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The Effects of Floral and Social Information on Bumblebee Forager Learning and Memory

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Abstract

Bumblebees rely on information gathered from their environment to make the best choices they can when foraging for pollen and nectar. The type of information gathered should influence how a bee learns and remembers it, but other factors such as the size of the bee's brain may also play a role in the learning and remembering process. While social information learned from other organisms and information gathered directly from flowers can each be used alone to improve both the efficiency with which a bee learns to forage from a flower and how accurately and how long the bee remembers these foraging skills, combining more than one type of information should reduce the uncertainty bees have about their choices and further improve their learning and memory. We examined how information can influence learning and memory in bumblebees by training them to associate social or floral information paired with flower color with a nectar reward and then testing their memory after either one or twenty-four hours. We found a clear improvement in forager learning and memory from the different types of compound stimuli when compared to the control treatment of simple color difference, but the nectar guide treatment had the most significant influence on both learning and memory. Although brain size and retention interval also had no significant effect, interactions between flower color and treatment type were found, and differences in how bees used floral and social information were found when the results were compared to other experiments.

Chapter 1

Introduction

Bumblebee foragers encounter many different types of information as they search their environment for nectar and pollen to bring back to their hive. The different forms of information about the flowers they harvest nectar from can help bees to forage more efficiently, maximizing the amount of nectar and pollen they collect and minimizing wasted energy and time on flowers that are less rewarding. The type of information foraging bees collect can influence the rate at which they learn about their environment and the length and accuracy of their memory. One type of information that bees collect, known as floral information, comes from the flower itself. Floral characteristics perceptible to bees like the shape or color of the flower can help bees learn to harvest nectar from the flower more efficiently and remember the more rewarding flowers for longer. Another type of information collected by foraging bees is social information. This is information gained from observing other organisms like foraging conspecifics and learning from their behavior. For example, the absence or presence of a forager on a flower could indicate whether that flower is rewarding or not. Floral and social information should allow bees to both increase the efficiency with which they learn about the reliability of flowers and to remember which flowers are reliably rewarding for longer, but in some situations one type of information is preferred over the other. In this chapter, I will examine the literature on bumblebee learning and memory as well as the different forms of information bees collect and how this may affect the learning and

memory processes. I will also review the literature on bumblebee brain size and how this relates to their cognitive abilities when it comes to foraging.

I. Learning and Memory

i. Learning

Learning how to navigate and harvest resources from an environment is a skill vital to the survival of most animals. Without learning, an animal won't know where to best find food, what areas are safe from predators, or how to locate potential mates. This skill is even more important when an organism's ability to learn is supporting the survival of hundreds of others, like in the case of a bumblebee forager. Eusocial insects like bumblebees live in large groups like hives, and each member of the group cooperates with each other and has a specific job (Anderson 1984). In bumblebee hives, foraging bees have the job of leaving the hive and seeking sustenance in the form of nectar and pollen from flowers (Rehan & Toth 2015). To successfully leave the hive, navigate the outside world to find food, and return to feed the other hive members, bees must be able to learn about their environment and especially about the food sources they utilize.

Learning in animals is a process shaped by different stimuli encountered throughout the animal's lifetime (Shettleworth 2010). For bees, this means that to learn about how to gather food, they must go out into their environment to forage. But many aspects of a bee's environment can influence how they learn to forage. One important part of an environment that influences learning is how reliable a food source is. If the food sources in an environment are unpredictable, the animal is more likely to follow

environmental signals indicating food sources, and in a more predictable environment they are less likely to follow signals (McLinn & Stephens 2006). For a bee, this could mean that they pay attention to flower color if food is constantly changing, but not if a food source is predictable. But this relationship is only beneficial if the signal is reliable. If the information an animal gathers does not reliably indicate a reward, they are more likely to make errors (Koops 2004).

The way an environment changes also has an influence on learning. How quickly an environment changes can affect the speed at which an animal learns. For example, if blue jays are in an environment that is constantly changing, they tend to collect information and learn at a higher rate (Dunlap & Stephens 2012). But environmental change doesn't always promote learning. Learning is promoted with some types of environmental change like when the best action an animal can do is continuously changing but reliably linked to the information they gather, while organisms tend to stick with their initial preference and avoid learning when confronted with other types of environmental change where experience isn't always linked with consequence (Dunlap & Stephens 2009). In other words, a changing environment can select either for or against learning depending on the relationship between the information an animal receives and whether the information is reliable (Dunlap & Stephens 2009).

As with all types of learning, learning to locate and forage from flowers takes time. But bees generally become more efficient at foraging as they practice and learn. For pollinators in general, the more attempts made at foraging on a flower, the less time it takes them to locate and harvest the nectar (Lewis 1986). The time spent moving between flowers can also rapidly decline throughout even a single day of foraging, as shown by

Keasar et al. (1996), but in this experiment it took much longer than a day for the bees to build a preference for certain types or patches of flowers. Learning to forage from a flower is not always straightforward, since learning to forage from a second type of flower can sometimes interfere with learning on the first one. A species of butterfly was able to quickly learn how to forage from one type of flower, but when a second flower was introduced the efficiency of foraging on the first one decreased, suggesting a cost of switching between multiple types of flowers (Lewis 1986). This phenomenon does not necessarily apply to all foragers, however, as Raine & Chittka (2012) found that bumblebees learning to forage quickly on one type of flower were able to change to another type quickly.

ii. Memory

The learning process would be impossible without the ability to store and retrieve the information gathered from the environment. Memory allows animals to use previously collected information to change their behavior and become more efficient at their tasks. Foraging bees must remember a long list of information, including the location of their hive and food sources and which food sources are worthwhile and which ones aren't. They also must remember how to access the nectar and pollen in these food sources, or they will waste time and energy and miss out on valuable nutrition.

Memory is shaped by information gathered during the learning process (Shettleworth 2010), but the accuracy and storage time of the memories can vary significantly depending on a number of internal and environmental factors. For example, how reliable the information is can play a role in how foraging bees store and remember

it. Information that reliably predicts a reward or absence of reward is more efficiently stored and retrieved when compared with less reliable information (Brea et al. 2014, Dunlap et al. 2009). Information will not be reliable for long if the environment changes rapidly, so a highly variable or quickly changing and unpredictable environment might not support efficient memory storage and retrieval. This means predictable environments lead to memories being stored for longer periods of time (Dunlap et al. 2009, Brea et al. 2014, McNamera & Houston 1987). The cost of living in an environment, defined here as the metabolic demands placed on an organism that it must meet in order to survive, also has an influence on their memory. If there is a high cost of living in an environment, an organism should store things in its memory for much longer because not doing so could be detrimental, while a low cost of living in an environment should lead to short memory storage since forgetting is not as costly (Dunlap et al. 2009). The storage and accuracy of formed memories in animals can also depend on the animals' life history traits. An animal's age, energy reserves, and sex can alter its ability to store and retrieve memories (Dunlap et al. 2006, Dunlap et al. 2009). Memory performance tends to change throughout an organism's lifetime, with a notable decline near the end of life, possibly as a result of deleterious mutations (Dunlap et al. 2009). This life history influence on memory extends to animals outside of eusocial insects and behaviors other than flower foraging. For example, pinyon jay females that are unmated remember where their food caches are for significantly longer than jays with mates (Dunlap et al. 2006).

There is a metabolic cost to storing and retrieving memories (Dunlap et al. 2009, Dukas 1999), so it would be impossible for animals to remember everything they learn. Brain tissue has a high energetic cost to maintain, and to have accurate and long-lasting

memories, more brain tissue is usually required (Dukas 1999). This cost of storing memories is one of the reasons that animal memory decays over time (Hardt et al. 2013, Keasar et al. 1996). This memory decay serves the purpose of purging unimportant memories that can interfere with the important memories during memory retrieval (Hardt et al. 2013). Since it is costly to remember things, it follows that forgetting things can be advantageous if the memories are not valuable, a concept known as adaptive forgetting (Kraemer & Golding 1997). Memories can also be lost as a function of remembering other things. Remembering more important things is made possible by filtering out and forgetting less important things, and this ability to forget the unimportant is a useful trait and is linked with improved cognitive functioning (Storm 2011). How memories are valued can be a function of the time since the information was learned. In general, memories decline in value as they get older because the chance they are accurate declines with time (Koops 2004). But in less variable environments memories can stay valuable for longer (McNamera & Houston 1987) and older memories can be valued as much as new memories (Dunlap & Stephens 2012). For many animals, memory decay occurs largely overnight during sleep (Hardt et al. 2013). Some studies suggest that memory loss in bees overnight can be especially beneficial in variable environments by allowing bees to quickly change the focus of their foraging as rewarding resources rapidly change (Keasar et al. 1996). While memory loss does occur overnight in bumblebees, foraging-related memory decay might not be too significant over one night (Chittka 1998). For *Bombus impatiens*, a notable memory decay is noted after multiple weeks, but the performance on memory tests weeks after learning is still better than baseline levels, suggesting the memories have not been completely purged (Chittka 1998).

II. Floral Information

While foraging, animals encounter two main different types of information to learn from and remember. Animals collect information themselves by experiencing things firsthand, a type of information known as personal information, and they also gain social information by watching what other animals do (Dall et al. 2005). Since foraging bees encounter personal information mainly from flowers, this information will be referred to here as either personal or floral information interchangeably. Nectar foragers like bumblebees encounter flowers that can be incredibly complex in terms of the different floral information they provide. Flowers have been described as sensory billboards, projecting multiple signals of sight and smell components to attract pollinators (Raguso 2004). The types of floral information perceptible to bees include flower color, shape, size, presence of nectar guides, olfactory, and thermal components (Patiny 2012).

i. Color

One type of floral information perceptible to bees is the color of a flower. Bees can readily perceive multiple colors of flowers and will discriminate between colors to choose the more rewarding flowers (Dukas & Real 1993). Once bees make an association between flower colors and nectar sources, they can use the color to more reliably find additional good sources or avoid poor ones. While not all flower colors are easily perceptible to bees, flowers of certain colors can lead to an increase in foraging efficiency among certain species of bee (Dukas & Real 1993, Sanchez & Vandame 2012). Color can be a more important form of floral information than some other traits because, unlike

information such as scent, bees can detect color from a significant distance (Giurfa et al. 1994). Color also might be preferred by bees and other foragers over other types of floral information. A study by Orth & Waddington (1997) showed that carpenter bees likely have a hierarchy of information they prefer while foraging, with color being the most important cue, and other information like spatial positioning being used only when color is not available. And a Cepero et al. (2015) study showed that foraging butterflies were able to learn to forage based on flower color more efficiently than flower shape, but they were also potentially able to forage by linking color and shape together.

While developing an association between a color and a reward or lack of reward can raise foraging efficiency, bees also tend to have an innate bias towards certain colors before any information is gathered (Gumbert 2000). This bias can potentially interfere with other types of information like other floral information or social information gained from other foragers (Worden & Papaj 2005). Color preference can also make bees forage less efficiently by causing them to stick with a preferred color when switching would be more efficient. An experiment by Jones et al. (2015) showed bees sticking with low-quality flowers that they were trained to forage on and only moving to higher quality ones if the more rewarding flowers were of a color that matched the bees' innate bias. Another experiment showed bees being hesitant to switch to unfamiliar colors unless they had strong social information indicating it was worth making the switch (Worden & Papaj 2005). This innate preference for certain colors diminishes as the bees learn which flowers are rewarding, and a color that reliably signals a rewarding flower will eventually be preferred by the bee over its original preferred color (Gumbert 2000).

In some species, this color preference can lead to a behavior known as flower constancy, where a forager focuses on a small number of species in an area where other equally rewarding flowers are available (Raine & Chittka 2005). While this behavior has been observed in multiple species of bumblebee (Raine & Chittka 2005), it has also been seen in other foraging insects. When honeybees were trained that flowers of a certain color offered a higher reward than flowers of a different color, they kept foraging on the flower associated with a higher reward for days after the rewards were switched to become equal (Amaya-Marquez et al. 2017). The honeybees learned to associate one color with a reward rapidly but took significantly longer to unlearn this association and move to a different color (Amaya-Marquez et al. 2017). This observation does not always translate to bumblebees, however, as another experiment comparing flower constancy between honeybees and bumblebees showed bumblebees switching between colors much more readily than honeybees (Gegear & Laverly 2004).

ii: Shape

Another form of floral information is the shape of the flower bees are foraging from. The shape of a flower refers to the outline of the flower formed by the corolla, the presence or lack of symmetry in the flower, and the morphology of the nectar and pollen-storing areas. A flower's shape can influence how often foragers visit it, with one study showing that multiple bee and fly species alter their number of visitations to certain flowers based on shape (Campbell et al. 2013). This trend extends to other foragers, as monarch butterflies are able to recognize flowers visually by shape alone and could also link flower shape and color together when foraging (Cepero et al. 2015). A flower's shape also influences foraging efficiency. One study on a species of stingless bee

demonstrated an increase in foraging efficiency with distinctly shaped flowers, although the study showed color to be a more significant factor in foraging efficiency changes (Sanchez & Vandame 2012). While certain shapes may help increase landing rates and foraging efficiency, bees are proficient at differentiating between multiple flower shapes, and are able to rapidly form a preference for flower shapes that are more rewarding than others (Lamb & Wells 1995). This preference for certain shapes may be purely reward driven, as shown in the Lambs & Wells (1995) experiment, since no strong preference for flower shape was formed when the flowers were equally rewarding.

The outline of a flower seems to be one of the floral traits that is more noticeable to foraging insects. Simple circular flowers seem to be less memorable, with one honeybee experiment showing that bees prefer flowers with distinct “disruptive” outlines over the circular models that the bees were trained on (Free 1970). This idea was supported in another study on pollinating bees and beetles that showed not only a preference for flowers with distinct shapes over circular ones, but that foragers were more likely to land on the flower closer to the nectary if the flower had a distinct outline (Dafni & Kevan 1997). Flowers with larger outlines are also more easily detected by bumblebees, lowering the bee’s flight time while searching for flowers during foraging trips and therefore the energetic costs of using them (Spaethe et al. 2001).

Flower shape has more aspects than simply the outline of the flower petals, and much of the variation in flower shape has to do with the structure of where and how the nectar is stored. This aspect of flower shape can influence how bees learn to harvest nectar, with bumblebees in one experiment locating nectar and learning to harvest it faster on flowers with shallow corolla tubes and exposed nectar when compared with

flowers with deeper corolla tubes and harder-to-access nectar (Lavery 1994). In general, flowers with more complex morphology correlated with significantly higher handling times among bumblebees (Lavery 1994). Some flowers have also evolved “anti-bee” traits that make it much harder for bees to access their nectaries. Flowers with smaller or absent petal lips and narrow corollas were a deterrent for bees, likely a specialization of the flower to attract other pollinators like hummingbirds (Zung et al. 2015).

How symmetric a flower is can also influence how foragers find and utilize it. Foraging bees have been shown to view flower symmetry as an important piece of floral information, as demonstrated in an experiment by Anders Moller (1995) where bumblebees showed a preference for more symmetrical flowers when foraging. This can be explained by data from the same study that showed symmetrical flowers have higher nectar contents than asymmetrical ones on the same plant (Moller 1995). The preference for different symmetrical flowers depends on the type of symmetry the flower displays. Honeybee foragers prefer flowers with radial symmetry (Free 1970), while bumblebees prefer those with bilateral symmetry (West & Lavery 1998). Although bumblebees would forage from flowers with both types of symmetry, the flower handling time and flight time between flowers were significantly lower on the bilaterally symmetrical ones when compared with flowers with radial symmetry (West & Lavery 1998).

iii: Nectar guides

Nectar guides are another form of floral information that bumblebees encounter while foraging. Nectar guides are colorful patterns on flowers that direct pollinators to a nectar source and have been shown to attract pollinators even if the pollinators have not previously encountered the pattern (Lehrer, Wehner & Srinivasan 1985). A study of

honeybees showed that adding nectar guides to model flowers increased the flowers' attractiveness to foraging bees despite the color, size, or shape of the flower (Free 1970). Nectar guides are so important to the pollination success of plants that most flowers pollinated by insects possess nectar guides and most of these nectar guides are in the insect-visible color spectrum (Penny 1983). It is even suggested that dichogamous plants have evolved nectar guides on both male and female flowers to prevent bees and other pollinators from discriminating between the flowers and ensure better pollination (Pohl et al. 2008).

While nectar guides increase the foragers' attraction to flowers, they can also affect foraging efficiency. Previous studies have demonstrated that the presence of nectar guides can increase the foraging efficiency of bumblebees by reducing the time it takes bees to locate food sources on the flower, reducing the time spent on the flower after nectar is harvested, and increasing the likelihood of bees locating the nectaries (Leonard & Papaj 2011, Leonard et al. 2013). Bees foraging from artificial flowers with nectar guides also remembered these flowers were rewarding more accurately when compared with flowers without nectar guides (Leonard & Papaj 2011). This enhanced learning and memory from the floral information nectar guides provided occurred regardless of the flower's shape (Leonard & Papaj 2011). The benefits of nectar guides are not unique to bees, as Dinkel & Lunau (2001) found that foraging flies were also able to locate nectaries more reliably and faster in the presence of nectar guides.

The color, pattern, and placement of nectar guides can influence how they attract and guide pollinators. The contrast between a nectar guide and the color of the flower is an important aspect since handling time of flowers by pollinators significantly decreases

with a higher contrast between guide and flower (Waser & Price 1985). When colored flowers with colorless albino nectar guides were compared with other flowers with darker nectar guides and a higher contrast between the guide and the flower, foraging bees and hummingbirds were found to highly prefer the non-albino variant, but visited the albino variant more when darker guides were added (Waser & Price 1985). The nectar guides' position on a flower in relation to the nectar and pollen source can also influence their effectiveness. Nectar guides work best when radiating symmetrically out from the center of a flower, where the nectary is most often found (Goodale et al. 2014). This becomes apparent when the nectar guides are no longer centered around the nectary, since doing this causes a dramatic decrease in foraging efficiency in *Bombus impatiens* (Goodale et al. 2014). The pattern on nectar guides also affects their attractiveness, since guides in the form of dotted lines were more attractive than solid lines, leading to an increase in pollinator visits (Free 1970).

III. Social Information

While floral information is an important part of a bee's process of learning and remembering how to forage, social information can be equally if not more important. Social information is the information that bees gain from observing the actions or presence of other animals. This information can inform a bee which flowers are rewarding, which ones to avoid, and several other actions that can help them survive and thrive while out searching for food for their hive.

Studies have shown that many social insects can learn from conspecifics by “eavesdropping” and copying the behavior they observe (Chittka & Leadbeater 2005). This eavesdropping can inform on behavior outside of foraging such as the presence of predators (Coolen et al. 2005) or help with habitat selection (Danchin et al. 2004). Eavesdropping is not limited to conspecifics and can occasionally extend to other species (Chittka & Leadbeater 2005). Eavesdropping bees do not need a live subject to observe and can even eavesdrop on a model made of inorganic material (Kawaguchi et al. 2007, Leadbeater & Chittka 2007, Worden & Papaj 2005). Eavesdropping behavior is also not unique to insects and has been observed in other foraging species such as birds (Krebs & Inman 1992) and fish (Danchin et al. 2004).

For foraging bees, much of the social information collected for foraging purposes involves which flowers other pollinators are using. Bumblebees can use the presence of a conspecific on a flower as an indicator of which flowers could be rewarding (Baude et al. 2011, Jones et al. 2015, Worden & Papaj 2005). This can help bees make decisions in unfamiliar environments, as bees that have just started learning about the rewards in an environment tend to prefer flowers with conspecifics on them despite having no information on which flowers are rewarding (Leadbetter & Chittka, 2009). This behavior, known as “local enhancement,” is most noticeable when inexperienced bees are learning from experienced conspecifics (Leadbetter & Chittka 2007). Local enhancement in bees is not foraging-specific, and still occurs in the absence of foraging, suggesting a general attraction to conspecifics regardless of behavior (Leadbetter & Chittka 2007). Sometimes the presence of conspecifics will not cause local enhancement and foragers will view conspecifics as competition to avoid (Baude et al. 2011). The difference between

foraging bees viewing conspecifics as competition or sources of information may depend on the density of both resources and foragers (Baude et al. 2011).

IV. Floral vs. Social Information

It is apparent that bees gain both social and floral information while foraging, but which information do they prefer to collect and use? Previous research has shown that, for foraging bees, social information generally takes priority over personal information. But the information used by bees is highly dependent on the situation and can vary based on the environment and the knowledge the bee has about the environment.

When bees have little information about an environment, or their level of social and floral information is relatively equal, they tend to prefer social information. Naive bees that have no personal or social information about which flowers are best to forage from tend to gather social information rather than personal (floral) information (Leadbeater & Chittka 2009). The consistency of rewards in the environment can also influence which type of information is preferred. Social information seems to be preferred in environments where the rewards regularly fluctuate, suggesting that bees tend to copy other bees when they are unsure if a flower is rewarding or not (Smolla et al. 2016). And social information is preferred when both types of information indicate a reward reliably or when social information is more reliable than personal (Dunlap et al. 2016). Previous experience with poor rewards can also cause a shift in foraging habits. In an experiment by Jones et al. (2015), bees were far more likely to switch to flowers with conspecifics on them if the flower they were currently foraging on had low-quality

rewards. The cost of information can also affect this decision. For example, if floral information is costly to collect, some animals show a preference for social information (Kendal 2004). But even if the cost of information is equal, bumblebees still choose to follow social information more often than floral (Leadbeater & Chittka 2009). In some cases, certain animals prefer social information even if it goes against personal information that has been very reliable. An experiment by Rieucau & Giraldeau (2009) showed that nutmeg mannikins will trust social information gathered from conspecifics even if the personal information they have gathered on food sources has been completely reliable and following the social information requires switching food sources.

Social information is not preferred in every situation, and sometimes foraging bees collect and use floral information instead. Part of the decision-making process here has to do with how easy the information is to collect and the bee's current knowledge. Floral information might be gathered over social information if the floral information is easier to collect and might be followed over social information if the bee already has knowledge of it (Kendal 2004). If the bee has knowledge of the quality of rewards, it might also go with floral information. In one experiment, bees that were trained to forage on a flower with high-quality rewards were less likely to switch to different flowers despite the presence of conspecific demonstrator bees on the alternative flowers (Jones et al. 2015). If following social information leads bees to lower quality rewards, foragers might choose to rely on floral information instead (Leadbeater & Chittka 2009). Bees might also go with floral information due to innate preferences. The innate preferences for certain flower colors mentioned earlier can override social information (Jones et al.

2015). While innate color preference is not personally gathered information itself, finding flowers of the preferred color requires personal floral information.

Although bees are often forced to choose between utilizing floral or social information because the cost of collecting both is too high (Danchin et al. 2004), much of the time they use both types of information simultaneously. Foraging animals consistently combine both social and personally gathered information to make decisions on where to forage (Krebs & Inman 1992). Even if they have a preference for one type, bumblebees are successful at learning both types as long as the information is reliable (Dunlap et al. 2016) and even learn both types of information at a similar rate, although the time it takes a bee to begin learning social information is significantly faster than with floral information (Leadbeater & Chittka 2007).

V. Brain Size and Foraging Success

A less-studied aspect of foraging bees that can affect learning, memory, and foraging efficiency is brain size. Brain size in bees can be extrapolated from body size. Bee brain size correlates with bee body size, with larger bees having larger brains, and as the brain size increases each individual part of the brain increases in size as well (Mares et al. 2005). In bumblebees, larger bees (with larger brains) tend to collect nectar at a higher rate than smaller bees (with smaller brains) (Herrmann et al. 2018, Spaethe & Weidenmuller 2002). This is visible in lab experiments where larger bumblebees were able to learn new foraging-related tasks more quickly than their smaller counterparts, including associating colors with rewards, an important foraging skill (Worden et al.

2005, Collado et al. 2021). This increase in learning ability was present in bees that had larger absolute brain size as well as a larger brain size to body size ratio (Collado et al. 2021). Larger bumblebees also tend to collect more nectar within the same number of foraging trips in the same amount of time (Spaethe & Weidenmuller 2002). This increase in foraging efficiency can be seen at the colony level, with foraging performance increasing in a colony as the average size of the bees increases (Hermann et al. 2018). The correlation between brain size and performance is supported by other studies on invertebrates like *Drosophila*, finding a correlation between brain size and improved cognitive abilities (Burns et al. 2010). It should be noted that this link between brain size and function is not always a clear correlation, and some studies indicate bigger brains do not always lead to better cognitive functioning. A review by Chittka & Niven (2009) suggests that many cognitive functions can be performed efficiently with smaller brains with even a small number of neurons, and that larger brains can provide the opportunity for more specialization and increased cognitive abilities, but do not ensure higher cognitive functioning.

Although a general increase in foraging efficiency with increased brain size has been found, other aspects of larger bees could explain an increase in foraging efficiency. For example, body size in bees is correlated with eye size, which has implications for foraging success (Spaethe & Chittka 2003). Bees with bigger bodies, and therefore larger eyes, have improved visual traits like higher visual resolution that allow them to spot flowers more easily and could allow foraging earlier in the day and later in the evening (Spaethe et al. 2001, Spaethe & Chittka 2003). Larger bees are also able to carry larger amounts of nectar and pollen, so even when larger and smaller bees make foraging trips

at the same rate, the larger bees will bring in more resources (Spaethe & Weidenmuller 2002). Time spent on and between flowers could also be a factor. Larger bees may be able to fly in between flowers faster and would likely be able to harvest nectar at a higher rate than smaller members of their hive (Spaethe & Weidenmuller 2002).

Conclusion

Learning and memory are two abilities that determine how efficient a foraging bee is at collecting food. The ability to quickly form and store accurate memories can be influenced by factors like the rate of change in an environment, how reliable the information gathered in the environment is, and the physical traits of the bee. The type of information a bee collects can also influence how the information is learned and remembered. Social information is often preferred over floral information, but this is situationally dependent, and there are numerous situations where the bee will use floral information alone or use a combination of both. Cognitive functions might be better in a bee with a larger brain, but this is not always true, and life history factors like the bee's age can play an equally important role. One important gap in the research on these subjects was in bee brain size related to memory. Although multiple studies covered the relationship between brain size and cognitive abilities, the studies focused on foraging efficiency and learning with little information on memory. The link between bumblebee brain size and memory is one of the areas I examined in my experiments, which I will cover in chapter 2.

Chapter 2

Introduction

Bumblebees are bombarded with different types of information as they explore their environment on foraging trips. Flowers display a plethora of information while advertising their rewards like billboards through olfactory and visual components (Raguso 2004), and other foraging bumblebees or heterospecifics give hints at where to look for nectar or simply draw attention through local enhancement (Leadbeater & Chittka 2007). Although bees often collect and use both social information from other foragers and floral information from flowers, in some situations one type of information is more helpful in learning and remembering floral rewards than the other type. Understanding how bees collect and process this information requires some knowledge of their learning and memory processes and how these processes relate to different types of information.

Learning in most animals follows a general trend of knowledge and proficiency increasing as more information is gathered. This can be seen in pollinators when the time needed to harvest nectar from a flower decreases with successive visits to the flower (Lewis 1986). This is accompanied by a decrease in time needed to move between flowers (Keasar 1996), but the learning process can be hindered if the pollinators are learning about multiple flower types at the same time (Lewis 1986). The rate at which animals learn from different types of information is also tied into the environment they forage in and trends in the availability of the rewards they seek. In a rapidly changing

environment, for example, some animals will adjust the rate at which they gather information, collecting more information and learning at a faster rate (Dunlap & Stephens 2012). An environment that is constantly changing requires constant monitoring and rapid learning to ensure the information gathered is still relevant. A major exception to this idea is if the information is unreliable. If information in a rapidly changing environment does not reliably indicate a reward, learning will be selected against (Dunlap & Stephens 2009). This is related to the idea that errors in decision making in animals increase as their information becomes less reliable (Koops 2004).

Like learning, memory is also influenced by the degree to which information is linked to an actual reward. Information that consistently leads to a reward is stored and retrieved more easily (Brea et al. 2014) and is retained for longer when compared with a less consistent link between information and reward (Dunlap et al. 2009, McNamera & Houston 1987). Environments that demand high metabolic costs for survival should select for longer memory storage (Dunlap et al. 2009), and memory functions can also be related to an animal's specific physical traits like sex, age (Dunlap et al. 2006), and brain size (Dukas 1999). Memory storage is imperfect and, given its metabolic costs, information gathered and stored in an animal's memory tends to degrade over time (Hardt et al. 2013, Keasar et al. 1996). Memories are less likely to still be accurate and valuable as the time since they were formed increases (Koops 2004), and memory loss overnight can be beneficial if the environment requires rapidly changing foraging tactics (Keasar et al. 1996). But in environments that are relatively constant, older memories can have similar value to newer memories (Dunlap & Stephens 2012, McNamera & Houston 1987).

Both learning and memory in bumblebees are shaped by the type of information the bees collect. The information collected by foraging bees about floral rewards can be broken into two main categories. Social information is gathered by observing other pollinators, either conspecifics or heterospecifics, and floral (personal) information is gathered from observable floral traits. Many types of floral characteristics make flowers attractive to foragers. A flower's color can be used by bees to discriminate between flowers with good and bad rewards (Dukas & Real 1993) and can increase the foraging efficiency of bees that can perceive the color differences (Sanchez & Vandame 2012). A flower's shape can also be used by bees to judge the quality of the flower's nectar and pollen reward and can alter the visitation rate (Campbell et al. 2013) and lead pollinators to form preferences for certain shapes (Lamb & Wells 1995). Multiple aspects of floral shape are memorable to pollinators, including the symmetry of the corolla (Moller 1995) and the size of the flower's outline (Free 1970). The presence of patterns on a flower known as nectar guides can also influence bumblebee forager learning and memory. Nectar guides can increase a flower's memorability (Leonard & Papaj 2011) and decrease its handling time (Waser & Price 1985). Much of the effect of nectar guides comes from their contrast, pattern (Waser & Price 1985), and symmetry, as well as their placement in relation to the nectar source (Goodale et al. 2014).

The source of social information gathered by bumblebees can vary. Bees observe the foraging behavior of other bees or heterospecific pollinators to identify reward sources through "eavesdropping" (Baude et al. 2011, Chittka & Leadbeater 2005), but bees can also be simply attracted to the presence of other bees through "local enhancement" (Leadbeater & Chittka 2007). This socially learned behavior does not

require a live subject to learn from; bees have been shown to learn from fake inorganic or dead and pinned foragers (Kawaguchi et al. 2007, Leadbeater & Chittka 2007, Worden & Papaj 2005). The type of information bumblebees prefer can depend on the situation, with aspects like level of previous knowledge, the cost of collecting information, and consistency of rewards in an environment all influencing which type of information bees collect (Dunlap et al. 2016, Jones et al. 2015, Kendall 2004, Leadbeater & Chittka 2009, Smolla et al. 2016).

The rate of learning and memory retention in bumblebees is potentially related to the size of their brain. Bee size is correlated with body size (Mares et al. 2005), and larger bees tend to have higher foraging rates than smaller bees (Herrmann et al. 2018, Spaethe & Weidenmuller 2002). Some studies argue this could be due to aspects of larger bees other than brain size such as improved foraging from larger eyes (Spaethe & Chittka 2003) or the ability to carry more nectar and pollen (Spaethe & Weidenmuller 2002), but other studies have shown the learning rate in larger bees is increased when compared with that of smaller bees (Worden et al. 2005, Collado et al. 2021).

My experiment will test the hypothesis that the learning rate and level of accuracy on a foraging memory test is affected by floral and social information. Combining information from flower color with nectar guides, specific flower shapes, and a pinned conspecific should allow bees to learn foraging accuracy criterion at a faster rate and allow them to remember this information more accurately and for longer when compared with a control test of simple color association. The effects of time on memory retention will also be tested, with two separate retention intervals used between the training and testing portions of my experiment to see whether memory decay increases with time.

When focusing on the compound stimuli of floral and social information compared to two types of floral information, I predict that the combination of floral and social information will have a more significant influence on learning and memory. Finally, I will test the hypothesis that bees with larger bodies, and therefore larger brains, will remember floral and social information more accurately and longer than those with smaller brains. To my knowledge, this aspect of memory in relation to brain size in bumblebees has not been tested.

Methods

Husbandry

Bumblebee test subjects were *Bombus impatiens* bees obtained from Koppert Biological Systems. The bees were kept in a 17in x 9in x 4in wooden box with two chambers and a plexiglass lid on top. The bottom of the hive boxes was covered in cat pine litter to control moisture, and the hive box also had screened windows in the sides for moisture control. Bees were given free access to the testing arena (Figure 1) where they could feed from containers with a 15% sucrose solution. Bees were also manually fed roughly a tablespoon of pollen three times a week directly into the hive box. To keep track of test subjects, queen bee tags from the Betterbee company were attached to foraging bees on their thorax with superglue. Since I glued the tags close to the base of the wings, any bees that had their mobility compromised during tagging were omitted from testing. Lights set to timers in the lab ensured the bees had fourteen hours of light and ten hours of darkness to imitate summer foraging conditions.

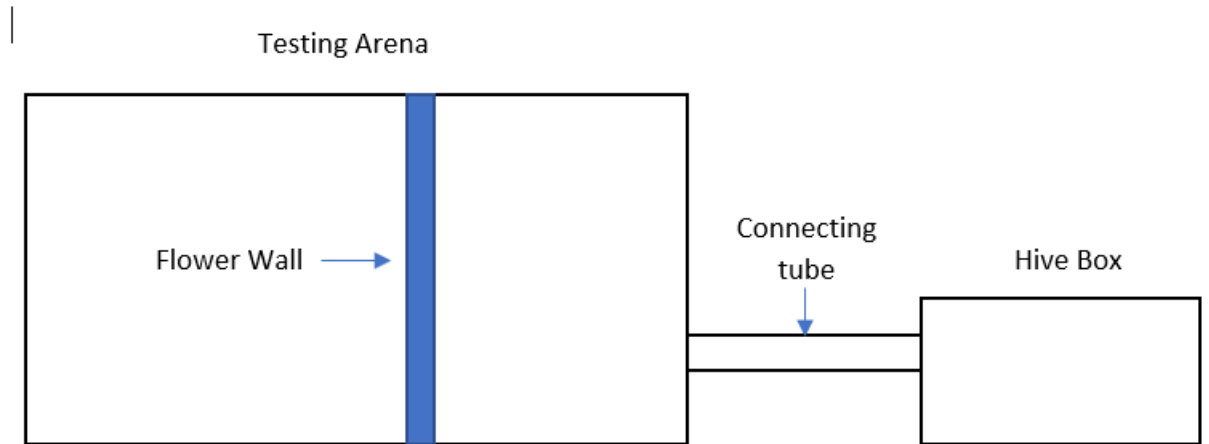


Figure 1: Side view of the setup of the hive box, connecting tube, flower wall, and testing arena. Bees moved from the hive box through the tube into the arena where the training and test sessions took place.

Experimental Design

I evaluated the learning and memory abilities of bumblebees by training the bees with a cue for them to associate with a reward, and then testing them on how well they remembered the association between the cue and the reward. The cue was the form of information, floral or social, that accompanied the rewarding flower. The rewarding flowers always had the tested floral or social information accompanying them, while the unrewarding flowers never did. Each test included a color component, with the focal flower that had the cue all being one color and the non-focal flowers being the other color. After the bees trained to reach a certain accuracy level in their choices, they took a memory test of these choices after a retention interval. The memory test included the cues from the training portion, but none of the flowers were rewarding. I repeated this process with four treatment groups (control, shape, nectar guides, and social), two different focal colors (yellow or orange), and two different retention intervals (one hour or twenty-four hours). I recorded the number of correct (focal flower) and incorrect (non-focal flower) choices for

training and test sessions, and then analyzed videos to calculate foraging efficiency in terms of how much sucrose was gathered per second. I tested twenty bees for each of the four treatments, with ten bees for each focal color (yellow and orange) and ten bees for each retention interval (1 hour and 24 hours) in each treatment for a total of 80 bees tested.

Experimental Setup

The training and test arena was a 48in x 24in x 15in wooden box connected to the hive box with a 1.5in diameter clear plastic tube. The arena had a plexiglass lid on top for observing bee choices and for a camera to record trainings and tests. I inserted a plastic wall containing fake flowers for bees to forage from during training testing and pre-training (Figure 2). This wall had twelve evenly spaced holes to hold the artificial flowers, with the back of the wall being accessible to refill the flowers with sucrose during tests without interfering with the test subjects. I constructed flowers from 2in diameter foam disks with a “nectar” well in the middle that contained the sucrose solution or water used during testing and training. I made nectar wells from Eppendorf tubes, with holes cut in the back of them so they could be refilled via pipettor. I cut flowers for the “shape” tests using a flower-shaped paper puncher, while the other flowers were cut with a simple circular puncher (Figure 3). Flowers used in the “nectar guide” tests had four solid lines centered around the nectar well and drawn on through a stencil with a sharpie. Flowers used in the “social information” tests had a dried, pinned *Bombus impatiens* bee stuck through the foam petal. I recorded all training and test sessions with a camera placed above the arena.

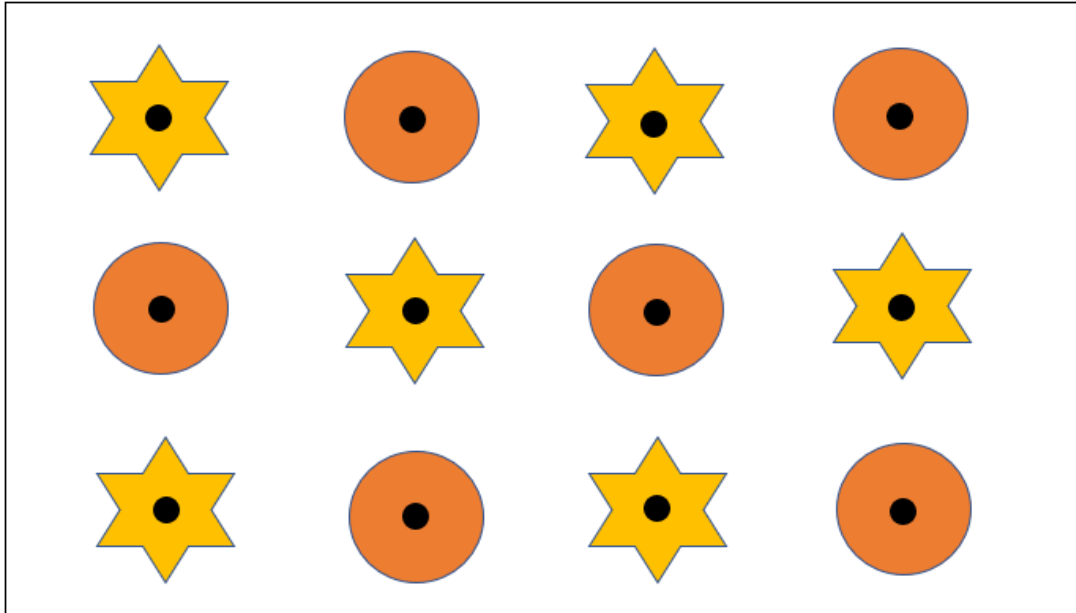


Figure 2: An example of a flower wall set up for a training or test session. The black circles in the middle of the flowers represent the nectar well. This test session is set up for the shape treatment with yellow as the focal color and orange as the non-focal.

Control	Social	Nectar guide	Shape

Figure 3: Graphic representations of both colors of flower from each treatment type. The bee figure on the social treatment flowers represents a pinned *Bombus impatiens* bee, and the blue circle in the middle of each flower represents the nectar well.

Experimental Procedure

Pre-training

I used pre-training sessions to familiarize the bees with the setup of flowers. I designed pre-training to have none of the cues in the experimental design so the bees did not learn anything that would affect their behavior during training and testing. I used purple flowers during pre-training so that the orange and yellow flowers used during training were novel to the bees at the beginning of training. None of the floral or social cues—shape, nectar guides, or pinned conspecifics—were present during pre-training. There was also no difference in reliability during pre-training, meaning every flower gave a reward. All the flowers in pre-training were identical, giving the bees no identifiable characteristics like shape, pattern, or color to create a preference for certain flowers over others. I placed rewards in the nectar wells of the flowers with a transfer pipettor. The reward in pre-training was a 60% by volume sucrose solution, a higher concentration than the 15% solution normally fed to the bees to encourage foraging during pre-training.

Training

During training flowers with certain characteristics (color, shape, presence of nectar guides, or presence of a pinned conspecific) were always rewarding, while flowers lacking these characteristics were never rewarding. The rewarding flowers contained 5 ml of a 60% sucrose solution inserted in the nectar well by a micropipettor. The unrewarding flowers contained 5 ml of water. A single bee, tagged previously as a forager, entered the testing arena through the connecting tube and foraged from the artificial flowers. Choices

were determined when a bee stuck any part of its head into the nectar well. Throughout the training session, bees were able to leave the arena to drop off sucrose at the hive and then return to continue foraging. To complete training, a bee had to reach a level of at least 80% accuracy in the last 20 choices. This means a bee needed to make at least twenty choices to complete training and that, of the last twenty, at least sixteen needed to be correct. Bees that did not reach this criterion after a certain amount of time were not allowed to attempt a training session again for a full week to ensure anything they learned did not interfere with the next training session. Bees that did reach the 80% mark were removed from the arena for their one- or twenty-four-hour retention interval and then tested.

Testing

After either one or twenty-four hours depending on the retention interval, I tested bees on how well they remembered the social or floral cues learned during training. I tested memory using extinction trials, where no flowers offered a reward, and every flower was filled with 5 ml of water regardless of whether they were filled with sucrose during the training. I administered testing in the same manner as training, but bees were only required to make twenty choices total, and they did not have to reach any accuracy level. After bees made their twenty choices, I freeze-killed them so I could measure their body and head size later. Bees that made less than twenty choices were not trained or tested again.

Video Analysis

I recorded all training and testing sessions for video analysis. I conducted video analysis using Behavioral Observation Research Interactive Software (BORIS) to timestamp each part of the recording where a bee exhibited a specific behavior. I recorded the times when bees landed on and left each flower as well as the time they entered and left the arena. I counted the time spent on each flower as the handling time and the total time the bee spent in the arena as the total foraging time. To calculate the sucrose per second gathered, I used the number of flowers visited throughout the active foraging time, with each flower handled by the bee counting as 5 ml of sucrose.

Statistical Analysis

I used ANOVAs and ANCOVAs to analyze the results of the experiment. I first used ANCOVAs to assess variance in learning by looking at differences in treatments and flower colors in the number of choices to reach the 80% accuracy level and in sucrose gathered per second of foraging time. The covariate for these ANCOVAs was head size. I compared body and head size measurements from bees but found no strong correlation between the two (Figure A1), so I used head size as a proxy for brain size. After I found head size to be an insignificant factor, I removed it from the analysis and conducted ANOVAs. After doing the initial learning ANOVA on trials to reach learning criterion, I looked at the pattern of learning using a repeated measures ANOVA, with the training broken up into four quartiles by number of choices. For all analyses, I blocked by colonies to account for any variation from colony-level behavioral differences and dropped colony from the analysis if it was found to be non-significant. I conducted the

same factorial ANCOVAs and ANOVAs for memory, examining memory test accuracy and sucrose per second. Since the tests occurred after one of two retention intervals, I added this to the ANOVA. Once again, head size did not have a significant effect in the ANCOVA, so I used an ANOVA instead. I compared the first ten and last ten choices separately, as well as a repeated measures ANOVA of both first and last choices. For memory, I conducted the same ANOVAs blocked by colony that were done for learning. After running the ANOVAs, I used Tukey's Honest Significant Difference (HSD) tests to examine significant differences among the groups being compared.

Results

Learning Results

Choices to Criterion

First, I conducted an ANCOVA to examine the effect of treatment type and flower color on the number of choices needed to reach training criterion blocked by colony with head size as a covariate (Table A1). The ANCOVA showed head size had no effect, so I dropped this from the analysis and conducted an ANOVA (Table 1). First, I found a significant effect of treatment. Using Tukey's HSD test, I found that nectar guide trainings were completed in significantly fewer choices than control and social trainings (Table 2). I also found a significant interaction between treatment at the color of the rewarding flowers. When examining the interaction between color and treatment present in the ANOVA, I found nectar guide treatments with orange flowers result in significantly fewer choices than control treatments with orange flowers and social treatments with orange flowers (Table 3). When looking at the difference between

colonies, I found colony 7 took significantly more choices to complete training than colonies 4 and 5 (Table 4).

	Sums of Squares (SS)	Degrees of Freedom	Mean Squares (MS)	F	<i>p</i>
Treatment	2827	3	942.4	3.989	0.01118
Color	813	1	812.8	3.441	0.06795
Colony	3775	4	943.7	3.995	0.00571
Treatment:Color	3445	3	1148.2	4.860	0.00402
Residuals	16064	68	236.2		

Table 1: ANOVA results of the effects of treatment type and flower color blocked by colony on choices needed to complete training.

	Nectar Guide	Shape	Social
Shape	0.59441		
Social	0.01433	0.25863	
Control	0.04636	0.49798	0.97087

Table 2: Results of a Tukey HSD test examining the difference between treatment types for number of choices to complete training.

	Nectar Guide: Orange	Nectar Guide: Yellow	Control: Orange	Control : Yellow	Social: Orange	Social: Yellow	Shape: Orange
Nectar Guide: Yellow	0.8645 5						
Control: Orange	0.00673	0.2385 7					
Control: Yellow	0.8857 1	1.0000 0	0.21627				
Social:Orange	0.00101	0.0667 7	0.99909	0.05869			
Social: Yellow	0.8636 2	1.0000 0	0.23953	1.00000	0.0671 2		
Shape: Orange	0.8690 1	1.0000 0	0.23393	1.00000	0.0650 6	1.0000 0	
Shape: Yellow	0.6320 2	0.9999 0	0.46773 3	0.99979	0.1717 1	0.9999 1	0.9998 9

Table 3: P values from results of a Tukey HSD test examining the differences in number of choices to complete training comparing an interaction between treatment type and flower color.

	Colony 3	Colony 4	Colony 5	Colony 6
Colony 4	0.51876			
Colony 5	0.63006	0.97484		
Colony 6	0.68907	0.90759	0.99813	
Colony 7	0.99242	0.00718	0.02466	0.06471

Table 4: P values from results of a Tukey HSD test examining the differences in number of choices to complete training comparing differences between colonies.

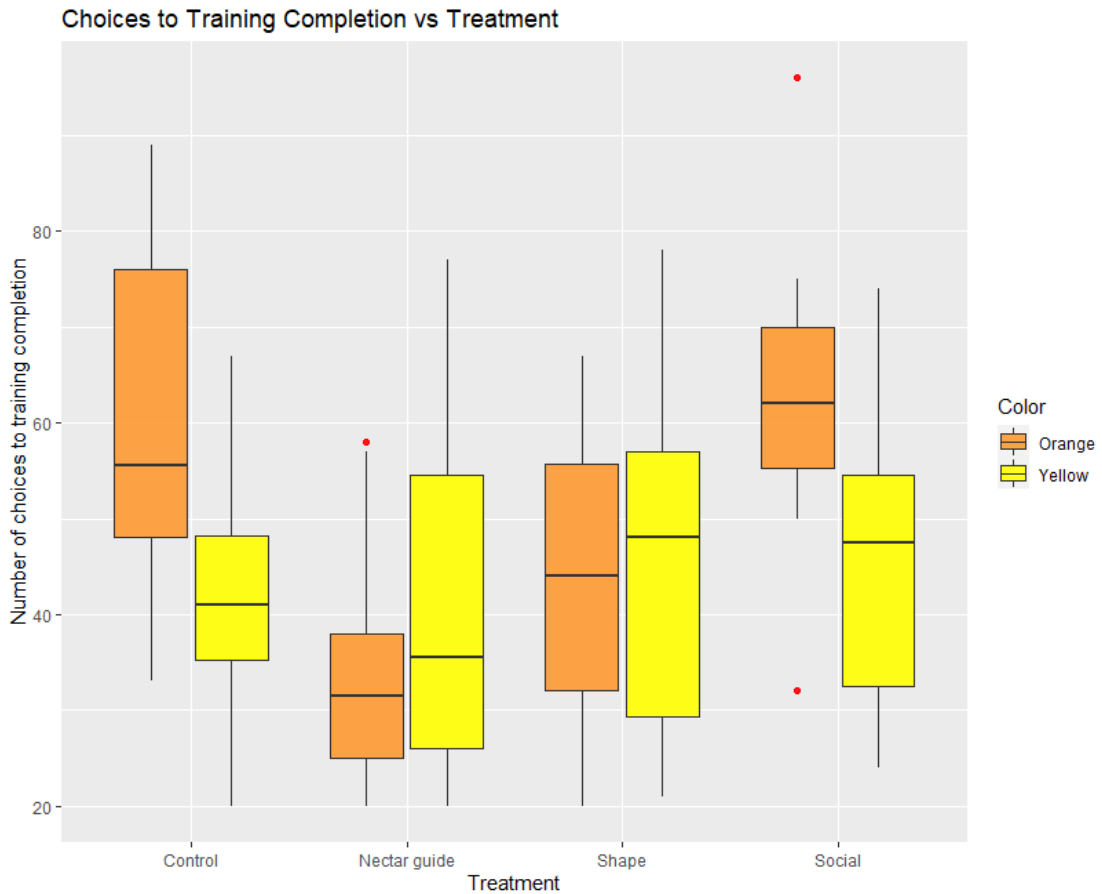


Figure 4: A comparison of the effects of treatment type on the number of choices needed to achieve 80% accuracy in 20 choices during training. Nectar guide treatments were completed in significantly fewer choices than control and social treatments. Red dots indicate outliers.

Pattern of Learning with Learning Score

Next, I looked at the pattern of learning with the percent of correct choices (learning score). Here I used a repeated measured factorial ANOVA blocked by colony to look at the effects of treatment on learning across training quartiles (Table 5). Treatment, quartile, and colony all influenced the learning score (percent of correct choices). I performed a Tukey's HSD test that showed each quartile had a significantly higher score than the previous one, an expected result as the bees improved their accuracy while they learned about the cues and rewards (Table 6). Another Tukey's test showed bees in the nectar guide treatment group performed better on this learning metric than those in the social treatments, but all other differences in treatment score were non-significant (Table 7). Although both treatment and quartile are significant effects, their interaction is not. Bees in the four treatment groups did not have a significant difference in performance from each other when comparing the scores between each quartile. Again, there was a significant difference in performance between the colonies, with colonies 4, 5, and 6 having a significantly higher average score than colony 3 (Table 8).

	Sums of Squares (SS)	Degrees of Freedom	Mean Squares (MS)	F	<i>p</i>
Treatment	0.167	3	0.0556	3.404	0.01814
Quartile	3.346	3	1.1155	68.324	<2e-16
Color	0.019	1	0.0581	1.186	0.27713
Colony	0.232	4	0.0581	3.560	0.00748
Treatment:Quartile	0.201	9	0.0223	1.366	0.20298
Treatment:Color	0.055	3	0.0182	1.116	0.34303
Quartile:Color	0.044	3	0.0146	0.897	0.44302
Treatment:Quartile:Color	0.115	9	0.0128	0.784	0.63107
Residuals	4.637	284	0.0163		

Table 5: ANOVA results of the effects of treatment type, focal color, training quartile, and bee ID on percentage of correct choices during training.

	Quartile 1	Quartile 2	Quartile 3
Quartile 2	0.00150		
Quartile 3	0.00000	0.04895	
Quartile 4	0.00000	0.00000	0.00000

Table 6: P values from results of a Tukey HSD test examining the differences in learning scores between different quartiles.

	Nectar Guide	Shape	Social
Shape	0.60012		
Social	0.02486	0.38215	
Control	0.05518	0.56156	0.99120

Table 7: P values from a Tukey HSD test examining the differences in number of choices in each quartile across learning phase between treatments.

	Colony 3	Colony 4	Colony 5	Colony 6
Colony 4	0.01130			
Colony 5	0.02191	0.94473		
Colony 6	0.02668	0.89419	0.99970	
Colony 7	0.08347	0.20875	0.56236	0.70450

Table 8: P values from a Tukey HSD test examining the differences in number of choices to complete training across quartiles comparing differences between colonies.

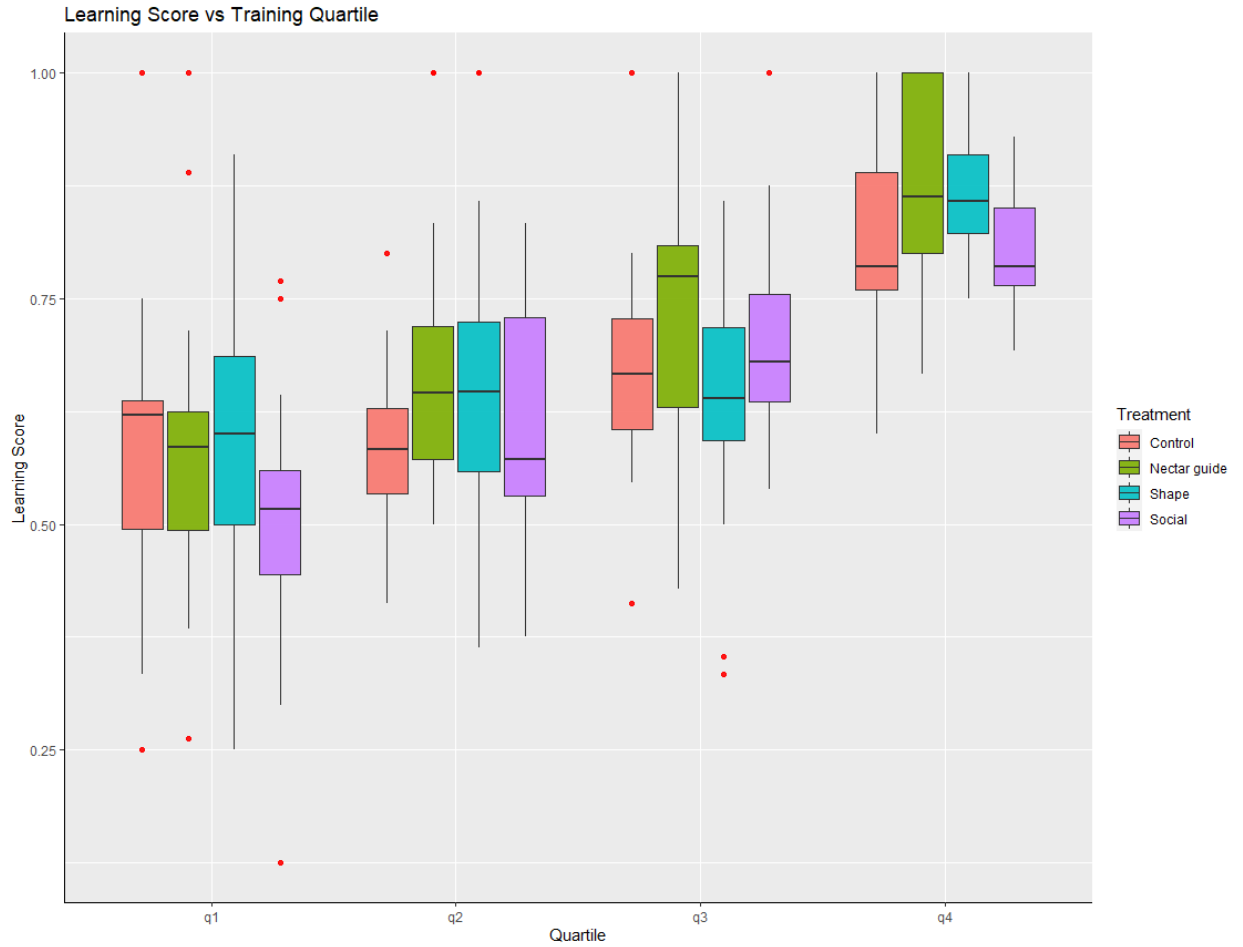


Figure 5: Learning scores during training by treatment type broken into quartiles. Learning scores increased in successive quartiles as bees came closer to the 80% training criterion. Red dots indicate outliers.

Pattern of Learning with Sucrose Per Second

I conducted the same repeated measures ANOVA blocked by colony and including quartiles while examining sucrose per second instead of learning score. This ANOVA showed a significant influence from the quartile and the colony (Table 9). A Tukey's HSD test showed that, apart from quartile 2 and 3, the quartiles were all significantly different from each other in their effect on sucrose gathered per second (Table 10). Like with learning score, sucrose per second increased from quartile 1 to quartile 4 (Figure 6). Another Tukey test showed bees from colony 5 having a higher

average score than bees from the other four colonies (Table 11). I found no other interactions between colonies or quartiles.

	Sums of Squares (SS)	Degrees of Freedom	Mean Squares (MS)	F	<i>p</i>
Treatment	0.0025	3	0.00082	0.450	0.7173
Quartile	0.1582	3	0.05272	29.008	< 2e-16
Color	0.0002	1	0.00017	0.094	0.7599
Colony	0.0517	4	0.01291	7.105	1.83e-05
Treatment:Quartile	0.0220	9	0.00244	1.342	0.2147
Treatment:Color	0.0139	3	0.00463	2.545	0.0563
Quartile:Color	0.0012	3	0.00039	0.217	0.8845
Treatment:Quartile:Color	0.0125	9	0.00139	0.763	0.6505
Residuals	0.5162	284	0.00182		

Table 9: ANOVA results of the effects of treatment type, focal color, training quartile, and bee ID on sucrose harvested per second foraging time during training.

	Quartile 1	Quartile 2	Quartile 3
Quartile 2	0.00018		
Quartile 3	0.00000	0.70596	
Quartile 4	0.00000	0.00001	0.00056

Table 10: P values from results of a Tukey HSD test examining the differences in sucrose gathered per second between different quartiles.

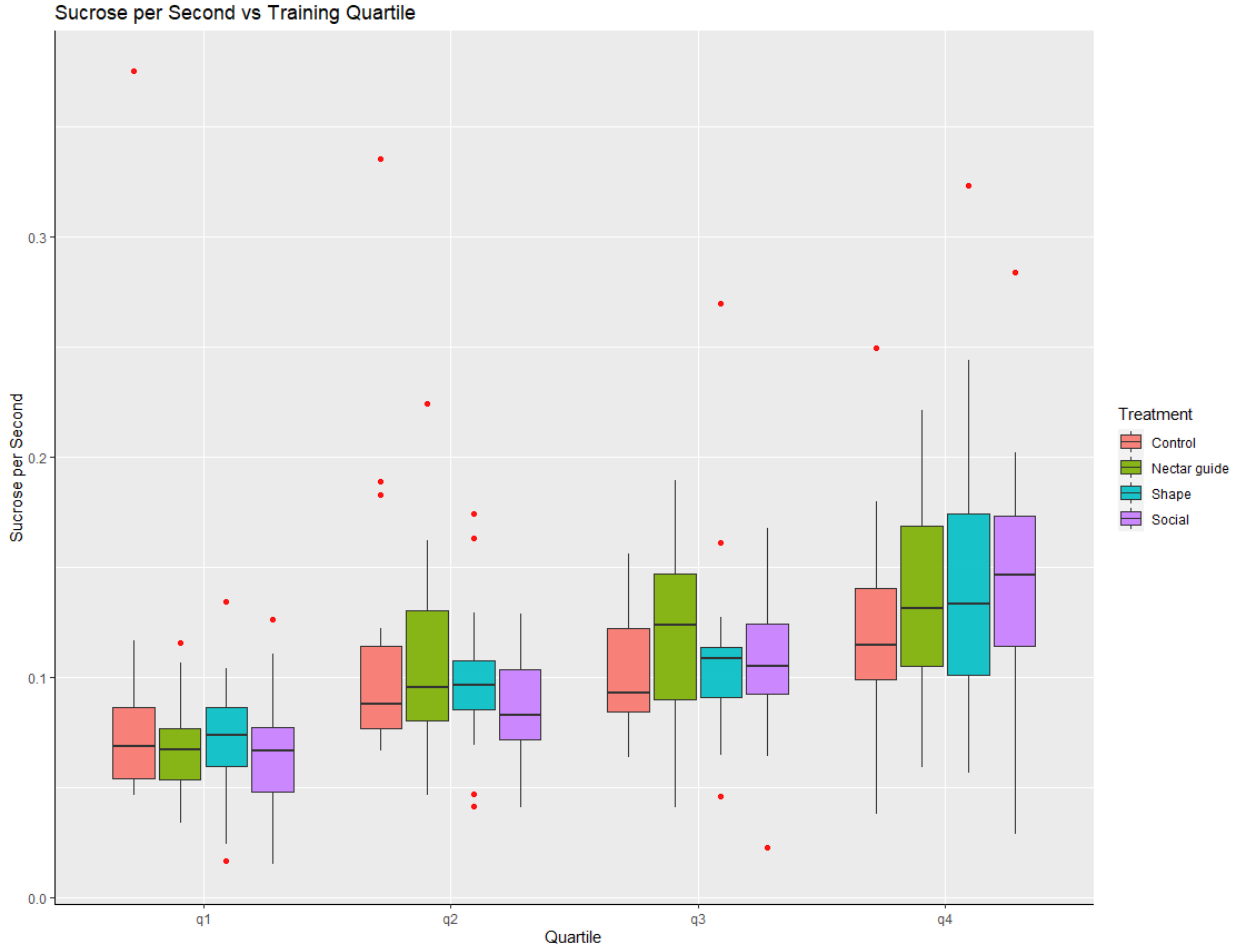


Figure 6: Sucrose gathered per second during training by treatment type broken into quartiles. Sucrose gathered per second increased in successive quartiles as bees came closer to the 80% training criterion, with the exception of no significant difference between quartiles 2 and 3. Red dots indicate outliers.

	Colony 3	Colony 4	Colony 5	Colony 6
Colony 4	0.28211			
Colony 5	0.01119	0.00037		
Colony 6	0.12136	0.67703	0.03684	
Colony 7	0.18995	0.96471	0.00765	0.97226

Table 11: P values from a Tukey HSD test examining the differences in sucrose gathered per second comparing differences between colonies. Colony 5 had a significantly higher average sucrose gathered than all other colonies.

Memory Results

Memory Score for First Ten Choices

I conducted a factorial ANCOVA to examine the effect of treatment and flower color on the first ten choices made during testing, with head size and colony being dropped after finding no significant effects from either (Table A2). Since this test focused on memory, retention interval was included. The ANOVA without head size (Table 12) showed a significant influence on memory score from treatment type, flower color, and treatment by flower color interaction. A Tukey's HSD test showed bees being significantly more accurate on nectar guide tests than control tests, with no other interactions found between treatments (Table 13). A Tukey's test examining flower color showed significantly more accurate choices on tests with a yellow focal flower compared with orange ($p = 0.0004285$). I found multiple differences in a Tukey's test examining treatment-color interactions. Every treatment-color combination led to significantly higher scores than control-orange except shape-orange, and yellow nectar guide treatments had significantly higher scores than shape-orange (Table 14). All other treatment-color interactions were non-significant. I found no significant difference between the two retention intervals. To determine whether the memory scores were better than chance, I performed one-sample t-tests against 0.5 to see if the treatments and flower colors led to bees remembering choices better than a random 50/50 selection. Tests with every treatment type and both flower colors had significantly better memory scores than 0.5 (Table 15).

	Sums of Squares (SS)	Degrees of Freedom	Mean Squares (MS)	F	<i>p</i>
Treatment	0.2314	3	0.0771	3.032	0.03559
Color	0.3511	1	0.3511	13.803	0.00043
Retention Interval (RI)	0.0281	1	0.0281	1.106	0.29698
Treatment:Color	0.2474	3	0.0825	3.242	0.02770
Treatment:RI	0.1224	3	0.0408	1.604	0.19726
Color:RI	0.0101	1	0.0101	0.398	0.53035
Treatment:Color:RI	0.0604	3	0.0201	0.791	0.50328
Residuals	1.6280	64	0.0254		

Table 12: ANOVA results of the effects of treatment type, focal color, and retention interval on the first ten choices made during testing. Treatment, color, and the interaction of treatment and color showed a significant influence.

	Nectar Guide	Shape	Social
Shape	0.29019		
Social	0.57337	0.95977	
Control	0.02103	0.63557	0.33979

Table 13: P values from a Tukey HSD test examining the differences in memory score for the first ten choices of the testing phase between treatments.

	Nectar Guide: Orange	Nectar Guide: Yellow	Control: Orange	Control: Yellow	Social: Orange	Social: Yellow	Shape: Orange
Nectar Guide: Yellow	0.85303						
Control: Orange	0.01795	0.00017					
Control: Yellow	0.99668	0.99668	0.00200				
Social: Orange	0.99999	0.78169	0.02676	0.98993			
Social: Yellow	0.99999	0.69829	0.03929	0.97551	0.99999		
Shape: Orange	0.78169	0.08051	0.51441	0.34086	0.85303	0.90931	
Shape: Yellow	0.99988	0.97551	0.00499	0.99999	0.99920	0.99668	0.51441

Table 14: P values from results of a Tukey HSD test examining the differences in memory score for the first ten choices of testing comparing an interaction between treatment type and flower color.

	t	Mean Score	p
Nectar Guide	9.5778	0.81	1.048e-08
Shape	5.3112	0.72	3.986e-05
Social	6.5633	0.745	2.772e-06
Control	3.3477	0.66	0.00338
Orange Flower	5.6568	0.6675	1.552e-06
Yellow Flower	12.093	0.8	9.05e-15

Table 15: P values from one-sample t-test results of test choice accuracy against random chance (0.5). All treatments and flower colors led to significantly higher scores than chance.

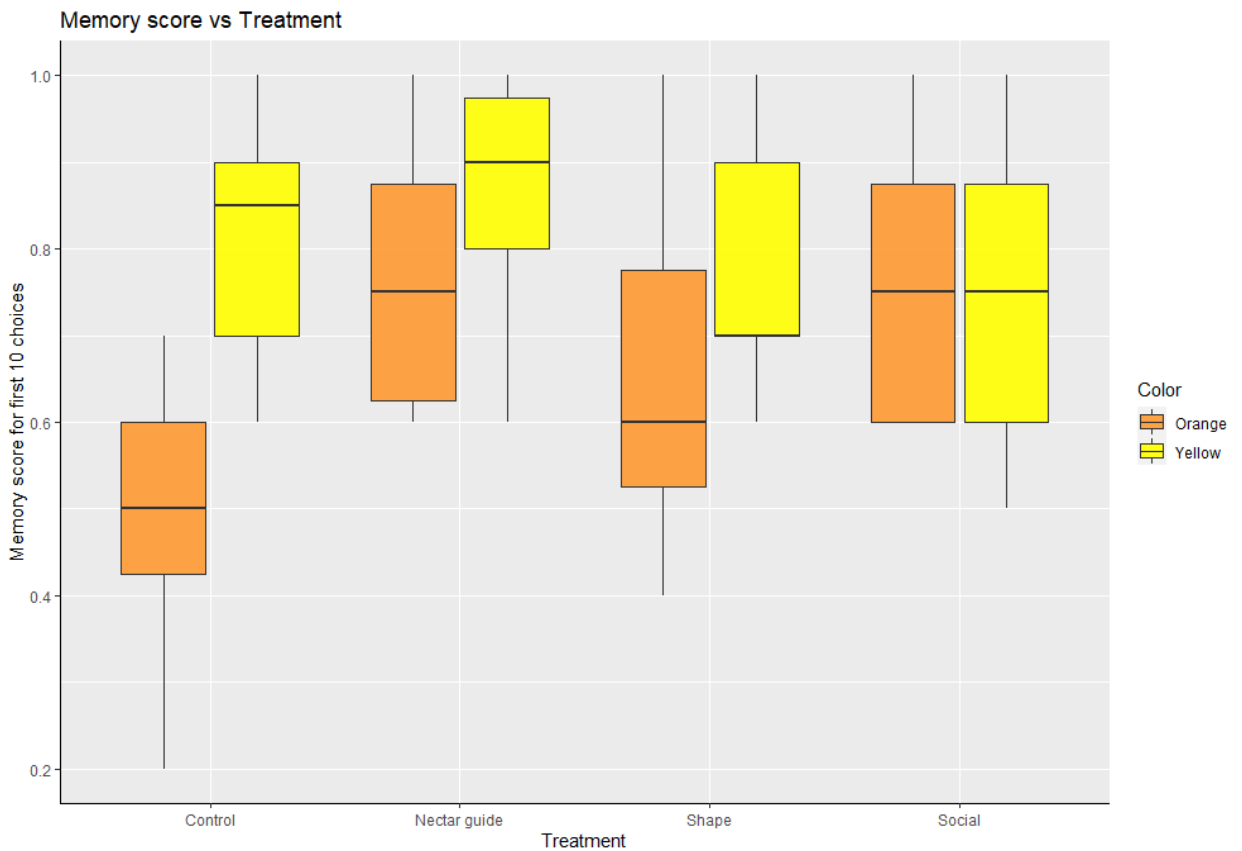


Figure 7: Memory score (choice accuracy) in both retention intervals for the first ten choices by treatment broken up into the two possible focal flower colors. For tests with orange as the focal color, every treatment except shape led to a higher accuracy than the control-orange treatment.

Memory Score for Both Test Halves

Next, I conducted a repeated measures ANOVA examining memory scores, blocking by colony, and factoring in both test halves as well as retention interval (Table

A3). After colony was found to have no significant effect, I conducted another ANOVA without it. The second ANOVA showed treatment, color, and an interaction between treatment and retention interval all have a significant influence on memory score (Table 16). I conducted a Tukey's HSD test on treatment that showed nectar guide treatments leading to significantly higher test scores than control and social treatments, with all other treatment comparisons being non-significant (Table 17). A Tukey's test on color showed tests with yellow focal flowers leading to significantly higher memory scores than tests with orange focal flower ($p = 0.0004509$). When examining the treatment–retention interval interaction with a Tukey's test, I found nectar guide treatments with both retention intervals and social treatment with a 1-hour interval leading to significantly higher scores than the control with a 24-hour retention interval (Table 18). I found no other significant interactions among treatment type and retention interval.

	Sum of Squares (SS)	Degrees of Freedom	Mean Squares (MS)	F	<i>p</i>
Treatment	0.427	3	0.1424	4.859	0.00311
Retention Interval (RI)	0.036	1	0.0360	1.228	0.26985
Color	0.380	1	0.3803	12.972	0.00045
Test Half	0.049	1	0.0490	1.672	0.19837
Treatment:RI	0.307	3	0.1025	3.497	0.01756
Treatment:Color	0.185	3	0.0618	2.107	0.10260
RI:Color	0.100	1	0.1000	3.412	0.06705
Treatment:Test Half	0.016	3	0.0052	0.176	0.91231
RI:Test Half	0.002	1	0.0022	0.077	0.78219
Color:Test Half	0.049	1	0.0490	1.672	0.19837
Treatment:RI:Color	0.030	3	0.0098	0.335	0.79971
Treatment:RI:Test Half	0.050	3	0.0168	0.571	0.63482
Treatment:Color:Test Half	0.132	3	0.0438	1.495	0.21895
RI:Color:Test Half	0.030	1	0.0302	1.032	0.31161
Treatment:RI:Color:Test Half	0.032	3	0.0107	0.367	0.77713
Residuals	3.752	128	0.0293		

Table 16: ANOVA results of the effects of treatment type, focal color, retention interval, test half, and colony on memory scores during testing. Treatment, color, and an interaction between treatment and retention interval showed a significant influence.

	Nectar Guide	Shape	Social
Shape	0.041272655		
Social	0.478981793	0.602205787	
Control	0.002603454	0.797297003	0.141623131

Table 17: P values from a Tukey's HSD test showing nectar guide treatments having significantly higher memory scores than both control and shape treatments.

	Nectar Guide: 1h	Nectar Guide: 24h	Control: 1h	Control: 24h	Social: 1h	Social: 24h	Shape: 1h
Nectar Guide: 24h	0.81791						
Control: 1h	0.99999	0.65150					
Control: 24h	0.02479	0.00012	0.05490				
Social: 1h	0.99999	0.71125	0.99999	0.04249			
Social: 24h	0.99995	0.58948	0.99999	0.07027	0.99999		
Shape: 1h	0.98326	0.24982	0.99811	0.24982	0.99561	0.99930	
Shape: 24h	0.86239	0.08908	0.95398	0.24982	0.93042	0.97128	0.99979

Table 18: P values from a Tukey's HSD test showing both retention intervals in the nectar guide treatment and the 1-hour interval in the social treatment leading to significantly higher memory scores than the control with a 24-hour retention interval.

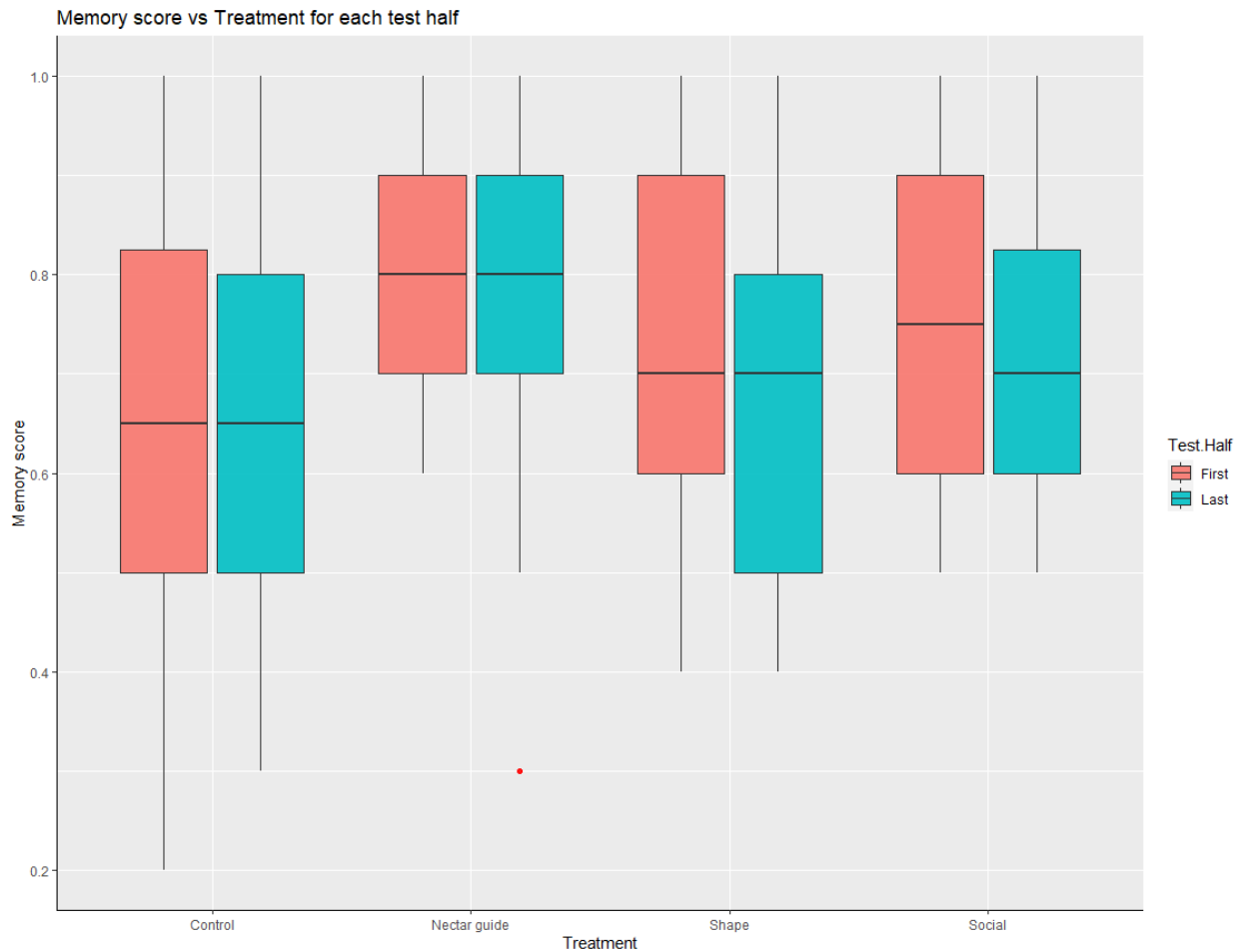


Figure 8: A comparison of different treatments to the memory scores for each half of testing trials. Red dots indicate outliers.

Sucrose Gathered Per Second for First 10 Test Choices

I conducted an ANOVA blocked by colony to evaluate the effects of treatment, color, and retention interval on sucrose gathered per second, dropping colony after the ANOVA showed it was non-significant (Table A4). The ANOVA without colony showed color was the only factor with significant influence (Table 19). I performed a Tukey's HSD test that showed tests with yellow flowers had significantly higher sucrose per second than tests with orange flowers ($p = 0.0040359$).

	Sum of Squares (SS)	Degrees of Freedom	Mean Squares (MS)	F	<i>p</i>
Treatment	0.1603	3	0.05343	1.990	0.12437
Color	0.2389	1	0.23895	8.898	0.00404
Retention Interval (RI)	0.0005	1	0.00055	0.020	0.88695
Treatment:Color	0.1043	3	0.03476	1.294	0.28400
Treatment:RI	0.0767	3	0.02555	0.952	0.42111
Color:RI	0.0552	1	0.05525	2.057	0.15633
Treatment:Color:RI	0.0777	3	0.02589	0.964	0.41519
Residuals	1.7186	64	0.02685		

Table 19: ANOVA results of the effects of treatment type, focal color, and retention interval on sucrose gathered per second during the first ten choices of testing.

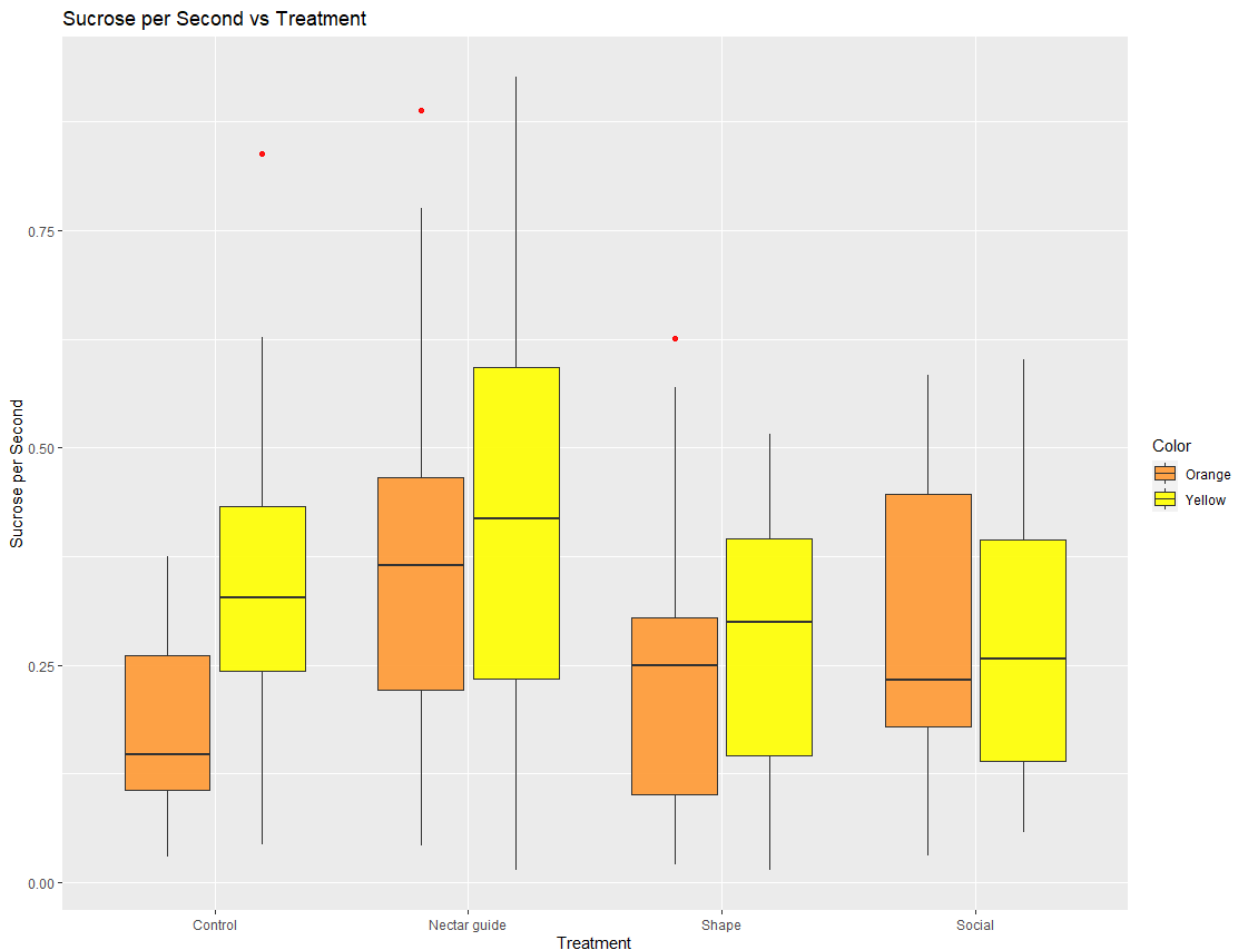


Figure 9: Sucrose gathered per second during test trials by treatment broken up into the two possible focal flower colors. Sucrose per second during tests with yellow focal flowers was significantly higher than in tests with orange focal flowers. Red dots indicate outliers.

Discussion

With my experiment, I aimed to assess a few main predictions. My two main objectives related to different types of informational cues that bees experience while foraging. First, I predicted that the compound stimuli of floral and social information would have a more significant impact on bumblebee forager learning than the compound stimuli of two types of floral information. Second, I predicted that the compound stimuli of floral and social information would also have a more significant influence on bumblebee forager memory than two types of floral information. A sub-objective of my experiment was to test the hypothesis that bee brain size is correlated with foraging performance, with larger bee brains allowing more efficient learning and more accurate memory. The results of my experiment did not support any of my hypotheses. The compound stimuli of flower color and nectar guides, two types of floral information, had a more significant influence on both learning and memory than the compound stimuli of flower color and pinned conspecifics. There was also no correlation found between brain size and foraging performance. My experiment showed some interesting results regarding the effect of flower color and retention interval on memory and some intriguing differences in learning and memory scores from the different treatment types.

The results from the learning portion of my experiment show that the type of treatment and an interaction between treatment type and flower color both had an influence on the number of choices the bees made to complete their training (Table 1). Trainings with nectar guide were completed in significantly fewer choices than social and control (Table 2), and trainings with nectar guides and orange flowers were completed in significantly fewer choices than social and control trainings with orange flowers (Table

3). There was a noticeable difference between the flower colors in control and social treatments, with trainings with yellow flowers having a greater influence than orange (Figure 4). Nectar guide trainings had a more significant influence on the percent of correct choices when looking at the pattern of learning (Table 7), and treatment type and flower color both had a nonsignificant influence on foraging efficiency in training measured by sucrose per second (Table 9). The results from the testing portion of my experiment show an influence from treatment type, flower color, and an interaction between treatment and color on the percent of correct choices in the first half of the memory test. Nectar guide tests had a significantly higher score than control tests (Table 14) and yellow focal flowers were associated with more accurate memory than orange flowers. With treatment-color combinations, all combinations had significantly better memory scores than control-orange except for shape-orange, and nectar guide-yellow led to better scores than shape-orange (Table 14). While every treatment and flower color overall led to significantly better memory scores than random chance as expected (Table 15), control treatments with orange flower were the only treatment-color combination that did not have memory scores significantly better than 0.5 (Figure 7). When looking at sucrose per second, flower color was the only significant influence, with tests with yellow focal flowers leading to higher sucrose per second than orange (Table 19). My experiment found no correlation between the bees' brain size and their performance on the learning and memory tests (Table A1, Table A2).

One interesting finding from my experiment is the lack of change in the choices bees made during their memory tests between the first and second half (Table 16). I compared the memory score in both test halves because previous literature has shown

that bees learning from social information tend to stop using the information and change their choices rapidly when confronted with a lack of reward, but this did not occur in my experiment. One explanation for this is that, since my experiment tested compound stimuli, the bees were not making judgements based on social information alone, and the additional information of flower color prevented the bees from rapidly changing their choices. I also wanted to examine whether a rapid change of flower choice happened if the forms of floral information like nectar guides were no longer rewarding, but the bees stuck with their initial choice in these treatments as well. It could be that the twenty choices made during the tests were not enough for the bees to switch based on the information they gathered, but some bees were able to build the association between cue and reward during training in as few as twenty choices, so twenty choices should be enough for them to build associations with new information. This result is less surprising than the lack of rapid change in choice using social information, since there is no literature to support such a change when using only floral information.

The differences in performance with flower color present in the experiment are also interesting. Trials with rewarding yellow flowers have consistently higher measurements of memory when compared with those with rewarding orange flowers. The differences are so significant that, for the control treatment, the association between orange flowers and rewards seems to be completely forgotten by the bees in their test sessions (Figure 7). For the beginning of the training sessions, this could be explained by the innate preference for certain flower colors found in some species of bee (Gumbert 2000). But innate color bias is a less convincing argument for color preference in memory scores since bees only follow color biases if the flower is also continuously

rewarding and ignore these biases as experience is gained if the flower is unrewarding (Maharaj et al. 2019). Another interesting aspect of color choice in my experiment is how it changes between learning and memory trials. With socially learned information, the color preference changes between training and testing. There is a significant difference between the two flower colors for social treatments during training (Figure 4), but during testing both flower colors have a relatively equal memory score in the social treatment group (Figure 7). Although this could be explained by a loss of innate bias with experience, it is unclear why this is only present in the social treatment.

The differences in learning pattern seen in the results of my experiment are also intriguing. Previous literature suggests that social information is learned at a similar speed to floral information (Abts & Dunlap 2022, Leadbeater & Chittka 2007), but my results show different patterns of learning between social information and floral information from nectar guides, with learning in the nectar guide treatments occurring in fewer choices (Figure 4) and with greater accuracy (Figure 5) than social treatments. The Leadbeater & Chittka (2007) paper also suggests learning may start earlier in the presence of conspecifics, but I would expect the social treatments to be completed in fewer choices than the floral information treatments if that were the case, and social treatment trainings have lower scores in my experiment at the beginning of training (Figure 5). One possible explanation for this is that the bees are initially viewing pinned conspecifics as competition and avoiding them like they have been shown to do in other studies (Baude et al. 2011). Results from my experiment would support this theory in the first three quartiles of training, as the social treatments start with a lower learning score than others in quartile one but improve over the next two quartiles (Figure 5). But in

quartile four the social treatment returns to a lower score than the nectar guide and shape treatment, drawing this avoidance theory into question as the bees likely would have learned to stop seeing the pinned conspecifics as competition by the end of their training.

One of the most interesting findings from my experiment is the effectiveness of nectar guides as a cue when compared with the other floral and social information the bees were trained and tested on. Previous literature has shown the effectiveness of nectar guides for learning, but the memory aspect has, to my knowledge, not been tested. One explanation for why nectar guides consistently lead to significantly better performances on different measurements of learning and memory compared with other treatments is the consistency of rewards. The consistent rewards might affect the social treatment choices since bees prefer social information in variable environments (Smolla et al. 2016) and this experiment provided no variation in rewards. For memory tests, the bees were no longer naïve like they were during the learning phase, and this would make them less likely to initially trust the social information, since bees prefer social information when they are unfamiliar with their options (Leadbeater & Chittka 2009). This does not explain why the shape treatment did not outperform social in learning and memory, since the shape treatment, like the nectar guide treatment, was a combination of two types of floral information. There are some potential explanations as to why shape treatments had similar results to the control. One explanation for this is that the aspect of flower shape that I used in the trainings and tests is not as memorable to bees as other aspects of floral shape. It could be that a distinctly shaped flower outline is not as influential to bee learning and memory as a flower that is significantly different in size. Distinct outlines should make flowers more memorable and easier to learn on (Free 1970), but perhaps

other memorable floral shape aspects like symmetry (Moller 1995) or size (Spaethe et al. 2001) would have a more significant impact. It is also important to note that the flowers in the nectar guide, control, and social treatments were all larger than those in the shape treatment and this could explain why shape had a poor performance compared to nectar guides. While the flowers in the shape treatment had the same diameter as those in other treatments, the cut-out shape of the petals caused them to have a smaller surface area. The size and depth of the nectar well can also improve learning (Lavery 1994) and could be a more memorable floral shape aspect that would have a more pronounced effect than the aspect of shape I tested in my experiment.

Part of my experimental design focused on completely reliable information to reduce uncertainty in the bumblebee's decision making. This provides an interesting contrast to experiments like that of Abts & Dunlap (2022), where unreliable information provided a higher level of uncertainty. In the Abts & Dunlap experiment, bees changed their choices across time as they discovered how reliable their rewards were, choosing to avoid conspecifics if floral information was more reliable or if previous information from conspecifics was unreliable. This difference in how bees value memory based on reliability was not present in my study, since the cues in my experiments were always reliable, so bees in my study should have valued both types of information equally in terms of reliability. This makes my findings on floral learning and memory compared with social learning and memory more intriguing, since the two resulted in clear differences despite their being equally reliable cues. I also found the difference in social information memory tests between the two studies interesting, since bees remembered floral information better than social information in my experiment, but the opposite was

true in the Abts & Dunlap study. This difference could be due to my use of compound stimuli. Perhaps social information has a more significant influence on memory by itself but is less influential than floral information when paired with another type of stimuli like flower color.

This experiment produced no evidence to support the idea of larger brains allowing improved learning and memory functions, since I found no correlation between head size and any measurement of learning and memory ability. This could simply be because of the sample size of this experiment and the low amount of variation I found in head and body size, and experiments with a larger sample size might show a clear correlation. It could also be evidence that brain size does not have a significant effect on insect learning and memory, as suggested in the review by Chittka & Niven (2009). It is still surprising to find no correlation between bee size and foraging performance, since, regardless of brain size, larger bees tend to have higher foraging rates (Herrmann et al. 2018, Spaethe & Weidenmuller 2002). This experiment also provided no evidence to support a difference in memory performance based on retention interval alone, although the memory test comparing test halves showed an interaction between treatment and retention interval. This difference involved both nectar guide retention intervals and the 24-hour social retention interval having a higher score than the control 24-hour interval. The low performance in the control group after 24 hours could be related to the tendency of bumblebees to lose memories overnight (Keasar et al. 1996), but other studies show a relative lack of memory decay in bumblebees in a single night (Chittka 1998). The lack of a significant influence from retention interval alone could be explained by the invariable foraging environment, since memories are stored for longer when rewards are

predictable (Dunlap et al. 2009, Brea et al. 2014). The experiment also found an influence from the colonies. Although some studies such as Raine & Chittka (2012) have found differences in learning at the colony level, significant influence on learning from colonies is rare, and this effect in my experiment could be due to unequal sample sizes in the colonies being compared since colony 3 had only one subject tested.

With this experiment, I present a novel comparison of social cues and different types of floral cues in relation to bumblebee forager learning and memory. When adding complexity to flowers, compound stimuli with two types of floral information had a more significant influence on learning and memory than compound stimuli with floral and social information. Bees were able to associate both floral and social cues with rewards and remember them better than chance, but the presence of nectar guides paired with flower color had the most significant influence on learning and memory. My experiment also showed some interesting dynamics in social information where bees unexpectedly continued to follow social information after it no longer indicated a reward, possibly due to the additional complexity provided by flower color. The focal flower color interacted with treatment in my experiment in different ways, with yellow focal flowers generally leading to better scores in learning and memory than orange focal flowers. My experiment did not show a correlation between brain size and foraging performance or a direct relationship between memory accuracy and time.

Future Directions

There are multiple directions the next steps of this research could follow. One aspect of my experiment that could be explored is the certain relationship between cue

and reward. While cues being 100% reliable should mean the bees make fewer mistakes (Koops 2004), reliability could also influence how the information is remembered, since multiple studies suggest differences in memory storage when information is reliable compared with when it is not (Brea et al. 2014, Dunlap et al. 2009). Future experiments could manipulate the relationship between cue and reward to examine whether social or floral information has a greater effect or whether the effect of retention interval is more significant in an environment with less reliable information. Another direction would be to test the effects of different retention intervals on memory. Since foraging-related memory decay in bumblebees may not be significant over a single night (Chittka 1998), perhaps a longer retention interval would find more evidence of memory decay and potentially highlight how long the different types of information are accurately stored in bumblebee memory. The associations bees build between social information and reward could also be changed and tested. Bees possibly viewed pinned conspecifics as competition in my experiment, so maybe social information would have a greater influence on learning and memory if the pinned bees were placed on the non-rewarding flowers instead of the rewarding ones, so the foragers seeing them as competition would be beneficial to their learning rather than detrimental. One other potential direction would be to vary the types of floral information studied. While flower shape had few significant effects in this experiment, perhaps including other aspects of shape like corolla size and symmetry would more noticeably influence learning and memory. Alternative forms of nectar guides that have been shown to have significant effects in other studies could be tested as well, including those with dotted lines (Free 1970), different placement (Goodale et al. 2014), and levels of color contrast (Wase & Price 1985).

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Appendix

This appendix includes a graph showing the correlation between bee head and body size as well as tables of ANCOVA and ANOVA results of tests that I dropped due to head size and colony being non-significant in explaining variation as referenced in the results section.

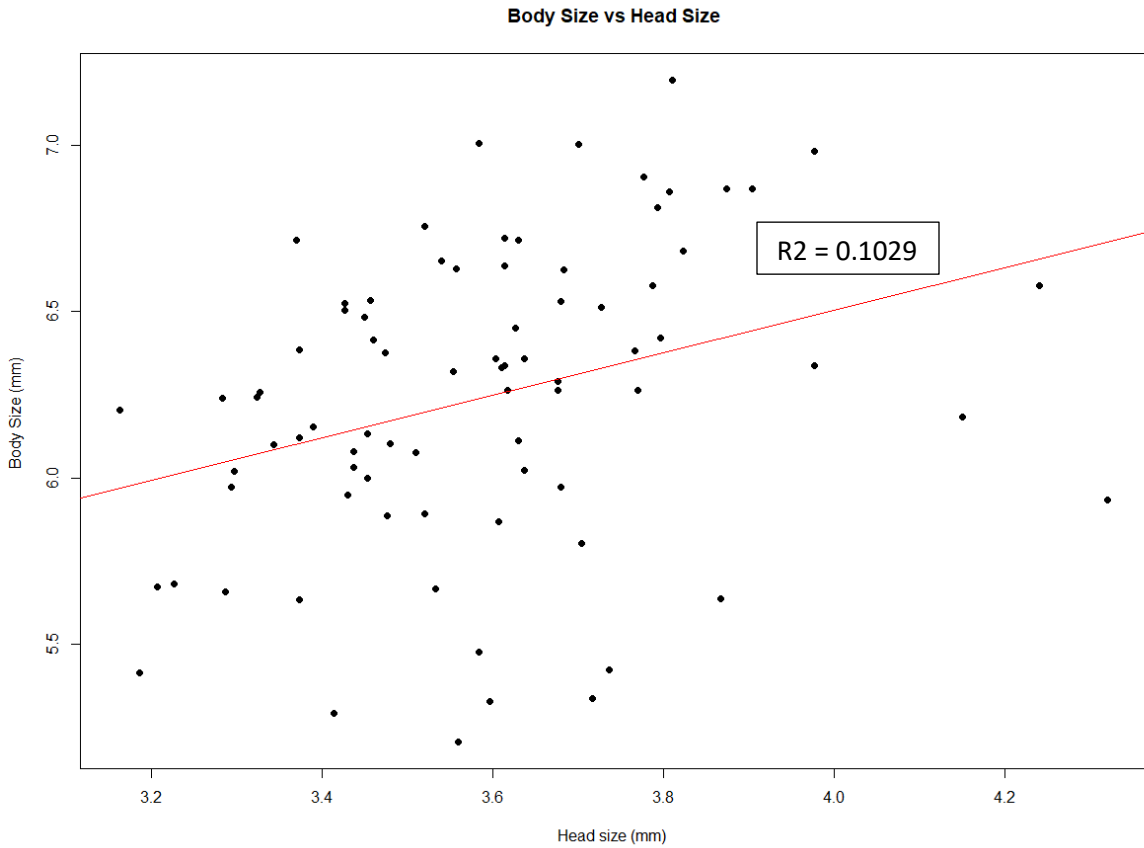


Figure A1: A comparison of the head size and body size in millimeters of the 80 tested bees during the experiment. Since the red regression line ($R^2 = 0.1029$, $p = 0.03715$) showed a low correlation between head and body size, head size was used as a proxy for brain size.

	Sums of Squares (SS)	Degrees of Freedom	Mean Squares (MS)	F	<i>p</i>
Head Size	327	1	327.4	1.367	0.24641
Treatment	2520	3	840.1	3.509	0.01990
Color	840	1	840.4	3.510	0.06537
Colony	3772	4	942.9	3.938	0.00625
Treatment:Color	3422	3	1140.5	4.763	0.00453
Residuals	16064	67	239.4		

Table A1: ANCOVA results of the effects of treatment type and flower color on choices needed to complete training. The ANCOVA is blocked by colony with head size as a covariate.

	Sums of Squares (SS)	Degrees of Freedom	Mean Squares (MS)	F	<i>p</i>
Head Size	0.0871	1	0.0871	3.547	0.06459
Treatment	0.2769	3	0.0923	3.759	0.01541
Color	0.3182	1	0.3182	12.963	0.00065
Retention Interval (RI)	0.0185	1	0.0185	0.752	0.38936
Colony	0.1206	4	0.0301	1.228	0.30870
Treatment:Color	0.2218	3	0.0739	3.012	0.03711
Treatment:RI	0.0922	3	0.0307	1.253	0.29896
Color:RI	0.0028	1	0.0028	0.114	0.73687
Treatment:Color:RI	0.0925	3	0.0308	1.256	0.29778
Residuals	1.4483	59	0.0245		

Table A2: ANCOVA results of the effects of treatment, flower color, and retention interval on memory score blocked by colony with head size as a covariate.

	Sums of Squares (SS)	Degrees of Freedom	Mean Squares (MS)	F	<i>p</i>
Treatment	0.427	3	0.1424	5.081	0.00237
Retention Interval (RI)	0.036	1	0.0360	1.284	0.25925
Color	0.380	1	0.3803	13.567	0.00034
Test Half	0.049	1	0.0490	1.748	0.18852
Colony	0.197	4	0.0492	1.757	0.14180
Treatment:RI	0.373	3	0.1243	4.436	0.00536
Treatment:Color	0.207	3	0.0689	2.459	0.06591
RI:Color	0.059	1	0.0594	2.119	0.14801

Treatment:Test Half	0.016	3	0.0052	0.184	0.90687
RI:Test Half	0.002	1	0.0022	0.080	0.77739
Color:Test Half	0.049	1	0.0490	1.748	0.18852
Treatment:RI:Color	0.063	3	0.0210	0.748	0.52565
Treatment:RI:Test Half	0.050	3	0.0168	0.598	0.61771
Treatment:Color:Test Half	0.132	3	0.0438	1.564	0.20151
RI:Color:Test Half	0.030	1	0.0302	1.079	0.30087
Treatment:RI:Color:Test Half	0.032	3	0.0107	0.384	0.76502
Residuals	3.475	64	0.02581		

Table A3: ANOVA results of the effects of treatment type, focal color, retention interval, test half, and colony on memory scores during testing.

	Sum of Squares (SS)	Degrees of Freedom	Mean Squares (MS)	F	<i>p</i>
Treatment	0.1603	3	0.05343	2.044	0.11727
Color	0.2389	1	0.23895	9.141	0.00367
Retention Interval (RI)	0.0005	1	0.00055	0.021	0.88546
Colony	0.1407	4	0.03517	1.346	0.26365
Treatment:Color	0.1031	3	0.03435	1.314	0.27812
Treatment:RI	0.0744	3	0.02480	0.949	0.42292
Color:RI	0.0399	1	0.03992	1.527	0.22135
Treatment:Color:RI	0.1059	3	0.03531	1.351	0.26650
Residuals	1.5684	60	0.02614		

Table A4: ANOVA results of the effects of treatment type, focal color, retention interval, and colony on sucrose gained per second during the first ten choices of testing.