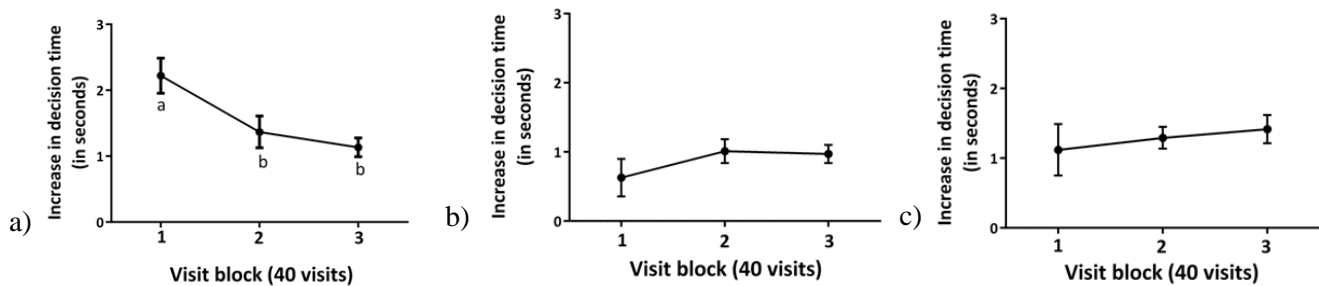


Figure 7: Switch Costs over 120 visits for (a) young chronological and foraging age, (b) old chronological and young foraging age, (c) old chronological and foraging age. Data points with differing letters are significantly different (statistically, $p \leq 0.05$) from each other.

The two variables, chronological age and foraging age, were then examined independently of each other using an unpaired T test in the last block of 40 visits (used as an indication of bees' final foraging behavior; Figure 7). When controlling for foraging age, there was no significant difference in switch cost between chronologically young and old bees (Figure 7a; $t_{29} = 0.85, p = 0.40$). When controlling for chronological age, there was a near significant difference ($t_{19} = 2.68,$

$p = 0.06$) in switch cost between bees with young and old foraging ages (Figure 7b). Bees with a young foraging age had a lesser switch cost than bees with an old foraging age.

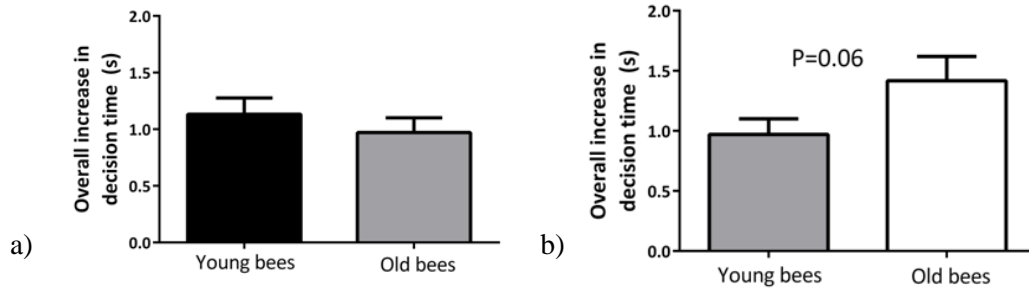


Figure 8: Switch Costs for Last Block of 40 Visits comparing (a) bees with young and old chronological ages, while controlling for foraging age, (b) bees with young and old foraging ages, while controlling for chronological age

Discussion

The objective of this experiment was to determine the separate effects of chronological age and foraging age on mental flexibility in foraging bumble bees. Surprisingly, we found that chronological age did not affect a bumble bee's mental flexibility. A higher foraging age, however, correlated with decreased mental flexibility.

After examining both task repetition and switch cost data, several interesting trends were observed. Bees with young chronological and foraging ages had to learn that there is a cost to switching; they had the highest switch cost seen among any group in their first block of 40 visits (Figure 7a). Higher switch costs signify that the bee is spending more time deciding which flower to visit, detracting from the time they could be collecting food, meaning they are foraging less efficiently. They learned to minimize this cost, cutting it in half by the third block of 40 visits, by increasing the number of repetitions they performed (Figure 5a). Bees with an old chronological age and a young foraging age did not need this learning period; in their first block of 40 visits, they exhibited the lowest switch cost seen among the three groups and showed no significant change in their behavior over the course of testing (Figures 7b and 5b, respectively). Bees with old chronological and foraging ages were also aware of the switch cost from the beginning and showed no significant change in it as they foraged (Figure 7c). While maintaining their switch cost, they were also able to increase how often they switched between tasks (Figure 5c).

Chronological age and foraging age were examined independently to determine which was accounting for the observed differences. Chronological age has been found in many species to negatively affect cognitive performance (Gage *et al.*, 1984; Engle & Barnes, 2012; Christensen *et al.*, 1997; Wecker *et al.*, 2005). Interestingly, in bumble bees chronological age was found to have no significant effect on either the proportion of repetitions or the switch cost. This implies that chronological age has no impact on mental flexibility, which refutes our hypothesis that chronologically older bees would be less mentally flexible. This trend was previously seen in honey bees; however, this study also showed that increased foraging age resulted in decreases in mental flexibility (Behrends *et al.*, 2007). Similarly, our study demonstrated that bumble bees experience mental decline with increased foraging experience. Foraging age was found to have an effect on both the proportion of repetitions and the switch cost, with bees of a greater foraging age switching between tasks more often with a higher switch cost. These results were not statistically significant using a significance level of $p = 0.05$ but they neared significance ($p = 0.08$ for percent repetitions and $p = 0.06$ for switch cost). We suspect that if the sample size for the group of bees with old chronological and foraging ages had been larger than 8, statistical significance would have been attained. These findings suggest that an increased foraging age correlates with decreased mental flexibility, which supports our hypothesis that cognitive decline begins in bumble bees once they start foraging.

All three groups demonstrated a degree of flower constancy; they repeated tasks more often than expected by random chance (proportion of task repetitions higher than 0.5, Figure 4), indicating that there is a time cost to switching between two tasks. Considering this switch cost, bees exhibit flower constancy because it is an economic foraging decision. Leigher and Rinaldo (2012) found that when the inter-flower distance decreases bees have less time to decide to switch, and therefore repeat more often. Gegear and Laverty (2005) also found that the switch cost is higher and bees are more constant when switching between dissimilar flowers because the brain has to process more floral traits. These studies suggest that floral constancy is based, at least in part, on bees' working memory limitations.

However, none of the groups showed complete specialization, indicating that there is a significant travel cost to consistently bypassing different flower tasks of equal reward. It appears that the bees recognized that there is a balance between repeating tasks and switching between them that allows for optimal foraging. In looking at our results (Figure 4), this balance was attained when the bee repeated 65-75% of the time and switched the rest of the time. This fact was supported because, even though the bees with a higher foraging age were able to switch between tasks more often, they demonstrated a higher switch cost than the bees with a lower foraging age, indicating that this was not an optimal behavior.

Proficient foraging behavior maximizes the efficiency with which foragers return food to the colony. This is important because colony health is directly dependent on its foragers' success (Wells and Wells, 1983). If these behaviors are not performed, it could negatively impact the colony's health because less food is available to the bees. Proficient foraging is dependent upon the forager's ability to switch between different flower tasks, which is a representation of its mental flexibility. Environmental stressors, such as pathogens, pesticides and parasites, could be causing a decline in this mental flexibility in bumble bee foragers, making them less efficient foragers. Our study shows that increased foraging age also causes a decline in mental flexibility; bees with increased foraging age may be more susceptible to environmental stressors, amplifying the detrimental cognitive effects. This would result in increasingly inefficient foragers, which would negatively affect colony health and could contribute to the population declines.

This study could be reproduced with increased task complexity (either by teaching the bees more than two tasks or by making the flowers more difficult to handle) to verify if the extent of the cognitive decline relates to the degree of cognitive processing required. The next step would be to identify how the bumble bee brain changes, both physically and chemically, as the bee gains foraging experience. Additional studies should be conducted to determine the breakdown of a colony's foragers in terms of chronological age and foraging age to examine at what point in its life a bumble bee starts foraging and for how long a bumble bee forages in a natural setting.

Acknowledgements

We would like to thank Dr. Robert Gegear for allowing us to partake in his research and advising us throughout the MQP process. Special thanks also go to Melissa Mobley and Karen Tran for their guidance and support.

References

- Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Cox, P.A., Dalton, V., Feinsinger, P., Ingram, M., Inouye, D., Jones, C.E., Kennedy, K., Kevan, P., Koopowitz, H., Medellin, R., Medellin-Morales, S., Nabhan, G.P., Pavlik, B., Tepedino, V., Torchio, P. and Walker, S. (1998, Feb.). The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology*. 12: 8-17.
- Arrington, C.M. and Logan, G.D. (2004, Sept). The Cost of a Voluntary Task Switch. *Psychological Science* 15(9): 610-615.
- Behrends, A. and Scheiner, R. (2010, April). Learning at old age: a study on winter bees. *Frontiers in Behavioral Neuroscience*. 4(15): 1-11.
- Behrends, A., Scheiner, R., Baker, N., and Amdam, G.V. (2007, Sept). Cognitive aging is linked to social role in honey bees (*Apis mellifera*). *Experimental Gerontology* 42: 1146-1153.
- Bumblebee. (n.d). Encyclopædia Britannica. Retrieved March 14, 2013 from <http://www.britannica.com/EBchecked/topic/84571/bumblebee>
- Chittka, L., Thomson, J.D., and Waser, N.M. (1999, Aug). Flower Constancy, Insect Psychology, and Plant Evolution. *Naturwissenschaften* 86(8): 361-377.
- Christensen, H., Henderson, A.S., Griffiths, K., and Levings, C. (1997, Jul). Does ageing inevitably lead to declines in cognitive performance? A longitudinal study of elite academics. *Personality and Individual Differences* 23(1): 67-78.
- Committee on the Status of Pollinators in North America. (2007). *Status of pollinators in North America*. Washington, D.C.: National Academies Press. Retrieved from <http://site.ebrary.com/lib/wpi/docDetail.action?docID=10172665>
- Delaplane, K.S. and Mayer, D.F. (2000, August). Crop pollination by bees. CABI Publishing, Cambridge, MA.
- Dove, A., Pollmann, S., Schubert, T., Wiggins, C.J., Yves von Cramon, D. (2000, Jan). Prefrontal cortex activation in task switching: an event-related fMRI study. *Cognitive Brain Research* 9(1): 103-109.
- Dukas, R. (1995, Jun). Transfer and interference in bumblebee learning. *Animal Behaviour* 49(6): 1481-1490.
- Engle, J.R., and Barnes, C.A. (2012, Sept). Characterizing cognitive aging of associative memory in animal models. *Frontiers in Aging Neuroscience* 4(10): 1-9.

- Gage, F.H, Kelly, P.A.T, and Björklund, A. (1984, Nov). Regional Changes in Brain Glucose Metabolism Reflect Cognitive Impairments in Aged Rats. *The Journal of Neuroscience* 4(11): 2856-2865.
- Gegear R.J. and Thomson J.D. (2004, Oct). Does the Flower Constancy of Bumble Bees Reflect Foraging Economics? *Ethology* 110(10): 793-805.
- Gegear, R. J. and Laverty, T. M. (2001). The effect of variation among floral traits on the flower constancy of pollinators. Pp. 1-20 in *Cognitive Ecology of Pollination: Animal Behaviour and Floral Evolution*. Chittka, L. and Thomson, J. D. (eds), ed. Cambridge: Cambridge University Press.
- Gegear, R. J. and Laverty, T. M. (2005, Apr). Flower constancy in bumblebees: a test of the trait variability hypothesis. *Animal Behaviour* 69(4): 939-949.
- Goulson, D. (2000, Mar). Are insects flower constant because they use search images to find flowers? *Oikos* 88(3): 547-552.
- Goulson, D., Lye, G.C. and Darvill, B. (2008). Decline and conservation of bumble bees. *Annual Review of Entomology*. 53: 191-208.
- Grüter, C. and Ratnieks, F.L.W. (2011, Nov). Flower constancy in insect pollinators: Adaptive foraging behavior or cognitive limitation? *Communicative & Integrative Biology* 4(6): 633-636.
- Leigher, K. and Rinaldo, B. (2012, Apr). Neuroeconomics of task switching in the bumblebees. Major Qualifying Project, Worcester Polytechnic Institute.
- Lewis, A.C. (1986, May). Memory Constraints and Flower Choice in *Pieris rapae*. *Science* 232(4752): 863-865.
- Møller, A.P., Barnier, F., Mousseau, T.A. (2012, Dec). Ecosystems effects 25 years after Chernobyl: pollinators, fruit set and recruitment. *Oecologia* 170(4): 1155-1165.
- Monsell, S. (2003, Mar). Task switching. *Trends in Cognitive Sciences* 7(3): 134-140.
- Ollerton, J., Tarrant, S. and Winfree, R. (2011). How many flowering plants are pollinated by animals? *Nordic Society Oikos* 120: 321-326.
- Waser, N.M. (1986, May). Flower Constancy: Definition, Cause, and Measurement. *The American Naturalist* 127(5): 593-603.
- Wecker, N.S., Kramer, J.H., Hallam, B.J, and Delis, D.C. (2005, May). Mental Flexibility: Age Effects on Switching. *Neuropsychology* 19(3): 345-352.
- Wells, H. and Wells, P.H. (1983, Oct). Honey Bee Foraging Ecology: Optimal Diet, Minimal Uncertainty or Individual Constancy? *Journal of Animal Ecology* 52(3): 829-836.