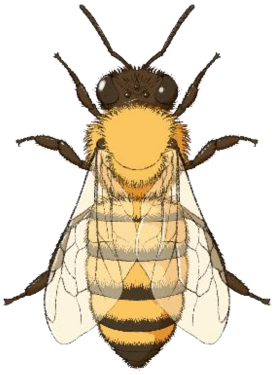
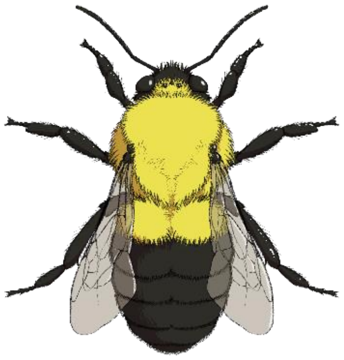
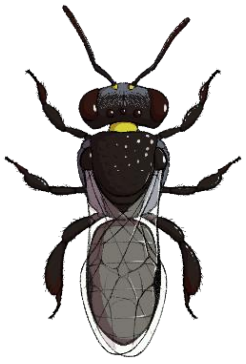
A glass half empty: Assessing the impact of empty flowers on foraging behaviour in three bee species







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Declaration

I hereby certify that the content of this thesis is my own work. This thesis has not been submitted for any other degree or diploma at any other university or institution. I consent to this thesis being made available for photocopying and loan under the appropriate Australian copyright laws.

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Mum and Dad, thanks for putting up with me being an eternal student

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Abstract

Flower visiting insects face the difficult choice of selecting which flowers to visit and which to ignore. Foraging becomes more complicated because flowers can sometimes stop offering nectar, either due to removal by other visitors or because of physiological changes in the plant. These flowers may act as ‘phantom decoys’, items that are unexpectedly unavailable at the time of choice that has been shown to influence preference relationships between other available items in the choice set. In this thesis, I aimed to understand the role of empty flowers in foraging choice of three bee species. In chapter 2, I reviewed the literature on artificial flowers in experiments on floral visitors, to understand how to best use artificial flowers to test the effects of empty flowers. In my experimental chapters, I then tested the effects of empty flowers on three social species of bees, *Apis mellifera, Bombus impatiens* and *Tetragonula carbonaria.*

After reviewing the literature of artificial flowers, I found that they are a particularly effective tool in cognition-based experiments using honey bees and bumble bees in enclosed laboratory environments. I also identified potential ways of increasing the use of artificial flowers in the field to increase the taxonomic range of studies.

If phantom decoys occur in pollinating insects, then the presence of empty flowers could have community-wide impacts on visitation rates of neighbouring flowers. I performed three experiments using artificial flowers to test the effects of empty flowers on foraging behaviour in *A. mellifera, B. impatiens* and *T. carbonaria*. Overall, I found that there were minimal impacts of empty flowers on flower choice by these bees. I showed, however, that social behaviour is a key driver in allowing bees to make effective foraging decisions in the presence of empty flowers, and that empty flowers can result in the abandonment of patches.

Empty flowers are a common consideration for foraging in bees, and have potential to impact the pollination of nearby flowers. I showed that while empty artificial flowers did not

have strong impacts on foraging choice in bee species, there is potential that they can impact how bees move between and within patches, which can be a potential driver in pollination of neighbouring plants.

Chapter 1: Introduction

Insects such as bees, flies, beetles, butterflies and moths, visit flowers to feed on nectar and pollen. In the process, flower-visiting-insects vector pollen between flowers, facilitating the reproduction of plants. Pollination provided by insects is critical not only to the functioning of most terrestrial ecosystems but also to our food security. Approximately 75% of crop species benefit from pollination by animals (Klein et al., 2007), with many non-insect-dependent plants gaining yield benefits from insect pollination (Garibaldi et al., 2013; Klein et al., 2007). Globally, pollination services are estimated to be valued at approximately AUD 230 billion (Gallai et al., 2009). In Australia, pollination services from honey bees alone were worth $14.2 billion in 2014 (Karasiński, 2020).

Much of modern agriculture across the world rely heavily on planting monocultures. Monocultures can have detrimental effects on pollinator habitats (Varah et al., 2020), while also producing minimally nutritious, transient food sources for floral visitors (Cole et al., 2022). Where insect pollination is required for the crop, managed bees must then be brought into crops during bloom periods to provide pollination services. However, there is increasing recognition that floral diversity in agroecosystems can benefit both the fruit set of crops and insect biodiversity (Garibaldi et al., 2013, 2014; Ghazoul, 2006; Liao et al., 2011; Nicholls & Altieri, 2013; Norfolk et al., 2016). Floral diversity also provides increased nutrients to floral visitors, particularly after the crop stops blooming. This floral diversity can be achieved in a variety of ways including mixed cropping (different crops interspersed), planting of flower strips and hedgerows (Kovács-Hostyánszki et al., 2013) and allowing weeds to flower (Marshall et al., 2003).

Pollination of crops generally improves with increased visitation from wild insects (Garibaldi et al., 2013, 2014; Norfolk et al., 2016). While honey bees and other managed pollinators can offer significant benefits to the fruit set of some species, the combination of managed species and wild species for pollination services is considered more beneficial for some crops (Garibaldi et al., 2013). Wild pollinators -not honey bees for example drove the pollination of sweet cherry and almond orchards in Germany and Egypt (Holzschuh et al., 2012; Norfolk et al., 2016). Overall, multiple species visiting individual flowers can result in heavier fruit (Kendall et al., 2022). Including non-crop flowers in cropping systems may therefore be an effective strategy for encouraging floral visitors to crops, potentially increasing quality and yield.

Floral Rewards

Flowers contain rewards that attract floral visitors. The two main rewards are nectar and pollen (Muth, Francis, et al., 2016), but floral visitors also collect resin (Armbruster, 1984) and oils (Simpson & Neff, 1981) from plants. Pollen acts as a protein-rich nutrient source for floral visitors. However, it is also used to carry the gametes of plants, and sufficient pollen movement is necessary for fruit set to occur. Therefore, plants often provide other resources that can attract floral visitors. Oil is only collected by a small number of specialised oil-collecting bee species (approximately 370 species) (Schäffler et al., 2015). Resin, used as nesting material, is collected by a range of bee species, particularly those in the Megachilidae and Meliponidae groups (Armbruster, 1984; Leonhardt & Blüthgen, 2009), though the majority of resin comes from wounds in trees, as opposed to flowers, so it is not as linked with pollination in comparison to other rewards (Armbruster, 1984; Leonhardt & Blüthgen, 2009; Roubik, 2006). Nectar is the main reward associated with angiosperms and is collected by the majority of pollinators (Simpson & Neff, 1981). It provides a carbohydraterich food source to floral visitors. Given the importance of nectar for floral visitors, it is important to understand the impacts of variation in nectar quality on floral visitor behaviour.

I chose to focus on nectar for this thesis as it is possible to manipulate the quality of a flower based on traits related to nectar and nectar collection, allowing experimental testing of factors influencing floral visitor foraging choices. Floral visitors have preferences in nectar qualities such as concentration, where bees, for example, prefer higher concentrations of sugar in nectar until it reaches around 50% sugar concentration (Cnaani et al., 2006; Harder, 1986; Kim et al., 2011; Loo & Bitterman, 1992; Silva & Dean, 2000; Waller, 1972). Nectar volume is also important, with increased volumes being preferred over smaller volumes (Urbanowicz et al., 2020). Nectar composition is also important, with floral visitors showing preferences for different sucrose/glucose ratios (Pyke et al., 2020). The quality of a flower can be influenced by nectar accessibility where decreased nectar access can reduce preference for flowers (Inouye, 1980; Mallinger & Prasifka, 2017). Nectar is considered a ‘manipulator’ of pollinators as well as an attractant due to its capacity to impact foraging choices in floral visitors (Pyke, 2016), making it a useful tool for studying floral visitor behaviour.

The floral neighbourhood

Increased floral resources in a landscape mean that co-flowering plants may share floral visitors. Depending on the flowers, the relationship between co-flowering plants can be beneficial (Johnson et al., 2003; Liao et al., 2011; Ruttan, 2017; Yang et al., 2013), or lead to competition for pollination services (Landry, 2013; Levin & Anderson, 1970). Highly rewarding flowers can sometimes result in competitive interactions and can reduce the benefits of co-flowering plants (Mesgaran et al., 2017).

The simplest way that co-flowering plants can be beneficial to their neighbours is by increasing the display size and therefore attracting floral visitors. Visitation to patches increases with increased patch density (Grindeland et al., 2005; Thomson, 1981). However, when one species is much more abundant than the other, the more dominant species can out-compete other co-flowering species (Feldman et al., 2004).

There is evidence that flower density may not always impact the seed set of co-flowering plants. While multiple similar flowers can increase the detectability of flowers from afar, floral visitors will still often only visit a single flower species (Feldman, 2008). Similarly, if there is a mix of plants in an area, floral visitors are more likely to visit flowers whose nectar is easy to access (Essenberg, 2013).

Plants have adopted strategies to allow them to receive floral visitation when there is a whole neighbourhood of flowers that could be chosen by floral visitors. The similarity of floral traits between co-flowering plants can play an important role in visitation, as floral visitors are capable of learning to associate floral rewards with floral traits such as colour (Muth, Papaj, et al., 2016). For example, co-flowering plants that have evolved in the same habitats may produce similar-looking flowers that are difficult for flower visitors to distinguish from one another (Albor et al., 2020; de Jager et al., 2011, 2022; Giurfa, 2004; Giurfa et al., 2003; Msweli, 2018). Co-flowering plants with shared pollinators are also more likely to look similar to each other (Bergamo et al., 2020). Alternatively, flowers can look similar due to having shared pollinators, which means to be maximally attractive to pollinators, plants might end up within similar morphologies that match the preferred traits of their pollinator. Having many similar-coloured co-flowering plants in a patch can also be beneficial to flowers in an area as floral visitors often prefer the most abundant floral resource in the area (Ramos-Fabiel & Martorell, 2022), and reduces the ability to discriminate between similar-coloured flowers remotely may act as an attractant to the whole foraging area.

Yet being similar to your neighbour is not always beneficial for plants. Rates of heterospecific pollen transfer increase when plants are surrounded by morphologically similar co-flowering species (Ashman & Arceo-Gómez, 2013; Ha et al., 2021; Zhang et al., 2021). Plants must then evolve structures to prevent the collection of incompatible pollen. This includes preventing incompatible pollen from attaching to the stigma of flowers (Costa et al., 2017), or ensuring pollen is likely to attach to a different part of the body of the floral visitor than the pollen from incompatible species (Huang et al., 2015), or having variation in anther size and presentation to prevent pollen placement from the wrong plant species (Bergamo et al., 2018). Understanding how floral visitors interact with neighbouring plants ensures that the pollination benefits of increased floral resources in crops can be optimised.

The most well-studied phenomenon between co-flowering plants is the magnet species effect (Braun & Lortie, 2019). High-quality flowers that attract large numbers of floral visitors can eventually lead to increased visitation of surrounding flowers (Cuadra-Valdés et al., 2021; Horna Lowell & Murphy, 2022); these highly attractive plants are referred to as 'magnet plants'. In agricultural environments, magnet plants can increase visitation to co-flowering plants resulting in spillover pollination benefits (Gilpin et al., 2019a; Laverty, 1992). Floral visitors learn to associate floral traits with rewards, which are important in determining how co-flowering plants can benefit from magnet plants. Once magnet plants have attracted floral visitors to the patch, morphologically similar plants may be more likely to benefit from pollination spill-over (Gumbert, 2000; Peter & Johnson, 2008). This is referred to as the similarity effect.

In contrast to highly-rewarding flowers, many flowers contain little to no nectar. Most plant species will at some stage have nectarless flowers (Thakar et al., 2003), with many producing little to no nectar at all (Shrestha et al., 2020). There are typically three broad reasons why flowers don't contain nectar; flowers can be permanently empty, nectar depletion over time, or flowers that are temporarily empty (Figure 1.1). Each strategy has different ways of obtaining pollination benefits without the costly production of nectar.

Permanently empty and minimally rewarding flowers

One way for a plant to prevent the costs of nectar production, while still getting pollinated is to produce little or no nectar and instead deceive floral visitors into visiting flowers with the expectation of a floral reward. Many permanently 'unrewarding' flowers contain trace elements of nectar, though not enough to provide sufficient nutrients to floral visitors. These trace rewards appear to make floral visitors think they've experienced a 'reward' (Bogarin et al., 2018; Shrestha et al., 2020). Many orchids contain minimal rewards and still gain visitation (Shrestha et al., 2020). Beyond trace amounts of nectar, orchids frequently partake in deceptive pollination, by imitating rewarding plants, or potential mates of pollinators to gain visitation (Gaskett, 2011). *Erycina pusilla*, for example, produces no rewards but mimics the appearance of rewarding Malpighiaceae plants to attract visitors (Dirks-Mulder et al., 2017).

Colour is a common way of utilising deceptive pollination. Rewardless orchids, for example, experience increased pollination when they are surrounded by rewarding flowers of similar colours to the unrewarding orchids (Johnson, 1994). Visual similarities to rewarding plants in terms of shape and patterns can also result in visitation to deceptive unrewarding plants (Jersáková et al., 2012).

Nectar depletion over time

Many flowers utilise strategies that involve keeping old flowers that no longer contain nectar while producing new flowers that produce nectar. Up to 70% of flowers on a plant do not contain nectar (Thakar et al., 2003). Flowers that reduce nectar production with age often also change colour with decreased nectar production (for example *Lantana camara* and *Tibouchina pulchra* (Brito et al., 2015; Thakar et al., 2003; Weiss, 1991).

In the case of *L. camara*, yellow flowers are receptive to pollination and contain nectar. Over time flowers turn from yellow to pink to red and reduce nectar production while becoming non-receptive to pollination. Floral visitors may not be able to distinguish the floral colours from far away but can learn to only forage on the yellow flowers that are visible when closer to the flower. The increased flowers overall increase the display size, making flowers easier to detect from a distance (Brito et al., 2015; Weiss, 1991).

Temporarily empty flowers

Flowers can be temporarily empty, which can impact visitation differently than permanently empty flowers. Nectar in *Callistemon lanceolatus* for example is easier to access in the morning during its flowering period (Sawarkar, 2017). Temperature can also impact nectar production, with higher temperature resulting in reduced nectar production (Descamps et al., 2021). Flower visitation also results in temporarily reduced nectar availability, and many bees have adapted to avoiding previously visited flowers. For example, the bumble bees *Bombus terrestris* and *B. pascuorum* will frequently avoid foraging on plants that contain scent marks from other individuals to prevent finding empty flowers (Goulson et al., 1998). While temporal nectar availability may not necessarily be a strategy for pollination benefits from the plant's perspective, it may impact foraging choice in similar ways to other forms of empty flowers, which can be important for pollination. For example, temporarily unrewarding flowers can impact foraging on the flowers containing nectar, with *B. terrestris* choosing flowers that are similar to previously rewarding flowers if the rewarding flower becomes unavailable (Internicola et al., 2009).

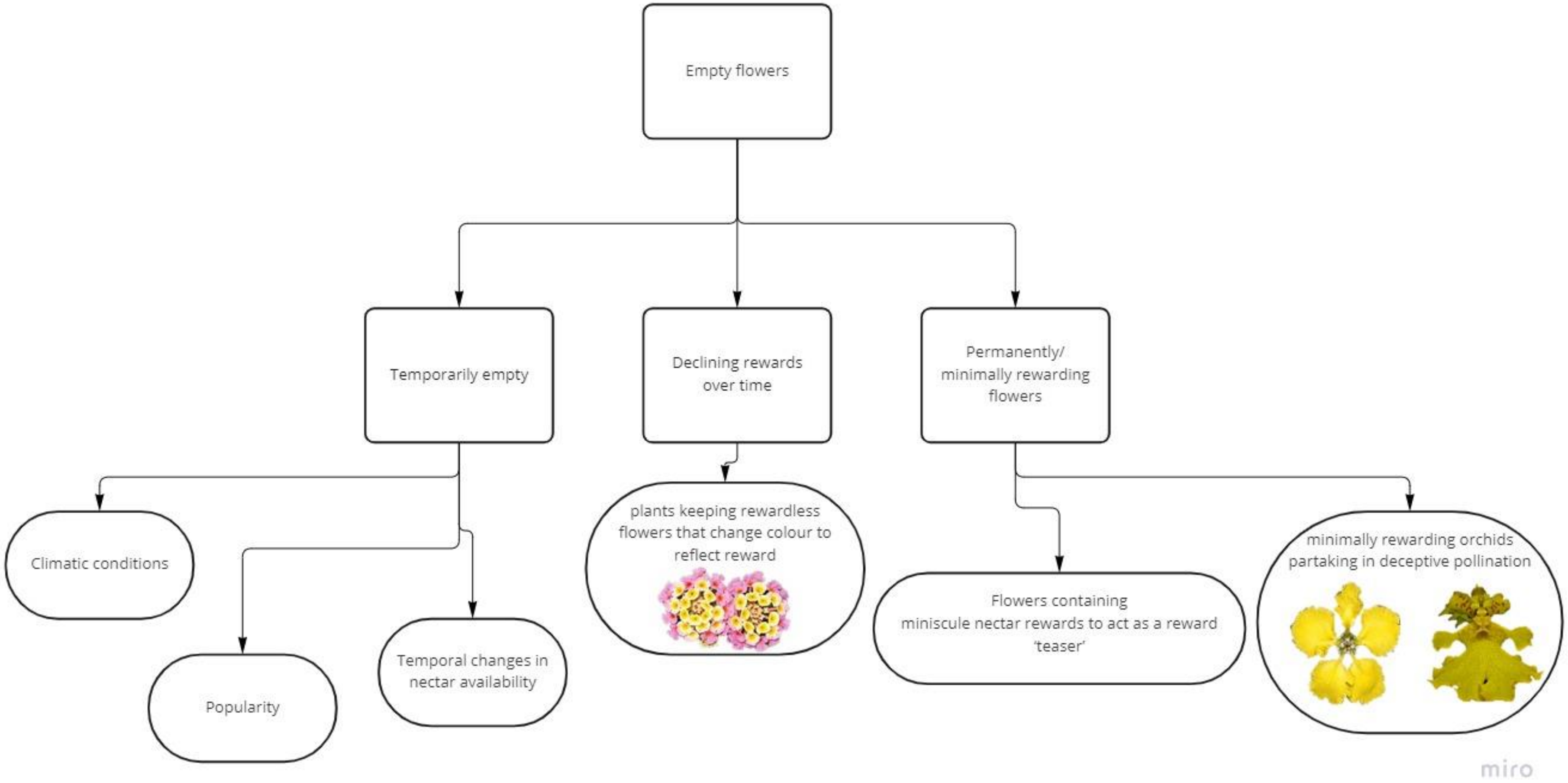


Figure 1.1: The different types of empty flowers

How do empty and unrewarding flowers impact foraging choices?

Floral visitors can learn which flowers are frequently empty, and reduce visitation to them (Simonds & Plowright, 2004; Smithson & Gigord, 2003). Permanently empty flowers must therefore rely on naïve individuals to find them for pollination purposes (Gigord et al., 2002). Due to bees learning to avoid permanently empty flowers, rare flowers that are permanently empty are more likely to receive pollination benefits than common ones, as floral visitors will have less experience with rare rewardless flowers (Gigord et al., 2001; Schiestl, 2005).

While floral visitors may abandon unrewarding flower patches, the next flower they choose to forage on may be influenced by the morphology of the empty flower. When there are many empty flowers in an area, *B. terrestris* is more likely to visit flowers of a dissimilar colour to the empty flowers (Smithson & Gigord, 2003), that is, they learn to associate a given flower morphology with a lack of reward. However, if flowers become empty, or disappear from a patch after a bee has learned it is rewarding, she may be more likely to pick a flower of similar colour to the previously rewarding flower (Gigord et al., 2001; Internicola et al., 2009). Foragers of the honey bee *A. mellifera* will also pick a known empty flower that was always unrewarding over a flower colour they have not previously been trained to (Dyer & Murphy, 2009).

Empty flowers can reduce visitation rates to patches (Biernaskie, Cartar and Hurly, 2002; Smithson and Gigord, 2003). As well as increased abandonment rates, bumble bees were more likely to move to a new inflorescence, and decrease overall floral probes on a plant when empty flowers were found (Ishii et al., 2008; Nakamura & Kudo, 2016). There are some benefits to the plants when there is a reduction in floral visitor movement, as this can prevent selfing, where a flower is pollinated by its own pollen (Biernaskie, Cartar and Hurly, 2002).

The use of behavioural economics to understand bee foraging choices

Optimal Foraging Theory predicts that animals choose items based on trade-offs between gains and losses (Pyke, 1984). Optimal Foraging Theory assumes that decision-makers use absolute valuation strategies when assigning value to items. Absolute valuation strategies involve putting a value on each item in the choice set (Rapoport, 1998). As a consequence of absolute valuation, animals are expected to have stable preference rankings between options in a choice set. For example, if given the options, A, B and C, where A is better than B and B is better than C, then A should always be chosen over C.; this is known as the principle of transitivity. Male *Drosophila* flies have been shown to use absolute valuation to choose between females of different genotypes (Arbuthnott et al., 2017). In contrast, comparative valuation strategies occur when the value of an item is determined through comparison with other items in the choice set. As a result, preference orders can become unstable. For example, if A is better than B and B is better than C, but C is preferred over A, comparative valuation is likely being used to make decisions. Multiple species have shown evidence of comparative evaluation strategies (Jackson & Roberts, 2021; Latty & Beekman, 2011; Shafir, 1994; Waite, 2001).

Comparative evaluation strategies are best illustrated by ‘decoy effects.’ Studies of human behaviour have shown that people can be impacted by unavailable and irrelevant options in choice sets (Colman et al., 2007; Highhouse, 1996; Park & Jang, 2018; Trueblood

& Pettibone, 2017). This is the “decoy effect”, the idea that the inclusion of a third irrelevant option in a choice set can cause changes in the way individuals value the remaining items in the choice set, resulting in alterations in preference by the decision-maker.

Decoys can be classified into two major groups: low-quality, available decoys and high-quality unavailable decoys. Low-quality decoys are items that are of lesser quality than the other items of the choice set. Since they are of lower quality than other items, a decision-maker using an absolute valuation strategy should ignore the low-quality item entirely. However, if comparative valuation strategies are used, the lower-quality item may impact the value of the other two items resulting in preference changes. For example, the decision-maker might choose an item more similar to the unattractive decoy (Heath & Chatterjee, 1995).

The second class of decoys are known as 'phantom decoys' and consist of very high-quality, but unavailable options that can impact the preferences for available options in a choice set. In humans, for example, sold-out options can cause people to pick options that are most similar to the unavailable option (Highhouse, 1996; Park & Jang, 2018). Alternatively, humans confronted by phantom decoys can become risk-averse and pick the option least similar to the phantom decoy (Scarpi & Pizzi, 2013). A good example of the impact of unavailable options can be seen in the choice of holiday packages, which frequently become unavailable when people book online. A sold-out holiday package results in people picking an available holiday package with a more similar star rating to the sold-out one (Park & Jang, 2018).

The effects of phantom decoys on choice are not limited to humans. In previous studies, both domestic cats and Asian honey bees have shown susceptibility to phantom decoys. Phantom decoys were tested on cats by testing using foods that varied in concentration of food (where cat food was diluted with bullion) and quality of food (higher quality food being chicken, lower quality being tuna). In the binary treatment, cats were given two bowls that contained two food items of equal value; one contained a diluted chicken-based feed, the other a less-diluted tuna-based feed. The phantom treatments consisted of two equal options, and a third, better food option (less diluted chicken-based feed). However, the food in the phantom decoy bowl was covered in transparent plastic, preventing the cats from feeding on it. In the binary choice set, there was minimal preference between the two food options. However, when a phantom decoy was present, cats were more likely to feed on the food option that contained chicken and was therefore similar to the decoy (Scarpi, 2011).

Phantom decoys have also been tested in the Asian honey bee, *Apis cerana*. *Apis cerana* workers were tested for their preference between flowers that differed in sugar concentration and sugar temperature. Bees prefer higher concentrations of sugar in nectar, and warmer nectar, so each of the two feeders containing nectar traded off between these two options (ie. one contained high nectar concentration, presented at a lower temperature, and the other contained lower quality, but warmer nectar). When presented with phantom decoys (a warmer flower with a high concentration of nectar), bees preferred flowers that were more similar to the phantom decoy flower. For example, a warmer flower was preferred if the phantom decoy flower was warmer. Interestingly, this study also used unavailable, but also unattractive phantom decoy flowers to test the foraging preferences of bees. Overall there was no effect of an unattractive, unavailable phantom decoy on the foraging preferences of bees (Tan et al., 2015).

Given that phantom decoy effects are prevalent in Asian honey bee choice, it is beneficial to see how widespread this effect is in other commercially relevant species. Empty flowers have the potential to act as phantom decoys in natural foraging contexts, and if the impact of phantom decoys on bees is predictable, we may be able to take advantage of empty flowers to optimise crop pollination.

Study species: honey bees, bumble bees, stingless bees

For effective pollination of crops, large numbers of floral visitors must visit crops. Historically, growers have relied on social species such as honey bees and bumble bees for pollination services (Garibaldi et al., 2017). In recent decades, there has been increased interest in the use of stingless bees and some solitary bees, particularly in the genus *Osmia* and *Megachile* for commercial pollination purposes (Bosch & Kemp, 2002; Garibaldi et al., 2017; Heard & Dollin, 2000). Eusocial species offer the advantage of being able to move large numbers of individuals to crops or glasshouses for pollination, as well as (in some cases) centuries of knowledge about breeding and husbandry.

For my thesis, I chose to focus on the foraging behaviour of three commercially available social bees; the Western honey bee *A. mellifera, the* Eastern bumble bee *B. impatiens* and the Australian stingless bee *Tetragonula carbonaria (Figure 1.2; Table 1.1)*

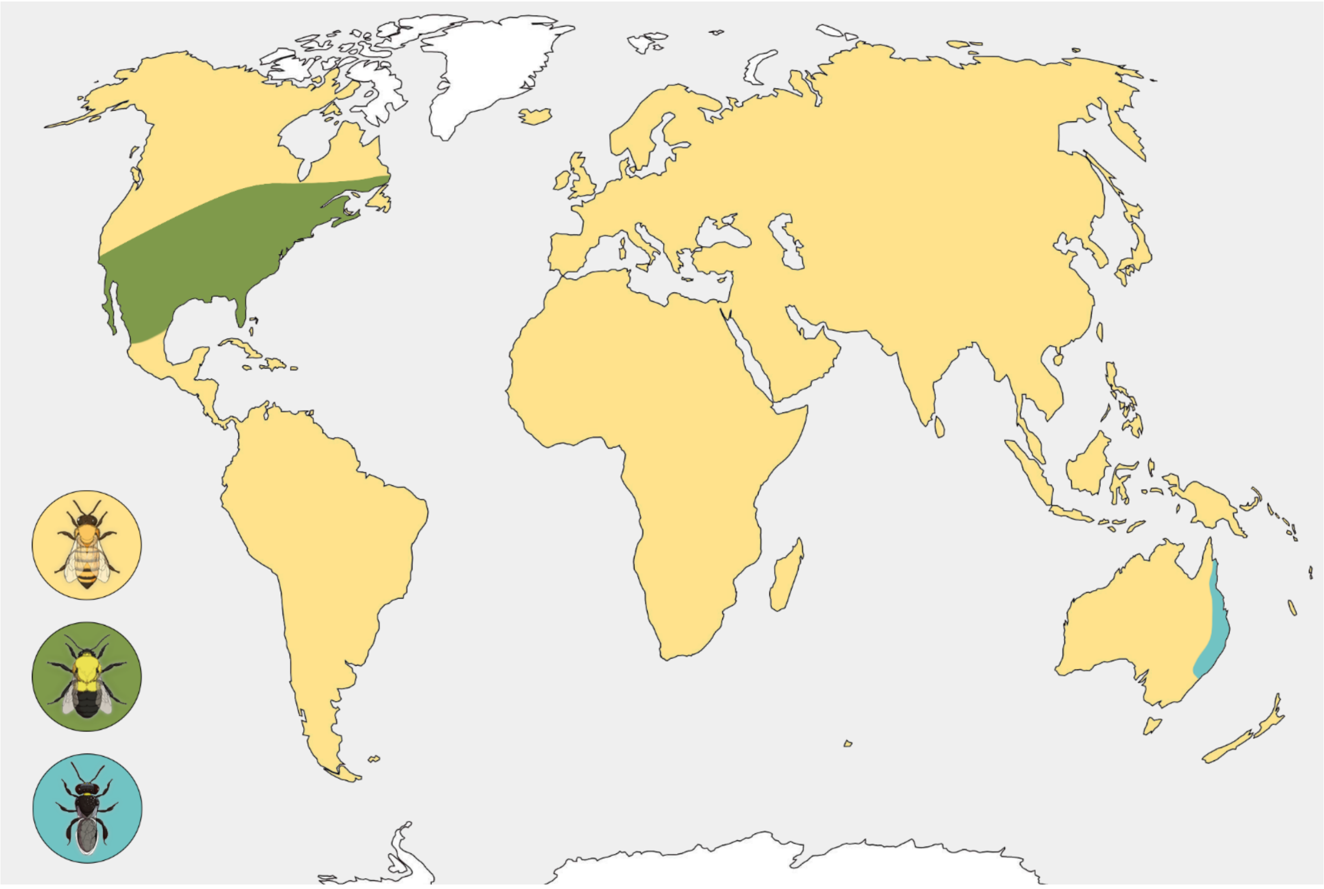


Figure 1.2: Distribution of study species *A. mellifera* (yellow), *B. impatiens* (green), and *T. carbonaria* (Blue)

Honey bees *Apis mellifera*

Honey bees are a group of bees belonging to the genus *Apis*, within the Apidae family. There are approximately 10 extant species of honey bees (Arias & Sheppard, 2005; Lo et al., 2010; Raffiudin & Crozier, 2007). Honey bees contain popular commercial pollinator species including *A.mellifera* and *A.cerana*. They are considered the most frequent floral visitor of all groups of bees (Garibaldi et al., 2013; Hung et al., 2018). Honey bees have a widespread distribution, with species native to Africa, Asia and the Middle East.

The Western honey bee, *A. mellifera*, is arguably one of the most studied species of insect, due to its widespread pollination benefits generalist foraging behaviour, complex behavioural abilities (Howard et al., 2019) and ecological dominance (Garibaldi et al., 2021). *A. mellifera* is native to Europe, Africa and the Middle east (Cridland et al., 2017) (Figure 2). They are now distributed across the globe in every continent except Antarctica due to the movement of hives by humans. They are common visitors and pollinators to many crops that require bee pollination.

Apis mellifera is a eusocial species of bee that makes foraging decisions with assistance from its nestmates. Honey bees create large hives, containing up to 50000 individuals. *A. mellifera* uses the waggle dance (Frisch, 1967; Hrncir et al., 2011) which is a form of communication that can transmit information on the quality and location of resources. They also use scent marks to choose flowers (Beekman, 2005; Stout & Goulson, 2001).

Considered a model species for insect behaviour and cognition, honey bees have also been studied extensively for their learning and cognition abilities. *A. mellifera* has shown the capacity to learn and remember a range of concepts associated with flowers (Menzel, 2012). They have been reported to understand numerical concepts (Howard et al., 2018, 2019), and have been studied extensively for their capacity to learn to associate floral traits with rewards

(Giurfa, 2004). Given their ability to associate rewards with flower traits, it is likely their foraging behaviour is influenced by empty flowers.

The effects of empty flowers on honey bee choice behaviour in co-flowering settings are important to study as they are often the most common visitor to flowers (Gilpin et al., 2019b; Landry, 2013; Ojija et al., 2019). *A. mellifera* have previously been shown to be susceptible to unattractive decoys, the effects of phantom decoys are still unknown. While the impact of phantom decoys has been investigated in related species *A. cerana* (Tan et al., 2015), the extent to which the phantom decoy effect influences *A. mellifera* foraging is unknown (Table 1.1).

Bumble bees, *Bombus impatiens*

Bumble bees (*Bombus spp*) are the only extant genus in the tribe Bombini. Currently, there are approximately 250 species of bees within the genus *Bombus* (Cameron et al., 2007; Williams, 1998). Bumble bees are generalist foragers (Williams et al., 2018), which has allowed species within this genus to be distributed across temperate regions across the globe, except for Oceania and Africa (Nascimento et al., 2022), though *B. terrestris* has been introduced to Australia and New Zealand. There has been increasing concern over bumble bee populations, with declines occurring globally due to disease spread and habitat fragmentation (Cameron et al., 2011).

Bumble bee behaviour is of interest to researchers as this group has strong cognitive abilities. Bumble bees can learn to associate flower colour with reward (Muth et al., 2016) and handle complex flowers to gain rewards (Laverty, 1994; Muth et al., 2015). *B. terrestris* have shown the capacity to use social behaviour to learn complex tasks, such as string pulling (Alem et al., 2016), and pushing balls to obtain rewards (Loukola et al., 2017), which have not been seen in other invertebrates.

Bombus impatiens are a popular commercial pollinator of crops in North America (Figure 2). In 2005, 55000 colonies of *B. impatiens* were reared for pollination purposes. Bumble bees are particularly important pollinators as they can buzz pollinate (Cooley & Vallejo-Marín, 2021). Buzz pollination occurs when bees vibrate on flowers to release pollen from plants. Some plant species require buzz pollination, including tomatoes, kiwi fruit, eggplants and potatoes (Arroyo-Correa et al., 2019; Cooley & Vallejo-Marín, 2021; De Luca et al., 2019). Unlike honey bees (A. *mellifera*) which cannot buzz pollinate, bumble bees can effectively pollinate plants that require buzz pollination.

Bumble bees are considered primitively social as they have a solitary phase in their life cycle (Sadd et al., 2015), and they use some social behaviour when foraging. Queens of *B. impatiens* initiate new nests each spring, during which time they forage alone outside of the nest. Once the first workers are produced, these workers then take over all foraging tasks for the colony. *B. impatiens* workers use scent marks to learn where nest mates have previously foraged (Saleh et al., 2006). Individuals also learn by foraging in locations where other nestmates are present (Worden & Papaj, 2005).

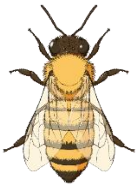
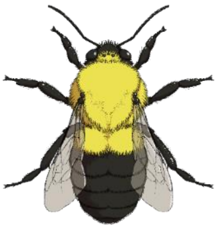
Bumble bees are capable of learning to associate colours with rewards (Muth, Papaj, et al., 2016; Riveros & Gronenberg, 2012). *B. impatiens* have also shown evidence of reversal learning (Strang & Sherry, 2014), where bees were trained to switch between flowers to gain a reward. Reversal learning indicates that *B. impatiens* can adapt well to changes in their environment, which implies changing floral resources may impact their foraging choice (Table 1.1).

Stingless bees, *Tetragonula carbonaria*

There are eleven species of stingless bee in Australia, with approximately 215 species globally (Bueno et al., 2021). These are important pollinators of native plants, and are increasingly used as managed crop pollinators for macadamia, avocado and blueberries (Heard, 1994; Heard & Dollin, 2000; Kendall et al., 2020). Similar to honey bees, they produce honey, albeit in smaller amounts. The capacity of stingless bees to provide greenhouse and open pollination benefits suggests their use as commercial pollinators will increase over time (Greco et al., 2011).

Tetrragonula carbonaria is one of the most widely propagated species of stingless bee in Australia, and are distributed on the northeast coastal regions of Australia (Figure 1.2). Colonies consist of a single queen and up to 10000 individuals. Workers of this species use scent marks when foraging to recruit nestmates to food sources (Bartareau, 1996; Gloag et al., 2021). The foraging behaviour of *T. carbonaria* remains poorly understood, compared to that of commercial *Apis* and *Bombus* species. Foraging choice on flowers can be temperature dependent (Norgate et al., 2010), but there are a range of other floral attributes that may impact foraging choice in *T. carbonaria.* While they have colour vision, they have a reduced ability to discriminate between colours in comparison to bumble bees and honey bees (Dyer, Streinzer, et al., 2016; Spaethe et al., 2014). They also have lower visual acuity in comparison to honey bees and bumble bees, likely due to their smaller size (Dyer, Streinzer, et al., 2016). They have been shown to have some innate colour preferences, with a preference for white over pink (Dyer, Boyd-Gerny, et al., 2016). Their colour preferences are also driven by green contrast (Dyer, Boyd-Gerny, et al., 2016). They show floral constancy when foraging on pollen, which my indicate floral constancy is a common part of their foraging strategy (White et al., 2001). Overall, there is no information on how neighbouring flowers can impact *T. carbonaria's* foraging choices (Table 1.1).

Table 1.1: Study species used for the thesis.

	Apis mellifera	*Bombus impatiens*	*Tetragonula carbonaria*
FAMILY	Apidae	Apidae	Meliponidae
SOCIALITY	Eusocial	Primitively social	Eusocial
RECRUITMENT	Scent marks, dance behaviour, local enhancement	Scent marks, local enhancement	Scent marks, local enhancement
TYPICAL COLONY SIZE	~ 80000 individuals	~ 300 individuals	~10000 individuals
NESTING HABITAT	Tree hollows, commercial hives	Ground nesting, commercial hives	Tree hollows, commercial hives
IMPACT OF EMPTY FLOWERS ON FORAGING CHOICE	Similarity effects	Similarity effects	Unknown
SIZE OF WORKERS	16 mm	20 mm	4mm
			

Social information when foraging

Eusocial species of bees need to forage as efficiently as possible, as the food they collect impacts themselves and their colony. Social species can use social information from their nestmates to assess floral resources and potentially pick more rewarding food sources. The use of scent marks at food sources, or along pathways to food sources, is common, and can be used to attract (Gloag et al., 2021; Roselino et al., 2016; Sommerlandt et al., 2014), or repel individuals (Giurfa & Núñez, 1992; Saleh et al., 2006). Scent marks can also be used as a sign of the previous visitation and can help bees work out which individual flowers to avoid (Giurfa & Núñez, 1992; Stout & Goulson, 2001). Social bees also use local enhancement, whereby bees learn to forage on different flowers based on the presence of other individuals on those flowers (Leadbeater & Chittka, 2007, 2009).

Given that eusocial bees are amongst the most important pollinators globally, there is value in considering how social behaviours might influence social bees' responses to empty flowers. Scent marks, for example, can result in preferences for options in the choice set being amplified. For example, when ants pick nest sites, trails may be laid by individual ants to reinforce the direction of a preferred nest. As increased numbers of individuals lay trails, feedback loops of recruitment occur, resulting in a quorum decision to use a particular nest site. The nest site choice may not necessarily be the best option, it was simply the one that received the most recruitment. In *Messor barbarus* for example, preferences for nest sites that would normally be considered of equal value result in clearly skewed preferences due to recruitment mechanisms (Jeanson et al., 2004).

Amplification of preferences caused by recruitment could be an important factor in foraging choice in the presence of ‘phantom decoy’ empty flowers. For example, individual ants are susceptible to decoy effects from a low-quality decoy, but groups of ants showed reduced susceptibility to decoys (Edwards & Pratt, 2009; Sasaki & Pratt, 2011). An open question, therefore, is whether decoys (e.g. empty flowers) impact foraging choice even in ecologically relevant contexts, where social bee species are foraging using social information.

Thesis Aims

This thesis aims to understand how social bees respond to empty flowers in a patch using a series of experiments deploying artificial flowers in three ecologically diverse bee species.

Specifically:

- In **Chapter 2,** I reviewed the literature on artificial flowers to determine how this experimental tool has been used in previous studies. I also developed guidelines and decision tools for choosing an appropriate artificial flower for various experimental contexts.
- In **Chapter 3,** I tested the impact of empty flowers (phantom decoys) on foraging choice by individual foragers of the Western honey bee, *A. mellifera.*
- In **Chapter 4** I tested if the presence of low-quality or phantom decoys could alter preference relationships between neighbouring flowers in a choice set using the bumblebee *B. impatiens. B. impatiens* use a range of social cues to choose flowers. I was therefore also interested in assessing foraging choices in a social foraging context.
- Lastly, in **Chapter 5** I determined if flower choice by the stingless bee *T. carbonaria* was influenced by the colour of an empty, but previously rewarding, flower.

Several chapters in this thesis include work that was significantly impacted by the COVID-19 pandemic. Therefore, this thesis is submitted and examined under emergency conditions as defined by the University of Sydney's Higher Degree by Research (HDR) Rule, Amended in December 2021. Due to public health orders, I was not able to complete the experiments in Chapter 4, and there were limitations to the methodology of Chapter 5. As such, I have included a COVID-19 impact statement outlining the scope of the intended work for these chapters.

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E. pusilla image from Geoff Gallice

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Chapter 2: Artificial flowers as tools to study animal-flower interactions

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Running headline

Artificial flowers for studying pollinators

Author contributions

CF: Conceptualization, methodology, investigation, data curation, writing

DFH: Conceptualization, editing

TEW: Editing

RG: Conceptualization, editing

TL: Conceptualization, methodology, editing

Abstract

1. The study of pollinator-flower interactions can be challenging due to the time and effort needed to maintain experimental flowers, intraspecific variation in flower attributes, the time needed to train individuals to flowers, and the need to synchronise flowering times. Artificial flowers are a promising solution to these challenges by allowing better control of floral characteristics, increasing experimental reproducibility, and decreasing maintenance time. Although artificial flowers have been used extensively to understand the behaviour of floral visitors, there are currently no guidelines for the selection and design of effective artificial flowers. We aimed to review the current literature on artificial flowers and provide guidelines for creating effective artificial flowers in future experiments.
2. We reviewed 160 papers that used artificial flowers to study flower visitation in bats, birds, and insects. We found that the majority of experiments involved commercially available social bees, in enclosed environments. To further the use of artificial flowers, we discuss effective ways to make multi-attribute flowers to attract wild flower visitors in the field. We suggest potential improvements to common designs that may facilitate experimental work on a wider range of taxa and research questions. We also discuss the potential uses of a range of emerging technologies for creating artificial flowers, including 3D printing, nectar refill automation and mass production methods.
3. Artificial flowers offer an effective solution to creating reproducible, low-maintenance flowers for ecological experiments. By taking advantage of current technologies and previously available designs, together with an understanding of floral visitor behaviour, it is possible to use them on a wider range of taxonomic groups beyond commercially significant social pollinators, and in field-based settings.

Introduction

Pollinator-flower interactions are an enduring subject of research across a range of fields, including ecology, agriculture, animal behaviour, and comparative psychology. A wide range of animal taxa including birds, bees, bats, butterflies and flies act as pollinators, with insects being particularly common flower visitors across a wide variety of angiosperm families (Ollerton et al., 2011). Most plant species globally (65-80%) rely on insects as their primary pollinators (Ollerton et al., 2011).

Understanding how floral traits such as shape, morphology, size, colour, scent, and nectar quality influence the behaviour of floral visitors is a key question in the field of pollination ecology. Although it is possible to investigate pollinator behaviour by manipulating real flowers (Galen & Cuba, 2001; McCall & Larsson, 2006; Midgley & Johnson, 1998; Peter & Johnson, 2008), flowers can be difficult to use in experiments due to the need for regular maintenance and the variability in blooming times. Further, the standardisation of real flowers is difficult because important characteristics such as colour, reward value, and chemical profiles vary from one flower to the next even within a species (Noe et al., 2019). Key floral traits such as nectar concentration are also subject to temporal changes (Wright, 1988)**.**

To overcome the challenges of working with living flowers, some researchers have turned to artificial flowers, which can be manipulated to address various questions about pollinator behaviour. In this paper, we use the term to include any experimental apparatus that is designed to mimic aspects of real flowers. Note that mimicry need not be exact; for example, a glass container offering sugar water could be considered an artificial flower as could coloured paper discs used to investigate colour preferences. Artificial flowers have been used to decode the waggle dance of honey bees (Frisch, 1967), investigate colour discrimination in honey bees (Frisch, 1967) and flies (Hannah et al., 2019) and determine innate colour preferences of butterflies, flies, and bees (Dyer et al., 2016; Giurfa et al., 1995; Goyret et al., 2008; Sutherland et al., 1999; Yoshida et al., 2015)**.**

Although artificial flowers have been used to address key questions in pollination ecology (Frisch, 1967; Giurfa et al., 1995; Goyret et al., 2008; Hannah et al., 2019; Sutherland et al., 1999; Yoshida et al., 2015), there are currently definitive frameworks available for researchers interested in incorporating artificial flowers into their research. The lack of guidelines and diverse range of flower designs makes it difficult for researchers to select the artificial flower best suited for their research questions and taxa. Researchers must instead re-design flowers from scratch which may take considerable time and effort.

Here we seek to simplify the design process by developing clear guidelines for selecting artificial flowers to meet the requirements of different types of experiments. We first summarise how artificial flowers have been used in the literature and the types of questions they have been used to address. We then discuss the relative advantages of different artificial flower designs and provide a simple decision tool and a set of guidelines for key design specifications to assist researchers intending to use artificial flowers as an experimental tool.

Methods

Review of artificial flower designs

We used Google Scholar to find studies that used artificial flowers in their experiments. Artificial flowers are used across multiple disciplines and different researchers use different terms for the same tool, so our search terms were: ‘artificial flower*’ ‘model flower*’, ‘model inflorescence*’, ‘artificial inflorescence*’ and ‘dummy flower*’. We chose to use Google Scholar over other databases because Google Scholar allows a full-text search of publications rather than one limited to keywords, titles and abstracts (such as Web of Science). Searching the text was important because artificial flowers are rarely the main subject of research papers and are instead used as a tool to answer a wide range of questions. We included studies spanning from 1970 to 2019.

Our search strings yielded over 700 000 results. We, therefore, examined all papers returned on the first ten pages of Google Scholar for each search as we noted a significant decline in relevant papers after this point. The exception was the term “dummy flower*, for which only three pages of results were returned.

We chose not to define the features of an artificial flower *a priori* and instead included studies where the authors themselves have used the terms ‘artificial flower/inflorescence, model flower/inflorescence and dummy flower/inflorescence. We then used the citation lists of included papers to find additional references. Only articles that were published in peer-reviewed journals were considered, which were cross-referenced using Web of Science and Scopus databases. We excluded papers that did not contain repeatable descriptions of the artificial flowers and the methods used to create them. For example, we did not include papers that simply stated ‘artificial flowers were used for the experiment’.

For each paper, we recorded: the focal animal species, a brief description of the method used to create the artificial flower, whether the flower was modelled after a real flower species, the main taxa being studied, and which reward (if any) was used in experiments. Where possible, we manually collated the supplied keywords for each article. Based on our review of artificial flowers, we developed a flowchart to help researchers find existing artificial flower designs that meet their experimental requirements. We also created a checklist of key considerations when choosing the attributes of artificial flowers.

Results

Review of Artificial flower designs

We identified 160 studies that used artificial flowers (examples in Figure 2.1). Most artificial flowers involved simple 2D flower shapes with some form of nectar receptacle (119 studies: 74%). The remaining studies used designs that we categorised as 'basic shape, no reward' (nineteen studies; 12%), 'visually complex, no reward' (twelve studies; 8%) or 'visually complex, nectar/pollen reward' (ten studies; 6%). Basic shapes are 2D shapes usually discs or circles with minimal resemblance of flowers to the human visual system. Complex flowers contain 3D shapes and look visually like a flower.

Some studies used artificial flowers that were visually simple but employed sophisticated designs in other aspects. For example, there were several instances of "electronic flowers". One was designed to study the effects of wind or the movement of flowers on forager behaviour (Sprayberry & Daniel, 2007). Other flowers contained nectar rewards that were automatically refilled to precise volumes and/or concentrations (Cnaani et al., 2006; Hartling & Plowright, 2011; Keasar, 2000). Three studies used radio-frequency identification (RFID) tag-enabled flowers to detect and record visitation (Ohashi et al., 2010).

In the studies we reviewed, ten orders of animals were used as target species; however, the majority of studies focused on social bees *Apis mellifera* (n=34), *Bombus impatiens* (n = 28) and *Bombus terrestris* (n=27). (For a list of study species, see Supplementary Table 1).

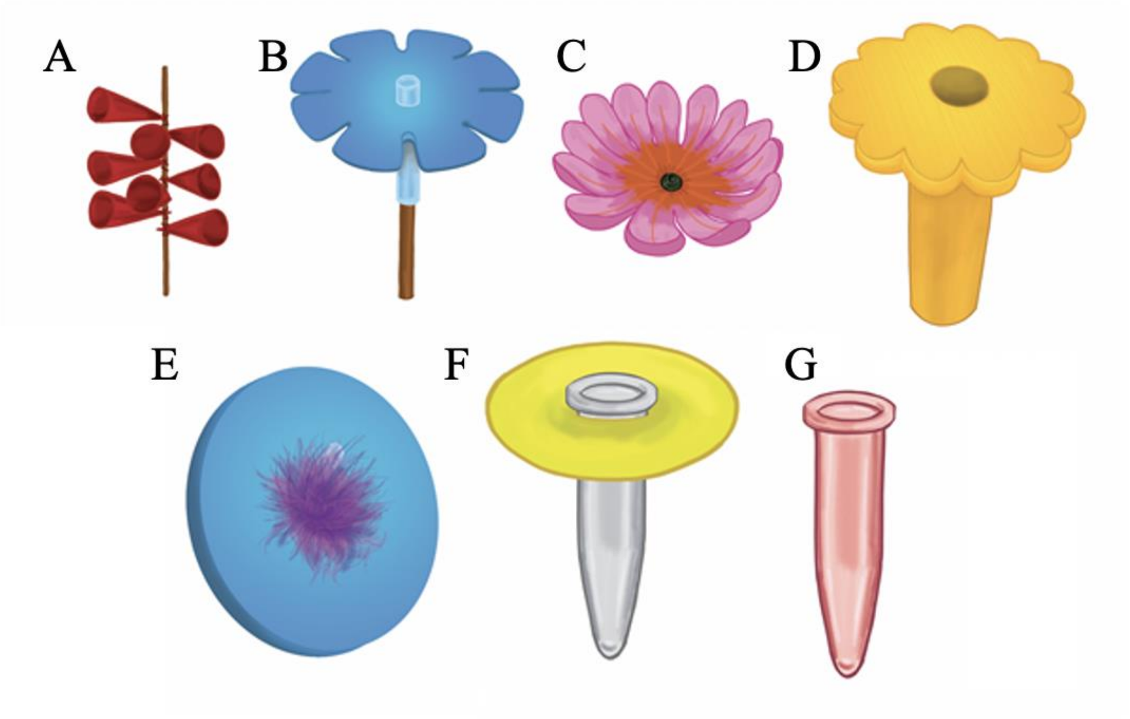

Figure 1.1: An example of the diversity of artificial flower types, including A) acetate inflorescences (Jersáková et al., 2012), B) flowers shapes with central nectaries, C) manipulated silk flowers (Mainali & Lim, 2008), D) 3D printed flowers (C Forster, unpublished), E) feathers used to hold pollen (Konzmann & Lunau, 2014), F) Basic circular disc containing an Eppendorf tube, and G) Eppendorf tube containing nectar.

Most studies (n = 114) were conducted in enclosed environments, where artificial flowers were offered to flower visitors inside flight cages, greenhouses, or Petri dishes. Only

28 studies were completed on free-living animals in open environments such as fields and meadows; 22 of these involved social bees (usually Western honey bees) where experiments were conducted near hives or apiaries (Supplementary Table 1). Note, some studies involved multiple test species, so totals add up to more than 160.

The majority of artificial flowers in our review provided a nectar substitute (n = 114). Five artificial flowers provided pollen, six contained both pollen and nectar, and thirty-four contained neither pollen nor nectar. One study used pollen and nectar in separate flowers.

Finally, 48% (77) of experiments involved a pre-training phase where floral visitors had to be trained to use flowers before any testing occurred. Where training to flowers did occur, it took anywhere from three minutes to four days (though 57 studies did not list the training duration).

Discussion

Given the diversity of successful artificial flowers that exist in the literature, there is often no need to reinvent a flower from scratch. However, the literature itself is difficult to search and is spread across several disciplines. To allow researchers to quickly investigate relevant artificial flower types, we have developed a decision tool that compiles examples of flower types used in experimental contexts (Figure 2.2). While this is not an exhaustive list, it highlights papers that contain methods that can be easily repeated for future experiments. In the sections below, we make suggestions for improved use of artificial flowers in a wider range of experimental contexts.

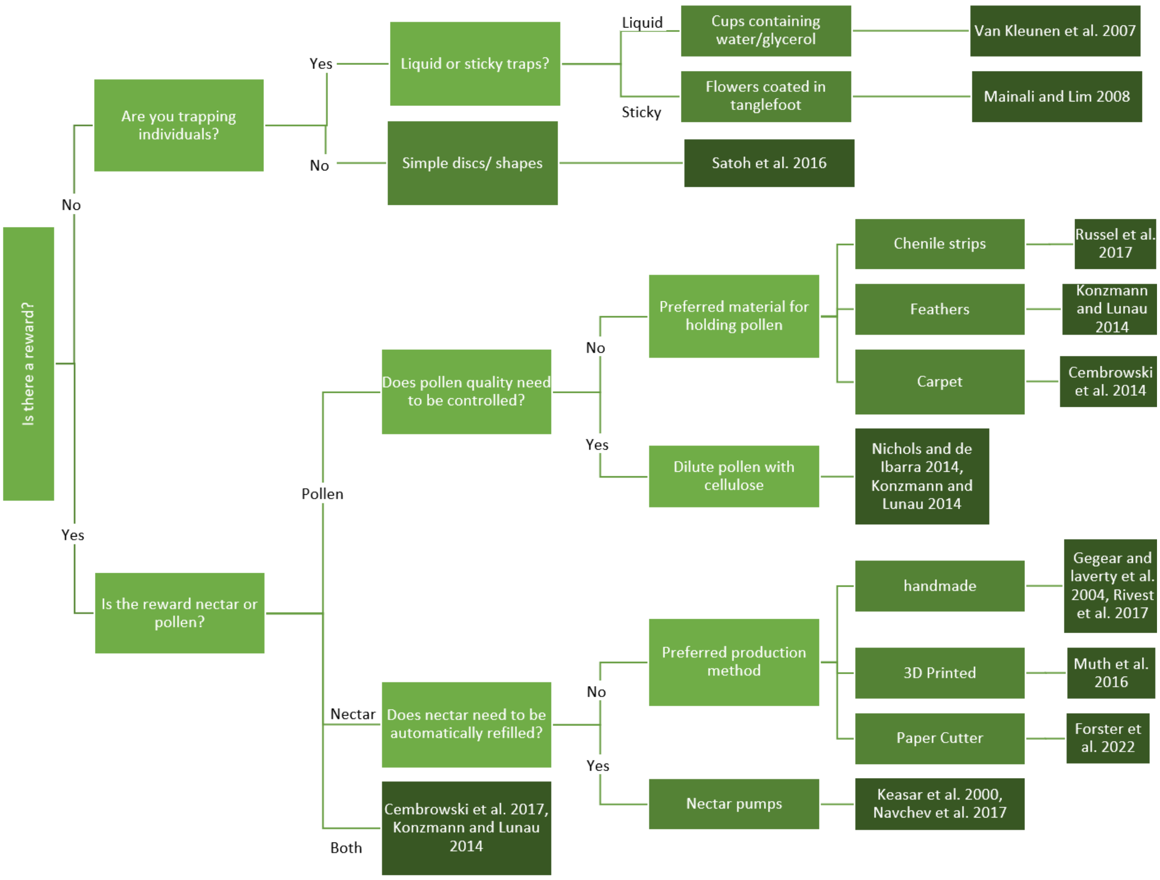

Figure 2.2: Flowchart guiding ways of manipulating and using artificial flowers in experiments

Increasing the range of taxa in artificial flower experiments

Bumble bees and honey bees are by far the most common taxa studied using artificial flowers. However, many insects including flies, butterflies, moths and beetles are known to visit flowers. Even predatory arthropods such as spiders, parasitoid wasps and lacewings may visit flowers to feed on nectar. To better understand plant-insect interactions, it would be useful to expand experiments beyond honey bees and bumble bees. However, doing so faces logistical challenges. For one, the focus on honey bees and bumble bees is likely driven by the commercial availability of these species which greatly simplifies experimentation. A possible solution to increasing the diversity of insects used in experiments would be to conduct experiments outdoors.

Artificial flowers in the field generally focus on a larger array of taxonomic groups than in experiments where artificial flowers are used in enclosed environments. There is evidence that wild floral visitors will visit artificial flowers in the field, including flies (Chen et al., 2015; du Plessis et al., 2018; Jersáková et al., 2012; Jersáková & Johnson, 2007; Jürgens et al., 2015; Policha et al., 2016; Whitehead et al., 2019), beetles (Dafni et al., 1990; Johnson & Midgley, 2001), spiders (Souza & Martins, 2004) and butterflies (Newman et al., 2012). Surprisingly, only three studies used artificial flowers to study free-flying hymenopterans, despite this taxon being the most studied overall (Møller & Sorci, 1998; Schemske & Agren, 1995), it is possible they are difficult to attract to artificial flowers and require training for successful artificial flower experiments.

More realistic artificial flowers may be more successful at attracting insects than less realistic flowers. Thrips were more likely to visit realistic-looking silk flowers, as opposed to artificial flowers represented by geometric shapes, suggesting there may be innate preferences for more ‘flower-like’ designs of artificial flowers (Mainali & Lim, 2011). Flies chose to visit real flowers over artificial flowers even when artificial flowers had the same visual and olfactory attributes as real flowers (Policha et al., 2016). Bumble bees are capable of recognising photographs of flowers they were trained to but are not as successful at recognising silhouettes or drawings of flowers (Johnson & Dafni, 1998; Slaa et al., 1998; Thompson & Plowright, 2014). *Lasioglossum* species are capable of recognising images of flowers, so they may use artificial flowers that look realistic (Howard et al., 2021). However, *A. mellifera* (Ladurner et al., 2005) and *Manduca sexta* (Raguso & Willis, 2005) showed little preference when given a choice between real and artificial flowers in enclosed lab experiments. Differences in responses to artificial flowers could thus be species-specific. To encourage adequate visitation to artificial flowers by wild species it may be helpful to more closely mimic the key visual or olfactory traits of preferred flowers.

Multiple studies suggest that bees will not visit artificial flowers without considerable training; this training imposes considerable logistical costs on researchers. Stingless bees, for example, require scent and training to forage on flowers (Dyer et al., 2016; Slaa et al., 1998). Similarly, bumble bees didn’t land on artificial flowers without significant training (Rivest et al., 2017). Western honey bees also require some form of scent and training to forage on artificial flowers (Giurfa et al., 1995). A study testing the effectiveness of artificial flowers on bees found that training was necessary for *Osmia lignaria* and *A. mellifera* to repeatedly visit artificial flowers (Ladurner et al., 2005). Overall, visitation to artificial flowers was reduced in comparison to real flowers (Ladurner et al., 2005). To study species that are less likely to visit flowers without significant training, it may be necessary to incorporate olfactory cues into flowers, irrespective of if the scent is a manipulated floral trait.

We suggest that artificial flowers could be a powerful tool for examining questions on pollination ecology, and floral visitor behaviour in free-flying insects, particularly in species that are difficult to keep in captivity or to address community-level questions. However, achieving this goal would require a better understanding of why some insect groups, most notably social bees, have previously failed to visit artificial flowers without training. To accelerate the use of artificial flowers in pollinator studies, we have made suggestions for floral traits that may be important for increasing their attractiveness (Table 2.1).

Use of scents to attract floral visitors

Making artificial flowers more ‘realistic’ and more attractive may be achieved by incorporating multiple floral traits into the design of artificial flowers. Hawkmoths, for example, required both odour and a leafy scent at the bare minimum to feed (Raguso & Willis, 2005). Most artificial flowers we reviewed consisted of a small number of flower attributes; often colour and nectar concentration or colour and scent. However, real flowers consist of an array of attributes including scent, flower shape, nectar and pollen composition. Multi-attribute flowers could help to attract species in field settings, where real flowers are also likely to be available, as the synergy of attributes can make for a more attractive flower (Nordström et al., 2017). At minimum, colour, scent and floral shape, and their interactions are likely to determine a flower's attractiveness (Nordström et al., 2017). Multiple flower cues are also important for learning including colour, shape, patterns and scent (Goyret, 2010; Riffell & Alarcón, 2013; Vergara et al., 2011).

Scent can play a key role in attracting floral visitors to flowers. Odours combined with visual cues have been associated with higher landing rates on artificial flowers by flies, cockroaches and bees (Policha et al., 2016; Roy & Raguso, 1997; Vergara et al., 2011). Without odour, artificial flowers may fail to attract individual flower visitors (Slaa et al., 1998). Scent and olfactory cues were used in 46 studies and were primarily used as attractants or to differentiate flowers for associative learning experiments. Examples of odour cues include clove oil (Hill et al., 2001), rose oil (Gegear & Laverty, 2005), peppermint oil (Slaa et al., 2003) and fruit juice (Tang et al., 2013).

There are several ways to incorporate odour cues into artificial flowers. Essential oils are frequently used to add floral scents to artificial flowers (Çakmak & Wells, 2001; Goyret & Raguso, 2006; Kulahci et al., 2008; Kunze & Gumbert, 2001; Petrikin & Wells, 1995). Essential oils should be added separately to nectar as essential oils may impact the taste of solutions (Burdon et al., 2020). We suggest that odours be added to flowers by putting scented solutions on a cotton bud next to the nectar receptacle of the flower (Figure 2.3). Essential oils can vary in their chemical composition, so it is important to cite the product used. To enhance replicability, researchers should consider using chemically pure odourants such as those available from chemical supply companies. Researchers could also use odour bouquets from real flowers for example by encasing whole flowers in cloths underneath artificial flowers (Roy & Raguso, 1997), or by removing the nectar from the chosen species and placing it under artificial flowers (Burger et al., 2010).

Figure 2.3: Example of using a cotton bud in an artificial flower to provide scent

Choosing rewards that suit your study system

Most artificial flowers contain nectar or pollen (real or artificial) as rewards. The presence of these ‘rewards’ stimulates learning and may trigger recruitment in social species (Simcock et al., 2018; Witjes & Eltz, 2007). Overall, the studies we reviewed largely used diluted honey or sucrose solutions as nectar substitutes with concentrations ranging from between 5 and 50%. Nectar in flowers is usually composed of sucrose, glucose and fructose (Pacini & Nepi, 2007). Rewards can be placed on or in the flower and provide rewards upon visitation (Keasar 2000).

Many species, including birds, bees and bats have shown preferences for sucrose-based nectars (Herrera M., 1999; Lotz & Schondube, 2006; Waller, 1972). When a nectar reward is offered to attract pollinators to an artificial flower, species-specific preferences for nectar concentration and composition may determine the effectiveness of the flower. Nectar in flowers typically ranges between 10-50% (w/w) sugar concentration (Basari et al., 2021; Chalcoff et al., 2006).

Pollen is used less frequently as a reward in artificial flowers in comparison to nectar, but is (Goyret et al., 2008; Muth et al., 2016; Nicholls & Ibarra, 2017) an important form of nutrition to bees, beetles and flies (Ruedenauer et al., 2016; Vaudo et al., 2016), offering a high-protein, nutritious food source to many insect visitors (Roulston & Cane, 2000). Pollen is also important as an olfactory cue for attracting floral visitors (Goyret et al., 2008; Nicholls & Ibarra, 2017) and can be beneficial for learning in insects (Muth et al., 2016).

There is some evidence that pollen quality and quantity can influence pollinator preferences (Nicholls & Ibarra, 2017; Ruedenauer et al., 2016). Researchers interested in investigating pollen-driven flower choice can manipulate pollen quality by adding indigestible cellulose (Nicholls & de Ibarra, 2014). Manually collecting pollen from flowers (Konzmann &

Lunau, 2014) is a potential way of obtaining pollen for experiments, but can be time-consuming (Nicholls & Ibarra, 2017). Commercially available bee pollen may offer an easier alternative to hand collecting pollen, however, this may carry the risk of disease transmission (Goblirsch et al., 2021). Researchers typically use ‘artificial anthers’ to provide pollen in artificial flowers. Materials to create anthers include feathers (Konzmann & Lunau, 2014), chenille strips (Muth et al., 2016) or carpet (Cembrowski et al., 2014) (See also (Nicholls & Ibarra, 2017; Russell & Papaj, 2016) for further examples).

Disease mitigation when using artificial flowers

When creating attractive, rewarding artificial flowers in outdoor environments there is also a responsibility on researchers to mitigate the potential spread of disease. Flowers in general provide an opportunity for parasites and diseases to be spread to floral visitors sharing flowers (Koch et al., 2017). Attractive artificial flowers may offer similar risks of disease spillover, especially if left out for long periods. However, unlike real flowers, it is possible to periodically clean artificial flowers with bleach or ethanol to prevent disease spread. The use of honey or honey bee-collected pollen may result in the spread of diseases if the honey or pollen is contaminated (Goblirsch et al., 2021).

Due to the risk of disease transmission, it is important to practice hygienic measures when using artificial flowers. For example, cleaning flowers with bleach between uses, and practising hygienic techniques when moving flowers across different field sites. Honey bee-collected products such as nectar and pollen should be used with great caution. Consider using artificial pollen, which can be purchased or produced (Paray *et al.*, 2021). For nectar, sugar syrups are a safer choice than honey wherever possible. If honey bee-collected pollen is used, researchers should ensure that it is sterilised by treatment with gamma radiation, pulsed UV or electron beam radiation (Goblirsch et al., 2021). It is important to note that while these sterilisation methods are effective at removing most pathogens, some pathogens are resistant (Simone-Finstrom et al., 2018). Pollen substitutes (See Paray *et al.*, (2021) for examples) should thus be used wherever possible.

Importance of considering the sensory system of the study species

Variations in spectral, spatial, and temporal acuity among floral visitors will shape the efficacy of floral designs. As a simple example, humans cannot see ultraviolet (UV) light, but most pollinators can, and so the unknowing inclusion or exclusion of UV cues may shape the attractiveness of artificial flowers in an unpredictable way. Human spatial acuity exceeds that of all insects (Hecht & Wolf, 1929; Land, 1997), so care is required when designing stimuli with intricate patterning, and when considering the size and spacing of stimuli concerning the viewing context in which they will be encountered.

Methods exist which allow researchers to take a 'subjective' view of the world, by incorporating information on the visual physiology of viewers (Johnsen, 2016; Kemp et al., 2015; Maia et al., 2019). Spectrometry and calibrated photography allow researchers to quantify the optical properties of stimuli and/or ambient lighting conditions through measures of surface reflectance and radiance. Data can then be combined with information on the spatial and spectral sensitivity of viewers within 'visual models' which can be used to estimate, among other things, the distinctiveness of particular floral designs within their viewing environment. Where the physiology and perception of viewers are well characterised including model species such as honey bees, blue tits, and chickens, the outputs of visual models represent broadly reliable guides to perception (Renoult et al., 2017)**.** In non-model species, however, data from closely related taxa may be drawn upon to generate predictions as to floral appearance, similarity, and discriminability, which should be coupled with behavioural experiments for validation (Kemp et al., 2015; Maia & White, 2018).

A suite of visual features may shape the attractiveness and efficacy of artificial flowers though a few are known to be of key importance and warrant particularly close consideration The chromatic properties of hue and saturation are among the most influential, with pollinators expressing strong innate preferences. Rich blues and yellows, for example, are attractive to honey bees, hoverflies, and some moths (Giurfa et al., 1995; Goyret et al., 2008; Kelber, 2003), while yellows and true ultraviolet-whites are preferred by several flies (Lunau, 2014).

Contrast is a similarly important cue as it influences floral visitors' abilities to detect flowers in their environment. Honey bees, for example, rely on contrast in the 'green' wavelength range, independently of colour to detect and view stimuli at a distance (Spaethe et al., 2001). Green contrast is also central to the processing of motion, shape, pattern, and size in many insects (Morawetz et al., 2013; Zhang et al., 1995). This has implications for the size of artificial flowers and the context in which they may be presented. Colour vision is typically used only when viewing stimuli of a large angular size (defined as the apparent size of an object to a specific visual system). It is therefore prudent, particularly in forced-choice experiments, to ensure that flowers are sufficiently large and/or encountered at close enough range to ensure colour vision is relied upon if the research question calls for it. Similar considerations are important when incorporating more complex patterns into the floral design such as floral guides, which focus the attention and orientation of some pollinators at close range. In such cases care should be taken to ensure that individual pattern elements are spectrally and spatially resolvable through explicit consideration of the acuity of likely viewer(s) and the context in which flowers will be encountered.

Choice of tactile surfaces may impact the viability of artificial flowers in field-based experiments, particularly for insects. Most flowers contain cone-shaped cells that impact the texture of flowers (Kay et al., 1981). The presence of flower epidermal cells can be a predictor of insect, bird or bat pollination (Costa et al., 2017; Papiorek et al., 2014). Bees have shown preferences for rougher floral surfaces over smooth surfaces (Alcorn et al., 2012; Whitney et al., 2009; Wilmsen et al., 2021). In general, plant surface is important to ensure adequate grip to consume food from plants (Voigt, 2019). The benefits of grip can be seen by the preference for artificial flowers containing conical cells are preferred by bees when flowers are moving (Alcorn et al., 2012).

While conical cells can impact the texture of flowers, these texture changes can also impact how flowers are seen in different light conditions, impacting the constancy of colour (Wilmsen et al., 2021). Previous experiments have shown that preferences for more tactile flowers can be observed before bees have felt the flowers, suggesting they have an impact on the visual properties of flowers (Alcorn et al., 2012). Bumble bees have shown preferences for artificial flowers mimicking conical structures found in floral petals when flowers were presented vertically (Wilmsen et al., 2021).

Electrical fields have the potential to be important in attracting species. Insects can gain positive electric charges by friction with surfaces. Movement of pollen is facilitated by the negative potential of flowers allowing for pollen to be moved to positively charged floral visitors (Clarke et al., 2017). Electrical fields also have potential importance in communication in honeybees (Greggers et al., 2013). Bumblebees and honey bees can detect weak electric fields using mechanosensory hairs and antennae (Clarke et al., 2017; Sutton et al., 2016). Electric information can be used by bees in associative cues (Clarke et al., 2013). It is possible to create artificial flowers with electric fields (Clarke *et al.*, 2013).

Artificial flowers are likely to be more effective if they are constructed with adequate consideration of the sensory systems of the target study species. To make more effective flowers with high consideration of sensory systems, we have provided a list of important sensory considerations for creating future flowers for particular study species (Table 2.1).

Reproducibility of floral designs

Despite the potential for artificial flowers to facilitate highly reproducible experiments, we found that many papers lacked the details needed for full reproducibility. Colour, as perceived by humans, was listed in most studies, with 55 only listing the human colour, 66 studies provided information on spectrophotometer details, 2 listed RGB or HSB coding for images and twenty-seven listed brands of paints or items used.

There are several ways that artificial flowers could be more reproducible. Hue is a particularly important trait that needs to be effectively reported to ensure reproducibility. Colours appear different to different species, and if the surface can reflect or absorb UV wavelengths, this will not be visible to human eyes, so qualitative descriptions of colours are not effective for reproducibility. At a minimum, researchers should report brand information that specifies the colour, type (e.g acrylic, oil) and finish (eg. gloss, matte) of paint or paper used. Hue should preferably be quantified and reported using methods of spectrophotometry or calibrated photography (White et al., 2015). When measuring colour using a spectrophotometer, it is important to use a stable light source that covers the relevant spectrum of colour associated with the artificial flowers being used (White et al., 2015). The direction of light also impacts the gloss and colour presentation of flowers to bees (Wilmsen et al., 2021). Light sources can also impact colouration, so should be reported. (White et al., 2015). Daylight is not recommended as a light source used for measuring colour due to increased noise, so experiments in controlled environments should involve artificial lights. However, this may not be possible in outdoor settings, so it is important to test if floral hues are impacted by daylight by using spectrophotometry or calibrated photography methods.

Researchers have until now largely used manual techniques (cutting, pasting, etc) that limit the extent to which flowers can be mass-produced, and that reduce consistency across flowers. The recent increase in the availability and affordability of tabletop manufacturing technologies such as 3D printers make it easier to create simple yet reproducible flowers (Muth et al., 2016). While there is no current evidence of using 3D scans, it would also be possible to 3D scan and print realistic-looking flowers.

The ability to easily share flower files also opens opportunities for citizen science and projects that span across continents. Particularly when using simple floral designs, they can be an effective tool for backyard experiments that promote inclusive, accessible science, particularly outside of laboratory settings. Flowers with prebuilt scale bars could be used to help with trait measurements of pollinators and identification.

Making use of previous artificial flower designs allows for consistency across experiments, especially if similar questions are being researched. We have provided some examples of basic flower designs for the Cricut machine including a disc and daisy-shaped flower (https://design.cricut.com/landing/project-detail/5f05ab4fdb066812f4d12caa) with a 3D printed base. We have also supplied a basic 3D-printed daisy-shaped flower (See Figure 2.2 for examples.

Table 2.1: Key attributes that should be considered when designing and using artificial flowers for research

	Description	Measurement, manipulation, and reporting	Example impacts on foraging choice	Can this attribute be used as an associative cue in behavioural settings?	Further reading
Visual Properties					(Maia et al., 2019; van der Kooi et al., 2016)
Hue	Fundamental 'colour'	Measured via calibrated photography or spectrometry and estimated either with or without explicit consideration of a viewer's visual system. Can be manipulated by using different coloured materials (paint, paper etc.), though this will also typically affect saturation and intensity to some extent.	Pollinators can express strong innate preferences for particular hues and may learn some more readily than others. Consideration of the sensitivity of viewers is also key, as no organisms are equally sensitive across the full visible range (300-700 nm). Well documented preferences include blue and yellow, and possibly ultraviolet, among bees, yellow and white among flies (Lunau, 2014), and yellow and blue among hoverflies and moths (Giurfa et al., 1995; Goyret et al., 2008; Kelber, 2003).	Hue is commonly used as an associative cue but can be impacted by other processes such as constancy (Gegear & Thomson, 2004), innate preferences (Giurfa et al., 1995), and viewing contexts, which may need to be 'overridden' with targeted training or behavioural experiments	

	Description	**Measurement, manipulation, and reporting**	**Example impacts on foraging choice**	**Can this attribute be used as an associative cue in behavioural settings?**	**Further reading**
Saturation or chroma	Purity or richness of colour.	Measured via calibrated photography or spectrometry and estimated either with or without explicit consideration of a viewer's visual system. Can be manipulated by diluting pigmentation applied to flowers (Papiorek et al., 2013), though this will also typically affect hue and intensity to some extent.	Pollinators may prefer greater saturation, though such effects can be difficult to examine independent of hue, contrast, and intensity preferences. Honey bees and Bumble bees favour higher spectral purity when trained to given hues (Rohde et al., 2013).	Limited evidence for its utility as an associative cue, with honey bees unlikely to use it as such (Kipp & Mason, 1982).	
Intensity	Stimulus brightness or luminance	Measured via calibrated photography or spectrometry and estimated either with or without explicit consideration of a viewer's visual system. Can be manipulated by using different 'coloured' materials, or through neutral-density filters, though this will also typically affect hue and saturation to some extent.	Limited evidence to suggest brightness is of interest to diurnal pollinators in natural settings, though some moths and birds make use of achromatic cues (Kelber, 2005). By contrast, brightness cues are not known to be significant for *macroglossum* (Kelber & Henique, 1999). It maybe more relevant to detection of flowers by nocturnal species	Limited and taxon-specific evidence for use as an associative cue among diurnal insects. Bees and parasitoid wasps do not use it (Desouhant et al., 2010; Ng et al., 2018). However, hawkmoths can use brightness as an associative cue. Nocturnal pollinators including moths may also be able to use and learn intensity cues (van der Kooi, Dyer, et al., 2019)	

	Description	Measurement, manipulation, and reporting	Example impacts on foraging choice	Can this attribute be used as an associative cue in behavioural settings?	Further reading
Pattern	The spatial arrangement of coloured elements.	Visual description when creating artificial flowers, the use of digital designs is preferable for reproducibility. Quantitative methods are also available which can consider the subjective perspective of a viewer through consideration of their spectral, spatial, and temporal sensitivity. Can be manipulated by printing/painting different patterns onto flowers.	Nectar guides are often important as attractants (An *et al.*, 2018), with 'radiating' markings on flowers often preferred by bees (Lehrer et al., 1995) and *Macroglossum stellatarum* (Kelber, 1997) Nectar guides can play a role in attracting species or acting as mimics Lunau (2000) The ecology and evolution of visual pollen signals. Nectar guides likely play more of a role in landing efficiency than for preferences, as the detectability of flowers with nectar guides is lower (Hempel de Ibarra et al., 2015). Nectar guides make it easier for pollinators to land more accurately on flowers and increase proboscis accuracy (Leonard & Papaj, 2011).	Patterns can be used as an associative cue, though care should be taken to consider the spatial acuity of the viewer and the context—including spacing and viewing distance—in which flowers are presented (Hempel de Ibarra et al., 2015).	

	Description	**Measurement, manipulation, and reporting**	**Example impacts on foraging choice**	**Can this attribute be used as an associative cue in behavioural settings?**	**Further reading**
Iridescence	Change in hue with the angle of viewing or illumination.	Measured via angle-resolved calibrated photography or spectrometry and estimated as the shift in hue (defined above) with viewing geometry. Can be manipulated by introducing nano-scale structuring (e.g. a diffraction grating) to the surface of the flower, such as via the use of a silicon mould of a compact disc (Whitney, Kolle, *et al.*, 2009)	Mixed and limited evidence for effects on foraging in natural conditions. Bumble bees may use iridescence (Whitney et al., 2009), while honey bees do not appear to (Garcia et al., 2019). Iridescence is also difficult to disentangle from other visual cues such as hue and saturation, thereby confounding tests of its significance (Morehouse & Rutowski, 2009).	Limited evidence for its utility as an associative cue, which is exacerbated by the difficulty of separating iridescence from other visual properties (van der Kooi *et al.*, 2019). The ability for animals to use it as a consistent signal depends on how it is presented to the animal, and the intensity of the iridescence (Stuart-Fox et al. 2021)	
Contrast	The difference in colour and/or brightness either between elements within a flower or between a flower and its viewing background.	Measured via angle-resolved calibrated photography or spectrometry and estimated as differences in colour and/or brightness, as defined above. Can be measured by varying the hue of the background or pattern against the main floral colour, depending on the question.	Contrast, colour and brightness is important for the detection of flowers (external contrast) or discrimination of pattern elements (internal contrast). Honey bees use ‘green’ contrast to detect flowers at a distance (Spaethe et al., 2001), while variation in viewing backgrounds can impact floral detectability and discrimination (Bukovac et al., 2017).		

	Description	Measurement, manipulation, and reporting	Example impacts on foraging choice	Can this attribute be used as an associative cue in behavioural settings?	Further reading
Angular size	‘Apparent’ size of a stimulus or pattern element to a specific viewer at a specific distance.	Can be manipulated by controlling the size of the flower and the distance at which it is viewed. To successfully test this, a setup that allows for forced perspective is necessary, such as a Y-Maze (Giurfa & Vorobyev, 1998)	Primarily influences foraging via its interaction with other visual properties. Honey bees, for example, exclusively use achromatic cues when viewing flowers of small angular size < 5^{o} (i.e. at a distance), and chromatic cues when viewing larger (or closer) flowers (Spaethe 2001).	Rarely used an associate cue unto itself but may shape the effectiveness of other cues in a context-dependent fashion (Lichtenstein et al., 2018).	
Symmetry	Invariance under some form(s) of reflection or rotation.	Defined by the number of planes of symmetry, with 0 (asymmetry/haplomorphy), 1 (bilateral/zygomorphy), and >2 (radial/actinomorphy) being particularly common. Can be manipulated by changing the floral shape.	Bees express preferences for symmetrical artificial flowers (Lehrer et al., 1995), as do crab spiders (Wignall et al., 2006). Symmetry impacts bees' preferences for floral patterns (radiating bars in radial symmetry and vertical lines in lateral (Lehrer et al., 1995).	Not used as an associative cue	

	Description	**Measurement, manipulation, and reporting**	**Example impacts on foraging choice**	**Can this attribute be used as an associative cue in behavioural settings?**	**Further reading**
Orientation	The angle a flower faces	Changing the angle of the flower presentation can manipulate this attribute.	Hovering insect flowers tend to be horizontal In radially symmetrical flowers, for hummingbirds, it is harder to approach a flower from all angles if it is semi pendant or horizontally orientated (Fenster et al., 2009).	A flower presented in different orientations may impact handling and nectar presentation due to gravity, it is possible floral visitors can learn to visit flowers of particular orientation to gain the greatest flower handling efficiency	
Corolla length	Corolla length is the measurement of the base of the flower to the tip of the lower lobes of a flower (Huang & Fenster, 2007).	Defined by the number of planes of symmetry, with 0 (asymmetry/haplomorphy), 1 (bilateral/zygomorphy), and >2 (radial/actinomorphy) being particularly common. Can be manipulated by changing the floral shape.	Corolla shape preferences are associated with functional groups, bees and flies having preferences for shape, but not beetles (Gómez et al., 2008). Hummingbirds prefer tubular flowers (Sutherland & Vickery, 1993). Bumble bees show a preference order of colour over shape (Sutherland and Vickery, 1993). Corolla length may increase the handling time for insect visitors. Corolla length can also encourage nectar robbing by some species.	Not used as an associative cue	

	Description	Measurement, manipulation, and reporting	Example impacts on foraging choice	Can this attribute be used as an associative cue in behavioural settings?	Further reading
Rewards					(Lotz & Schondube, 2006; Nicholls & Ibarra, 2017)
Pollen	Protein source associated with flowers	Pollen ‘quality’ is often assessed as crude protein, quantified via nitrogen content. Protein sources also should be reported for pollen composition (fat: protein, Amount of pollen, size of grains) (Stabler *et al.*, 2018). Pollen quality can be manipulated by mixing with cellulose (Nicholls and de Ibarra, 2014). It can also be manipulated by using pollen from different plant species or creating custom protein with different nutrients levels.	Most pollen is collected from honey bees. Pollen purchased may be of unknown and potentially non-native origin. It is unlikely that honey bees can discriminate between pollen quality via their antennae (Nicholls & Hempel de Ibarra, 2013) unless it is contained in a solution (Ruedenauer et al., 2016).	Bumble bees can be trained to high-quality protein containing flowers (Muth et al., 2016)	

	Description	**Measurement, manipulation, and reporting**	**Example impacts on foraging choice**	**Can this attribute be used as an associative cue in behavioural settings?**	**Further reading**
Nectar	The sweet solution produced by flowers as a reward to encourage floral visitation	Nectar volume, viscosity, and sugar can be manipulated using different sugar concentrations or compositions.	Nectar viscosity is impacted by temperature. Bees tap their tongues faster in warmer nectar (Shi *et al.*, 2020), and bumble bees prefer higher concentration nectar even if it is cooler (Whitney et al. 2008). Hummingbirds can distinguish between concentration differences of as little as 1% when foraging on lower concentration nectar, but this reduces at higher concentrations (Blem et al. 2000) There are species-specific preferences for different nectar compositions regarding sugar types. Artificial nectar containing micronutrients such as amino acids is also preferred.	Nectar is often a reward used in associative learning	

	Description	**Measurement, manipulation, and reporting**	**Example impacts on foraging choice**	**Can this attribute be used as an associative cue in behavioural settings?**	**Further reading**
Non-visual sensory cues					Methods for flora creation (Dötterl & Vereecken, 2010; Green & Linstead, 1990; Whitney et al., 2009)(Green and Linstead, 1990; Whitney *et al.*, 2009)
Olfactory cues	Scents associated with flowers	Olfactometers to measure odour dilution, proton transfer reaction mass spectrometry to measure volatile emissions, and thermal desorption gas chromatography-mass spectrometry (Powers et al., 2020). Can be manipulated by the addition or subtraction of different scents.	Olfactory cues play an important role in making artificial flowers more 'realistic' (Policha et al., 2016), and also influence visitors' discrimination of visually similar plants. For example, *Hoplitis adunca* uses olfactory cues to discriminate between *Echium vulgare* and *Anchusa officinalis*. Distilling scents from flowers, or using essential oils is common. Essential oils may be hard to replicate across brands**.** Odours can also be applied as individual components (eg. Gerinol and nonanol) or individual components can be mixed to create odour blends.	Olfactory cues are commonly used for attracting pollinators but are also successfully used as associative cues	

	Description	Measurement, manipulation, and reporting	Example impacts on foraging choice	Can this attribute be used as an associative cue in behavioural settings?	Further reading
Tactile cues	Textures on flowers, usually associated with shapes of epidermal cells.	Can be visualised using microscopy and manipulated by creating moulds of different floral surfaces (Whitney et al., 2009).	Tactile cues are likely important to allow for efficient landing, handling and movement on flowers (Whitney et al., 2009). There are also possible links between conical cells and increased depths of colour (Comba et al., 2000; Wilmsen et al., 2021). It is possible to use moulds to establish the texture of flowers (Bräuer et al., 2017) could potentially be used to recreate textures for artificial flowers. Bumble bees have more footholds in conical cell containing flowers, when presented vertically there was an increased preference for conical surfaces (Whitney et al., 2009).	Minimal evidence. However, it is likely landing would have to occur for the visitor to know the texture of the flower. Associative cues will likely be more linked with the intensity of colour increases with conical cells (Comba et al., 2000).	

	Description	Measurement, manipulation, and reporting	Example impacts on foraging choice	Can this attribute be used as an associative cue in behavioural settings?	Further reading
Temperature	The temperature of nectar/flowers.	Heat maps may be beneficial for temperature-specific studies. Can be manipulated by heating or cooling nectar or floral surfaces (Tan et al., 2015).	Flower temperature is likely more important for pollen. However, nectar texture and temperature may be impacted by floral temperature, which may impact visitation. From a floral perspective, temperature is often important for optimal pollen germination and pollen tube growth (van der Kooi, Kevan, et al., 2019). Bumble bees prefer warmer nectar, but not at the cost of sugar concentration (Whitney et al., 2008). Honey bee prefer warmer nectar, but do make trade-offs between temperature and concentration (Tan et al., 2015). Stingless bees prefer warmer nectar than ambient until it hits 34 degrees, then they start to prefer cool (Norgate et al., 2010).	Can be used as an associative cue (Whitney et al., 2008).	

	Description	Measurement, manipulation, and reporting	Example impacts on foraging choice	Can this attribute be used as an associative cue in behavioural settings?	Further reading
Aerial Electroreception	Electric fields are associated with flowers and pollinators	Electrostatic dusting to visualise electric fields, and electrometer for measuring electric charge (Clarke et al., 2017; Greggers et al., 2013).	Bumble bees and honey bees can detect weak electric fields using mechanosensory hairs and antennae which can assist in pollen transfer (Clarke et al., 2013)	Bumble bees can use electric fields as an associative cue. When trained with flowers containing an electric field and a colour, bees were faster to learn which flower was of higher quality in comparison to bees just trained on colour (Clarke et al., 2013).	

	Description	**Measurement, manipulation, and reporting**	**Example impacts on foraging choice**	**Can this attribute be used as an associative cue in behavioural settings?**	**Further reading**
Saturation or chroma	Purity or richness of colour.	Measured via calibrated photography or spectrometry and estimated either with or without explicit consideration of a viewer's visual system. Can be manipulated by diluting pigmentation applied to flowers (Papiorek et al., 2013), though this will also typically affect hue and intensity to some extent.	Pollinators may prefer greater saturation, though such effects can be difficult to examine independent of hue, contrast, and intensity preferences. Honey bees and Bumble bees favour higher spectral purity when trained to given hues (Rohde et al., 2013).	Limited evidence for its utility as an associative cue, with honey bees unlikely to use it as such (Kipp & Mason, 1982).	
Intensity	Stimulus brightness or luminance	Measured via calibrated photography or spectrometry and estimated either with or without explicit consideration of a viewer's visual system. Can be manipulated by using different 'coloured' materials, or through neutral-density filters, though this will also typically affect hue and saturation to some extent.	Limited evidence to suggest brightness is of widespread interest to diurnal pollinators in natural settings, though some moths and birds make use of achromatic cues (Kelber, 2005). By contrast, brightness cues are not known to be significant for *macroglossum* (Kelber & Henique, 1999). It may, however, be more relevant for nocturnal species, particularly concerning the initial detection of flowers.	Limited and taxon-specific evidence for use as an associative cue among diurnal insects. Bees and parasitoid wasps do not use it (Desouhant et al., 2010; Ng et al., 2018). However, hawkmoths can use brightness as an associative cue. Nocturnal pollinators including moths may also be able to use and learn intensity cues (van der Kooi, Dyer, et al., 2019)	

Conclusion

Artificial flowers are an exciting tool for future research on pollinator behaviour and ecology. Artificial flowers offer a range of advantages over real flowers including increased reproducibility, unlimited ‘flowering’ times and precise control over flower attributes. Creating flowers that contain traits relevant to the study species could allow for work on an increased range of species. It is also important to consider the range of traits contained in flowers to create effective artificial flowers. Artificial flowers hold tremendous potential for increasing our understanding of animal-plant interactions.

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Covid Impact Statement: Impact of empty flowers on foraging by the Honey bee, *Apis mellifera*

The following chapter was written under the University of Sydney's COVID-19 impacted thesis protocol.

Impacts to experiment

- *November 2019-January 2020:* significant interruptions due to smoke haze from bushfires that impacted NSW. Smoke haze was not only a safety concern but impacted the foraging behaviour of honey bees (Leonard et al., 2019), resulting in a slower-than-expected process of collecting pilot data.
- *March 2020:* My experiment had to be temporarily cancelled due to the first COVID-19 lockdown. The experiment was no longer permitted to be run at Macquarie University
- *October 2020.* The experiment was restarted at The University of Sydney due to entry restrictions to Macquarie University. Without controlled colonies in the flight cage, increased floral resources for honey bees in the vicinity meant training bees to feeders was less effective than in a flight cage. We also had issues with bees from other colonies recruiting to feeders, minimising my ability to collect dance behaviour.
- *April – June 2021:* Experimental data collection
- *23rd June*-11th October: Lockdown in Sydney meant the experiment had to be cut short.

Despite these circumstances and the way they impacted the final data set, I decided to include this chapter without multiple decoys and dance data. As such it provides different insights than those initially expected but is an important proof of concept for understanding decoy effects in honey bees.

In the section that follows, I present the introduction and abbreviated methods for the full experiment. In Chapter 3 I present the covid-adjusted experiment. Chapter 3, *Impact of empty flowers on foraging by the Honey bee, Apis mellifera* is intended for publication and has been written in full, including an introduction adjusted to suit the changes in methodology. The results from the actual experiment are reported in full in Chapter 3.

Introduction

Optimal Foraging Theory assumes that animals make food choices by trading off between nutritional losses (eg. energy expenditure) and gains (eg. caloric intake). Animals are therefore expected to assign a fixed 'value' to each food option; this concept is known as 'absolute valuation' (Rapoport, 1998). Animals that use absolute valuation strategies will show transitivity of preferences by ranking potential food options in stable preference order. For example, if item A is preferred over B, and B is preferred over C, then item A should be preferred over C. Transitivity of preferences has been observed in foraging pigeons (Siemann et al., 1996), chimpanzees (Boysen et al., 1993) and rats (Davis, 1992). In contrast, a lack of transitivity, ('intransitivity') of preference can indicate the use of comparative valuation strategies where the value of a food item is not a fixed property and instead can change due to other items in the choice set. Comparative valuation strategies have been observed in gray jays (Waite, 2001), crickets (Gabel and Hennig, 2016) and Western honey bees (Shafir, 1994).

The decoy effect is an intriguing example of how comparative valuation strategies can result in unexpected preference shifts. In humans, for example, if there are three holiday packages, and one becomes available, people are more likely to pick the holiday package that has similar amenities/climate to the sold-out one (Bateman et al., 2008; Colman et al., 2007; Heath & Chatterjee, 1995; Trueblood & Pettibone, 2017).

Decoys come in two common forms: low-quality decoys and phantom decoys. Low-quality decoys are decoys that are of lesser value than the other items in the choice set. Low-quality decoys are typically 'asymmetrically dominated', where an attribute of the decoy is similar in value to one of the attributes of the target option (Figure 1, Introduction). The addition of the third, lower-quality option usually results in people picking the item most similar to it in attribute space (Bateman et al., 2008). In contrast to low-quality decoys, phantom decoys are high-value items that are unavailable at the time of choice. For example, when reviewing job applicants, if a high-quality applicant dropped out of the application process, employers were more likely to pick the candidate more similar to the phantom ‘dropout’ candidate compared to when the decoy was absent (Highhouse, 1996). According to choice models based on absolute valuation, low-value or unavailable items should simply be ignored as they are of lesser value than other options.; therefore, they should have no impact on an individual's preferences for the other items in the choice set.

Decoy effects can result in two kinds of decision-making, dissimilarity effects and similarity effects. Similarity effects occur when the decision-maker chooses options more similar to the decoy (Colman et al., 2007; Highhouse, 1996; Park & Jang, 2018), whereas dissimilarity effects occur when the decision-maker chooses options less similar to the decoy (Trueblood & Pettibone, 2017). In honey bees, similarity effects have been seen using prior decoy tests (Shafir, 1994; Tan et al., 2015).

Phantom decoys are potentially relevant to bees, as they frequently experience unavailable items in the form of empty flowers. Previous studies on Asian honey bees show they are susceptible to phantom decoy effects when a flower is empty. In *Apis cerana,* multiple types of phantom decoys have been tested to understand how bees choose flowers. Attractive phantom decoys, where the phantom decoy is of higher value than the other choices available, induce a similarity effect, where bees were more likely to visit the flower most similar to their preferred but unavailable flower. Unattractive phantom decoys, where the phantom decoy is less attractive than the other available choices, did not affect *A. cerana* foraging choice (Tan et al., 2015).

Honey bees use both private and social information when making decisions about which flowers to visit. For example, Western honey bees use scent marks to understand which flowers have been previously visited and avoid them (Giurfa & Núñez, 1992). Western honey bees also communicate information about the perceived quality and location of flowers using the waggle dance (Frisch, 1967). The longer the dance lasts, the higher the individual rates the reward (Hrncir et al., 2011; Seeley et al., 2000). The waggle dance offers a unique opportunity in animal species, where we can gain a self-reported rating of a food source's perceived quality. Honey bees can thus express their food preferences in two ways: by what they choose, but also by what they choose to tell their nest mates about.

I tested the impact of decoys on Western honey bee preferences using 3 types of phantom decoys and two asymmetrically dominated decoys. We aimed to test if:

A) To determine how different types of decoys impact visitation rates on artificial flowers
B) To determine how different decoy types influence the 'perceived' value of artificial flowers, as expressed by the number of circuits during a dance

Methods

I determined how a range of decoy types influenced decision-making in honey bees using 3 phantom decoys and 2 unattractive decoys (Figure 3.1). I tested the effects of three phantom decoys and two unattractive decoys on the preferences of two other available flowers (A, which had better nectar quality and lower nectar accessibility and B, which had better nectar accessibility and lower nectar quality), using the honey bee *Apis mellifera.* Phantom decoy one was a decoy where the nectar quality was similar to flower A, and the nectar accessibility was similar to flower B. Phantom decoy 2 ‘dominated’ in the sense that its nectar quality was similar in value to the nectar quality of the flower A, but the nectar accessibility was better than flower A. Phantom decoy 3 consisted of a decoy that had similar nectar accessibility to flower B, but better nectar quality than flower B. We also aimed to test two unattractive decoys. The first unattractive decoy had similar nectar accessibility to flower A, but lower nectar quality. The second unattractive decoy had similar nectar quality to flower B, but lower nectar accessibility. We predicted that Western honey bees would in all cases be more likely to pick flowers more similar in attributes to the decoy they were presented with.

Experimental setup

I planned to conduct this experiment at Macquarie University (Sydney, NSW, Australia) in a flight cage. Flight cages consisted of a 25m x 10m mesh greenhouse.

The experimental colony was kept in an insulated wooden shed that held a three-frame observation colony. The observation colonies were filmed during experimental periods using Samsung tablets.

I chose to use flight cages because they forced bees to feed on our artificial flowers. Secondly, using a flight cage ensured we could track individual bees back to their source colony. Pilot studies started in September 2019.

Decoy types

Testing multiple decoys can be useful to establish if or when bees are likely to utilise similarity effects when making decisions. Flowers differed in two attributes that we predicted would influence bee choice (sugar concentration and accessibility) and one associative attribute (colour) that ensured bees could learn which flower they preferred. I used attributes that have previously been used in decoy experiments testing *A. mellifera* by Shafir (2002). To understand how individual honey bees chose flowers, I tested the effects of three phantom decoys and two unattractive decoys (Figure 3.1; Table 3.1). I used nectar concentration as one attribute because honey bees can discriminate between different nectar concentrations, and tend to pick higher concentrations over lower concentrations (Waller, 1972) (up to a threshold of 50% after which preference declines). The second flower attribute was tube length, where bees needed to crawl down a short (45mm) or a long tube (100mm long) to access the nectar. Previous research suggests that bees prefer short tubes over longer tubes (Shafir et al., 2002).

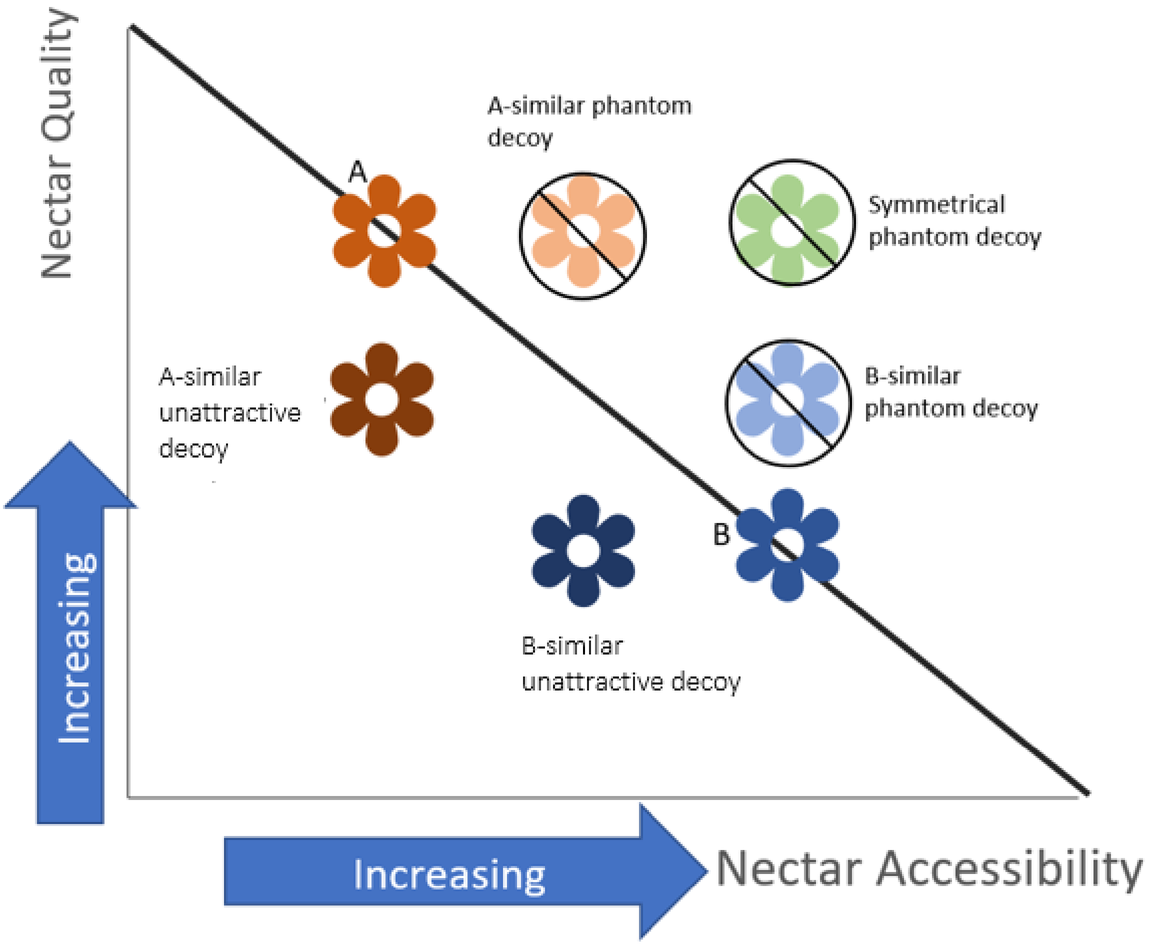

Figure 3.1: Attribute space used for the experiment. In decoy experiments, the aim is to have two flowers of equal value (orange A, and blue B), and test the preferences of these flowers against decoys that are either attractive and unavailable (phantom decoy flowers) or unattractive and available (unattractive decoy flowers)

To determine if bees were using absolute decision strategies, we compared honey bee preferences for two flowers A (50% (w/w) sucrose solution, 100mm tube length) and B (15% (w/w) sucrose and 50mm tube length), in the presence (trinary set) and absence (binary set) of a decoy flower. The attributes of A and B were selected such that we expected them to be equally preferred. If bees are using absolute decision strategies, then the presence of the decoy should not change bee preferences, relative to preferences in the binary choice set.

three phantom decoy flowers that were considered more attractive in the training, but contained no nectar in the test phase. We also tested the preferences of bees in the presence of two unattractive decoy flowers, which contained nectar during the test phase (Table 3.1; Figure 3.2).

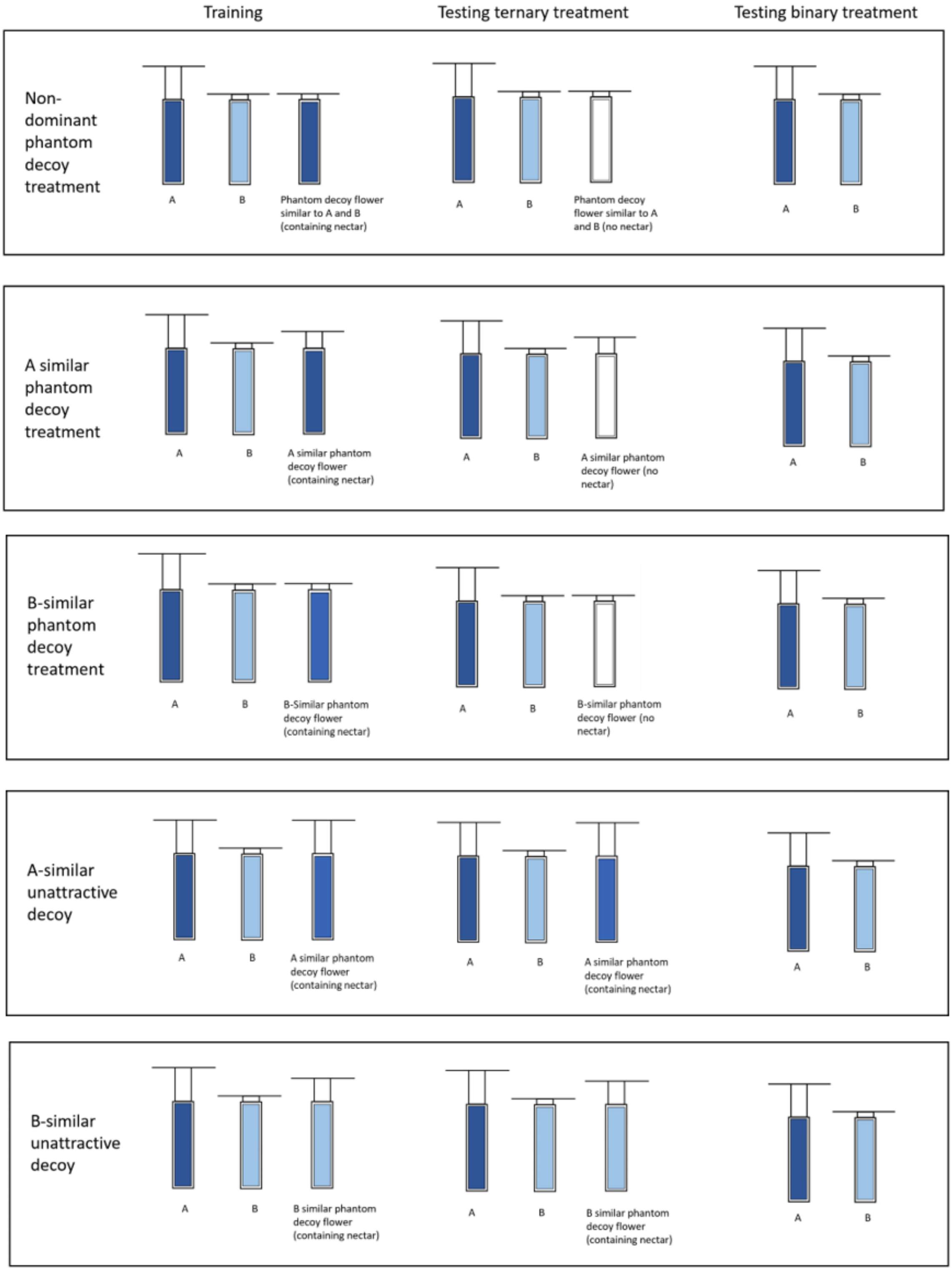

Figure 3.2: Layout of treatments for experiments. Flowers are represented as schematics, where a longer tube represents a longer tube length, and a darker blue tube contains a higher concentration of nectar

Table 3.1: Floral attributes used for the experiment

Flower Type	Sugar concentration (v/v)	Tube length (mm)	Nectar present
A	50	100	Yes
B	15	50	Yes
A similar phantom decoy	50	80	No
B similar phantom decoy	40	50	No
Non-dominant phantom decoy	50	50	No
A-similar unattractive decoy	40	100	Yes
B-similar unattractive decoy	15	80	Yes

Training and testing methods can be seen in the following chapter.

Tracking dance behaviour

Using dance behaviour, it is possible to get a rating of a food source from a bee that has just visited a flower. Bees that were trained to visit flowers were marked with paint pens. We followed bees' dance behaviour once they had visited our artificial flowers during the experiment. Upon returning to the colony after visiting a flower, the marked bee was located in the colony and timestamped for later video analysis. I checked if the bee was dancing, and if she was, counted the number of circuits she made during her dance. I only counted circuits as the flight cage did not allow for a long enough distance to get a full waggle dance. When foods are less than 200 m from the hive, honey bees do a round dance instead of a waggle dance. The number of circuits in a round dance still indicates the relative value of the food source from the bee's perspective (Waddington, 1982).

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Chapter 3: Impact of empty flowers on foraging by the honey bee, *Apis mellifera*

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Abstract

Floral displays often signal the presence of nectar, but nectar may not always be present due to previous visits by nectarivores or temporal changes in nectar availability. How does the presence of empty flowers impact Western honey bee preferences for the available flowers? We aimed to test if previously rewarding flowers changed the preference relationship between neighbouring flowers, and if empty flowers impacted overall visitation in the honey bee *Apis mellifera.* Using artificial flowers, we showed that although empty flowers did not influence foraging choices in bees, empty flowers did increase movement between flowers in the patch. The presence of empty flowers resulted in increased rates of patch abandonment. Our results suggest that while empty flowers may not directly impact foraging preferences in bees, they can have an impact on visitation within patches and in the surrounding area, which may have knock-on effects on the pollination success of both the emptied flower and neighbouring plants.

Introduction

When foraging, bees choose flowers in a landscape based on attributes associated with both the patch and with individual flowers. For example, floral attributes such as nectar quality (Roubik and Buchmann, 1984), ease of access to nectar (Zung *et al.*, 2015), and landscape attributes such as distance from the colony (Bruninga-Socolar *et al.*, 2022) may all potentially influence the flower choices of foraging bees. Many individual bees also have innate preferences for floral attributes such as, including floral colour (Giurfa *et al.*, 1995), shape (Lehrer *et al.*, 1995), and symmetry (Rodríguez *et al.*, 2004). Finally, flower choice can be influenced by neighbouring plants in an environment, such that preferences depend not only on the traits of each flower in isolation but their spatial context within a patch.

Flower visitation may be influenced by other plants in the area. Highly rewarding ‘magnet’ plants can encourage bee visitation and can lead to spillover effects where plants in the vicinity of magnet plants gain increased pollination benefits (Johnson *et al.*, 2003; Peter and Johnson, 2008). Bees generally learn to associate the colour of highly rewarding plants with nectar (Johnson *et al.*, 2003), Therefore, magnet plants may preferentially increase visitation to visually similar species (Gigord *et al.*, 2002; Johnson *et al.*, 2003; Peter and Johnson, 2008).

Flowers do not always contain nectar, and the presence of ‘empty flowers’ can impact bee foraging behaviour. Flowers can be empty of nectar due to nectar removal by other insects, temporal/physiological changes, or due to environmental conditions. Some ‘deceptive’ flowers are always empty and never offer nectar as a reward. Empty flowers often occur in reasonably high ratios on plants, as an energy-saving strategy, as the plant can maintain a large visual display while investing less in nectar production (Smithson and Gigord, 2003). Flowers also generally produce less nectar with age, (Gilbert, Haines and

Dickson, 1991; Weiss, 1991), but it can be beneficial to keep non-receptive flowers as they may save the plant energy while serving as a visual signal.

The impacts of empty flowers on bee foraging choice may vary depending on how often nectar is unavailable, and how many flowers in an inflorescence are empty. Increased frequencies of permanently empty flowers can result in floral visitors foraging on flowers that are morphologically dissimilar to the empty flowers (Smithson & Gigord, 2003). Smithson and Macnair (1997) found that bumble bees, *Bombus terrestris*, that had encountered an unrewarding flower (which were never previously rewarding) were more likely to visit flowers that were dissimilar in colour to the unrewarding flower. In this case, contact with the unrewarding flower may have caused learned avoidance of that flower type. In contrast, when a previously rewarding flower is no longer available, *B. terrestris* were more likely to seek out similar coloured flowers (Gigord *et al.*, 2002). Similarly, encounters with recently empty flowers can result in bees preferentially visiting flowers with more similar attributes to the unavailable flower (Tan *et al.*, 2015).

The presence of empty flowers can influence whether or not bees will return to flowers. For example, plants can contain multiple inflorescences, and bumble bees, (*B. terrestris)* will abandon an inflorescence if they visit too many empty flowers (Smithson and Gigord, 2003). Unrewarding flowers can also cause foragers to abandon patches altogether if they occur at high enough frequencies (Biernaskie, Cartar and Hurly, 2002; Smithson and Gigord, 2003). Patch abandonment can have knock-on effects on neighbouring flowers in the patch, which may no longer receive insect visits and may therefore incur decreased pollination.

Empty flowers could also influence their neighbour’s pollination success by altering preference relationships between other flowers in the patch. Although poorly studied in bees, in humans, choices can be influenced by unavailable options in choice sets, referred to as ‘phantom decoys’. For example, consumers picking holiday packages that varied in star rating and price were more likely to pick holiday packages that had similar star ratings to highly desirable but sold-out holiday packages (Park and Jang, 2018). That is, the presence of the phantom decoy leads humans to pick a target option more often than they would if the decoy was absent.

Phantom decoys can cause a decision-maker to shift preferences based on multiple attributes that are being considered (Figure 3.3). They can act in two ways: the presence of a phantom decoy can result in the decision-maker picking options more similar to the decoy (similarity effect), or less similar to the decoy (dissimilarity effect). Typically, there will be two attributes, and the two available options will be of equal value in preference space. Attractive phantom decoys will be better than both options in the choice set but will be more similar in attribute space to one of the available options (typically the target option). It is usually predicted that people will pick the option most similar in attribute space to the unavailable option (Highhouse, 1996; Tan *et al.*, 2015; Park and Jang, 2018).

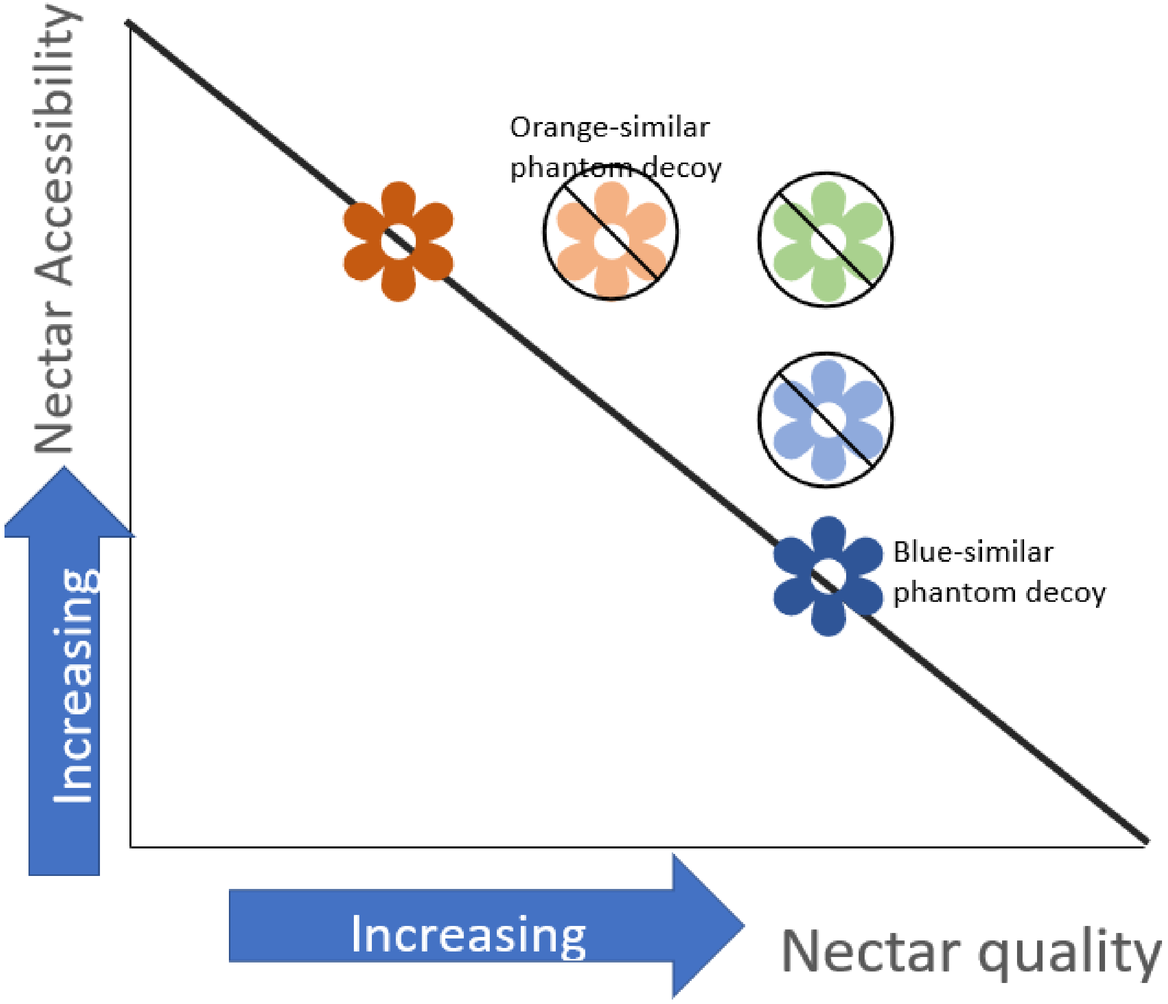

Figure 3.3: Visual representation of attribute space in phantom decoy experiments. The dark orange and blue flowers represent the available flowers. The light blue and orange flowers represent phantom decoys that are asymmetrically dominated by each of the available options. The green flower is a symmetrical decoy

Here, we investigated how empty flowers impacted floral preference, movement between flowers and abandonment of patches in *A. mellifera.* We hypothesised that:

1) The preference relationships between two flowers would change in the presence of an empty, previously rewarding flower

2) That the presence of an empty flower would increase movement between flowers and

3) That the presence of empty flowers would result in increased abandonment of the patch.

Methods

Honey bees

We used the Western honey bee, *A. mellifera* for our experiment. Western honey bees are native to Africa, Europe and the Middle East (Han, Wallberg and Webster, 2012), and were introduced to Australia in 1822. They are widespread across Australia and are used as crop pollinators due to their generalist foraging diet. Our bees came from six established Langstroth hives, containing between 2-3 boxes. Colonies were kept at the University of Sydney Camperdown campus, in New South Wales, Australia. Experiments were conducted between April and June 2021.

General Methods

To investigate the impact of empty flowers on bee behaviour, we compared bee visitation rates on the two focal options (herein, flowers A and B) in binary treatment where only the two options were available, and in an ‘empty flower’ treatment, where a third, unavailable option was added to the choice set.

We tested the effect of empty flowers using artificial flowers. Artificial flowers consisted of a 5cm diameter matte laminated paper shaped like a daisy (Figure 3.4). The flowers had a central Eppendorf tube containing sugar solution, with the tube length of the Eppendorf extended using a plastic tube. The flowers were coloured blue, white and yellow. We quantified the reflectance of blue, white and yellow papers using an OceanInsight JAZ reflectance spectrometer with pulsed PX-2 Xenon light, calibrated against a 99% diffuse white and 0% dark standard (Labsphere, New Hampshire). We then modelled the location of each sample in the hexagon colour space using the visual phenotype of the honey bee, *A. mellifera* and selected shades that were separated by a Euclidean distance of at least 0.11; the absolute discrimination threshold for honey bees (Chittka, 1992; Maia et al., 2019) (Supplementary Figure 1). Flowers were held by a 3D-printed triangular base that held the flowers 10 cm apart from each other. Each flower contained 5ml of sucrose solution.

Artificial flowers differed in two attributes that we predicted would influence bee choice (sugar concentration and accessibility) and one neutral attribute (colour) that ensured bees could learn which flower they preferred. We used attributes that have previously been used in decoy experiments testing *A. mellifera* by Shafir (2002**).** We used nectar concentration as one attribute because honey bees can discriminate between different nectar concentrations and tend to pick flowers containing nectar higher than 20%(Waller, 1972) (up to a threshold of 50% after which preference declines). We, therefore, had two different sets of nectar concentrations, 50% (w/w) and 20% (w/w). The second flower attribute was tube length, where bees needed to crawl down a short (45mm) or a long tube (100mm long) to access the nectar. Previous research suggests that bees prefer short tubes over longer tubes (Shafir, Waite and Smith, 2002).

We developed two flowers that were similarly preferred by bees (flowers A and B) and one flower that was the most preferred (Flower C). Flower A had a long (less preferred) tube containing the more preferred 50% sucrose (v/v) and flower B with a short tube (most preferred) containing 15% sucrose solution (less preferred). Our highly preferred option flower C) contained 50% sucrose solution with a short (45mm) Tube (Figure 3.3).

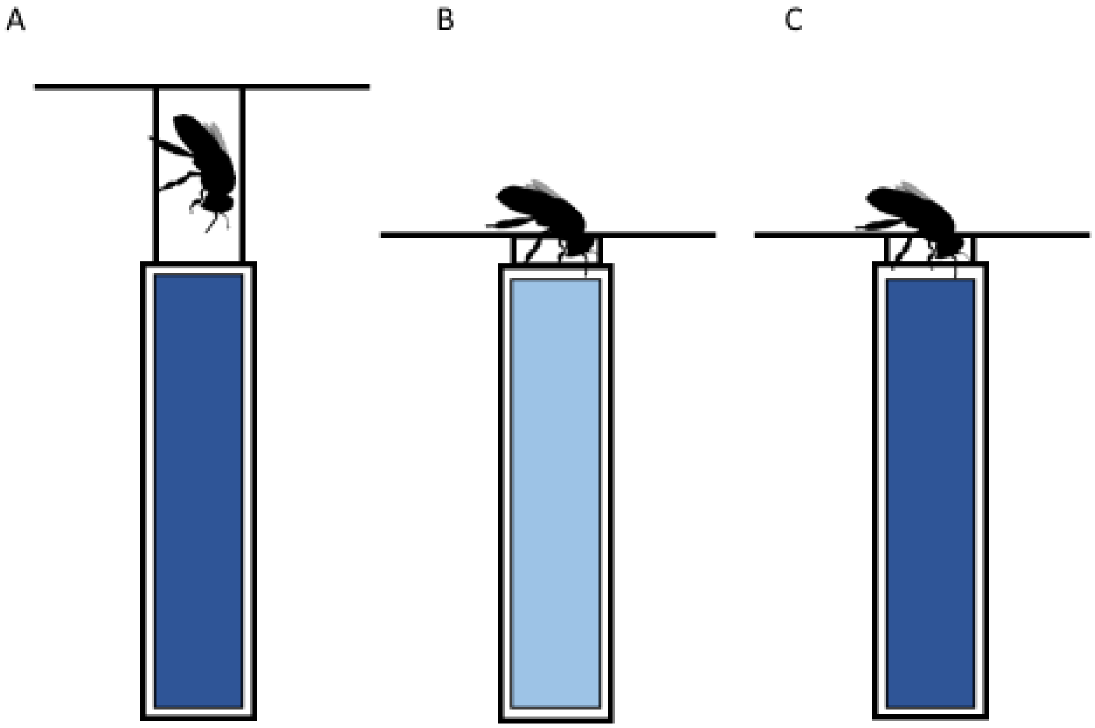

Figure 3.4: Flowers in the choice set. Flower A consists of 50% (v/v) sucrose solution and a 100mm tube, Flower B consists of a 15%(v/v) sucrose solution and a 45mm tube length. Flower C consists of a 50% (v/v) sucrose and a 45mm tube length. Image not to scale

Pre-training to artificial flowers and training bees to forage inside an experimental arena

Honey bees did not automatically recognize our artificial flowers as food sources. We, therefore, used a pre-training step designed to train groups of bees to forage on artificial flowers placed inside our experimental arena. To attract the interest of honey bees, honey bees were trained en masse to gravity feeders containing 7% sugar water that was placed directly at the hive entrance. Gravity feeders consisted of a plastic plate covered in a paper towel. An inverted cup containing sugar water was set on top of the plastic plate that allowed the sucrose solution to progressively leak out onto the paper towel. Individual bees were marked using paint when they arrived at the feeder.

Individuals that made at least five visits to the feeder were then trained to visit artificial flowers. To avoid creating potential experience biases, each artificial flower was coloured 1/3 blue, 1/3 white and 1/3 yellow (See Figure 3.5). This colour patterning was not used during the main training stage. We trained bees to visit artificial flowers by moving individuals from the feeder to the artificial flower using a cotton bud. At this stage, artificial flowers were located a short distance of approximately 5 cm from the feeder. Bees were moved while they were feeding, allowing honey bees to be moved with minimal stress.

Once individual bees were consistently visiting the artificial flowers (after approximately five returning visits) the flowers were moved incrementally into a wooden box (240mm x 400mm x 400mm) with a door (140mm x 130mm) that could be closed upon entering (Figure 3.6). Bees could only enter the box by flying through the door. Once bees finished foraging, the lid was lifted from the top of the box to allow bees to leave the box from the top (Figure 3.6).

Once honey bees were trained to enter the box, they were next trained to associate specific rewards with each of the three flower colours. Individual bees that entered the box were presented with one of the three flower types at a time in a randomised order. Once the bee had fed on all three flowers 5 times, it was considered ‘trained’. Because multiple bees were trained to flower types at a time, some bees might have been more experienced with individual flowers once testing began.

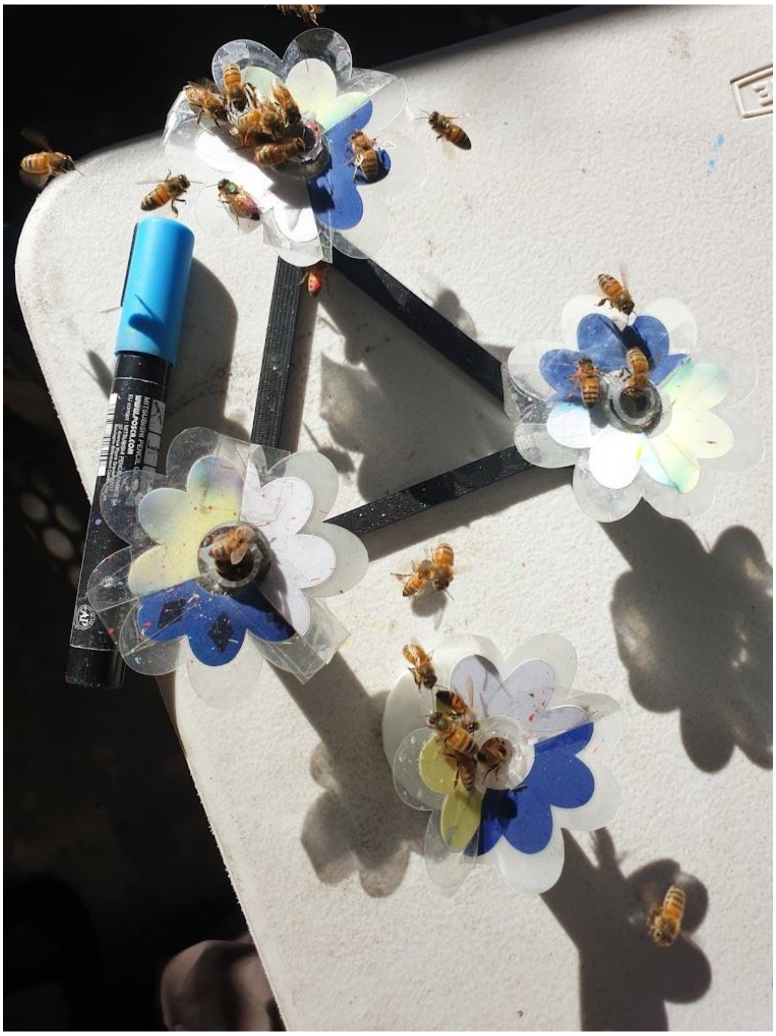

Figure 3.5: Training flowers used to train honey bees to artificial flowers, and for training to enter the box

Figure 3.6: Box used for experiments.

Training bees to associate floral colours with floral attributes

The main training step was designed to train honey bees to associate specific colours with each flower type (A, B and C). Once honey bees were trained to enter the box, they were shown each flower type in a randomised order by placing a single flower type in the box (three of these flowers were placed in the box at a time). When a marked individual entered the box, fed, left the box and returned to the colony, it counted as a visit. To be considered ‘fully trained’, the honey bee had to feed on all three flower types during this training session at least five times. Some honey bees did not visit all three flower types during the training session; these bees were classified as ‘ partially trained‘. Bees may have had experience with some of the flowers, just less than the total five visits to be fully trained. Trained or partially trained was considered a binary variable in the models during analyses (Figure 3.7).

Testing

Flowers were randomly assigned a solid colour each time the experiment ran. We chose blue, white and yellow as colours for the flowers as bees have previously been tested on these and blue white and yellow can be discriminated by bees (Giurfa, 1991; Giurfa *et al.*, 1997; Sanderson *et al.*, 2006). Colours were used as associative cues and were randomised between experiments to prevent any impacts on innate colour preferences.

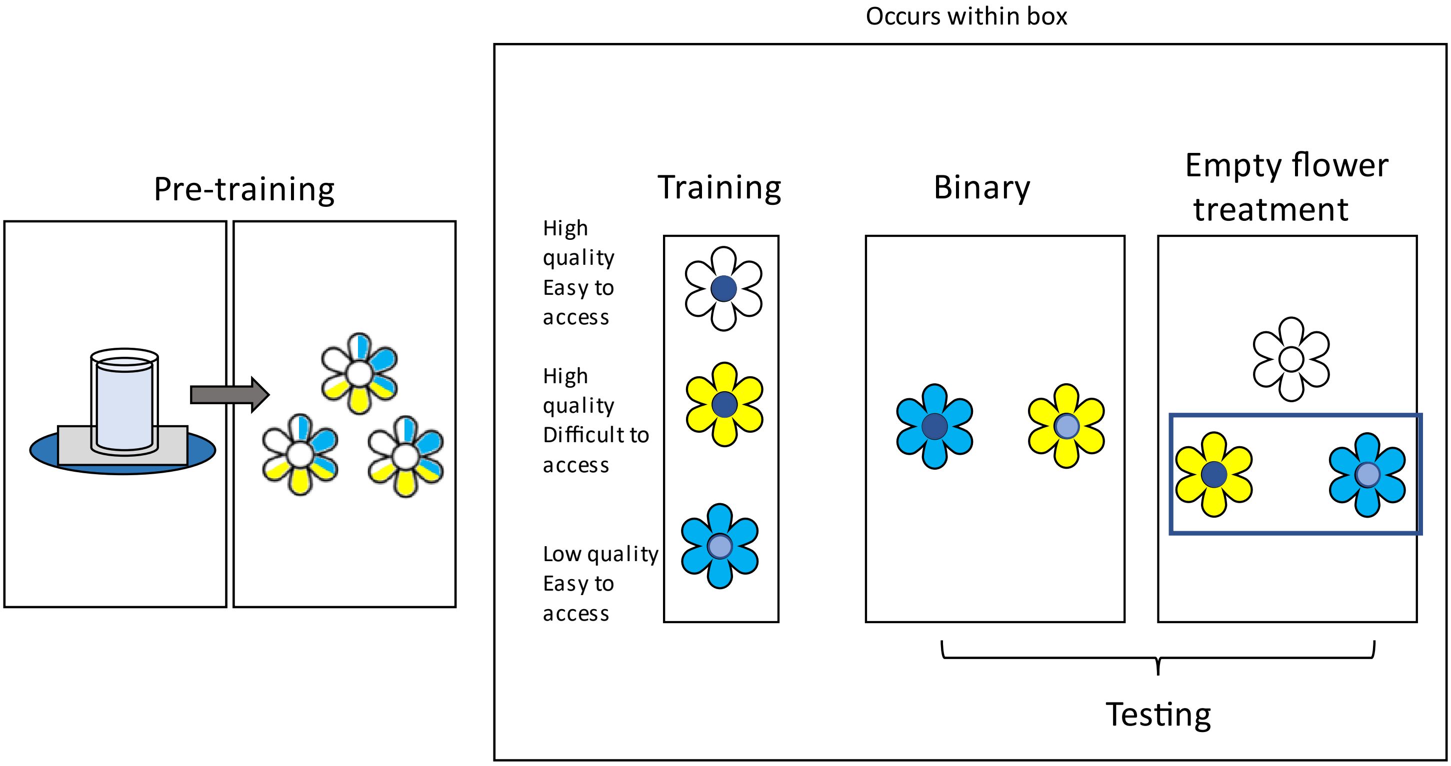

Figure 3.7: Pre-training, Training flowers, and treatments for experiments. Training flowers consist of three flowers: a high-quality, difficult-to-access flower (yellow), a low-quality easy-to-access flower (blue), as well as a high-quality, easy-to-access flower (white). Nectar concentration is denoted by shades of blue, where darker is a higher concentration, and lighter is less concentrated. white denotes no nectar. The binary treatment consists of 2 flowers, one containing a high-quality, difficult-to-access flower, and a low-quality easy-to-access flower. The empty flower treatment consists of a high-quality, difficult-to-access flower, and a low-quality easy-to-access flower, as well as a high-quality, easy-to-access flower with the nectar removed. Note, for experiments, colours associated with flowers were randomised each time an experiment was run.

We tested bees' preferences by letting a single individual honey bee enter a box that contained either the binary or the empty flower treatment. Bees were randomly assigned treatment and were only tested on one treatment. We recorded the identity of every flower the honey bees fed on, including the empty flower. We classified a feeding event as when a bee’s mouthparts made contact with the nectar solution or searched for solution when the empty flower was present for at least one second. Bees were allowed to re-enter the box, and we tested their preferences until the honey bee made 20 visits to the box. Flowers were cleaned with 70% ethanol after use in a foraging bout, and flowers were replaced with new ones for each bout to prevent residual ethanol scent impacting foraging. Each honey bee was only used once for experiments and was frozen at the end of the day to prevent her from returning to the colony. Some honey bees did not return to the flowers before all twenty trials were completed. Honey bees that took more than an hour to return were considered to have ’given up’. They were removed from the colony when they returned to the feeder the next morning to prevent re-entry to the box

Statistical Analysis

We tested the hypothesis that the presence of an empty flower would change preference relationships between the two neighbouring flowers by comparing relative flower preferences when the empty flower was present, to the binary trial when the empty flower was absent. We constructed a generalised linear mixed model (GLMM) using the lme4 package (Bates *et al.*, 2015) with the first flower that each bee fed on (either A or B, not including the empty flower C) during each visit to the box as a binary response variable, and treatment (‘binary’ or ‘empty flower’) and training status (‘trained’ or ‘partially trained’) as fixed effects. Honey bee identity was included as a random effect. The GLMM used a binomial distribution with a logit link function. Model assumptions were checked using the check_model function in the Performance package (Lüdecke *et al.*, 2021). We plotted this as a proportion of all visits by an individual as it was more visually representative of the choices made by bees overall.

We tested the impact of empty flowers on movement between flowers in individual honey bees using a GLMM with a binomial distribution and a link logit function, with the number of flower visits per bout as the response variable, and treatment (binary or empty flower treatment) as the predictor variable. Honey bee identity was included as a random effect. Model assumptions were checked using the check_model function in the Performance package (Lüdecke *et al.*, 2021).

Lastly, we determined if the presence of an empty flower increased the rate of abandonment of the floral patch. We compared the number of completed trials in the empty flower and binary treatments using a Kruskal wallis test (function: kruskal.test( )) , with number of trials completed as the dependent variable, and the treatment as the independent variable. Kruskal-Wallis test was used due to lack of normality in data, which can increase the chances of Type I errors when using single-factor ANOVA tests (McKnight, 2010)
All analysis was conducted using R version 4.1.2 (R Core Team, 2021).

Results

Twenty individual honey bees experienced the binary treatment, and twenty-five experienced the empty flower treatment.

There was no evidence of a change in flower preference in honey bees exposed to empty flowers (Table 3.2), compared to those foraging in the absence of an empty flower. In the binary trials, individual bees visited the long high-quality flower (flower A) 41% of the visits, compared to 35% of the time in empty flower trials. The level of training (trained or partially trained) had no impact on honey bee flower preferences (Figure 3.8; Table 3.2).

Individual honey bees moved back and forth between available flowers more frequently if the empty flower was present (Table 3.3) (Figure 3.9). There was a mean 0.8 (±0.05) flower visits per bout in the binary treatment, and a mean 1.21 (±0.06) flower visits per bout in the phantom treatment**.** When I excluded visitations to the decoy option the treatment effect was no longer significant, suggesting that most visitations were to the empty flower (Table 3.3, Table 3.4). There was a mean of 0.8 (±0.05) visits per bout in the binary treatment and a mean 0.72 (±0.04) visits per bout in the phantom treatment (Figure 3.9).

Honey bees were less likely to finish all 20 foraging visits if they were in the empty flower treatment: $\chi2(1) = 6.767$, p=0.009. On average, honey bees completed 17.9 (±4.12) visits to the arena in the binary treatment and 13.88 (±5.67) in the presence of the empty flower.

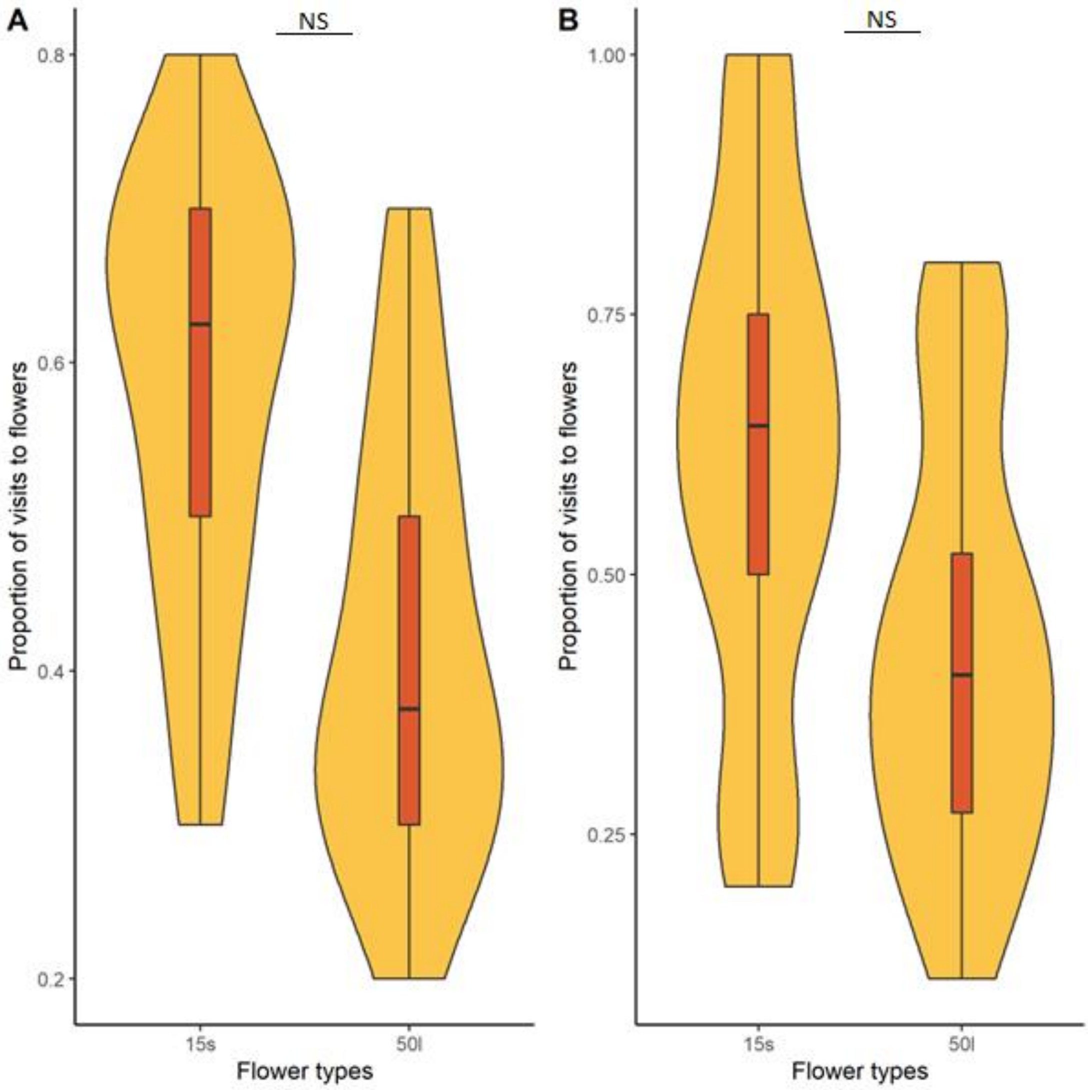

Figure 3.8: Median proportion of first floral visit by individual bees during the test phase in the binary (a) and empty flower (b) treatments (n=45). Violin plot shows distribution of choices made by bees. '15s' refers to the short flower with 15% (v/v) sucrose solution, and 50l refers to the long flower with 50% (v/v) sucrose solution (n=45). Bees made between 5 and 20 visits throughout the experiment. 'NS' refers to non-significant differences between proportion of visits to flowers

Table 3.2: GLMM outputs for preferences by individual honey bees

		First Floral Choice		
Predictors		*Odds Ratios*	*CI*	*p*
(Intercept)		1.40	0.87-2.25	0.166
Treatment [Empty flower]		1.32	0.84-2.08	0.234
Trained		1.08	0.67-1.75	0.750
Random effects				
σ^2	3.29			
$^{T}00$ bee identity	0.28			
ICC	0.08			
N bee identity	45			
Observations	705			
Marginal R^2/Conditional R^2	0.005/0.083			

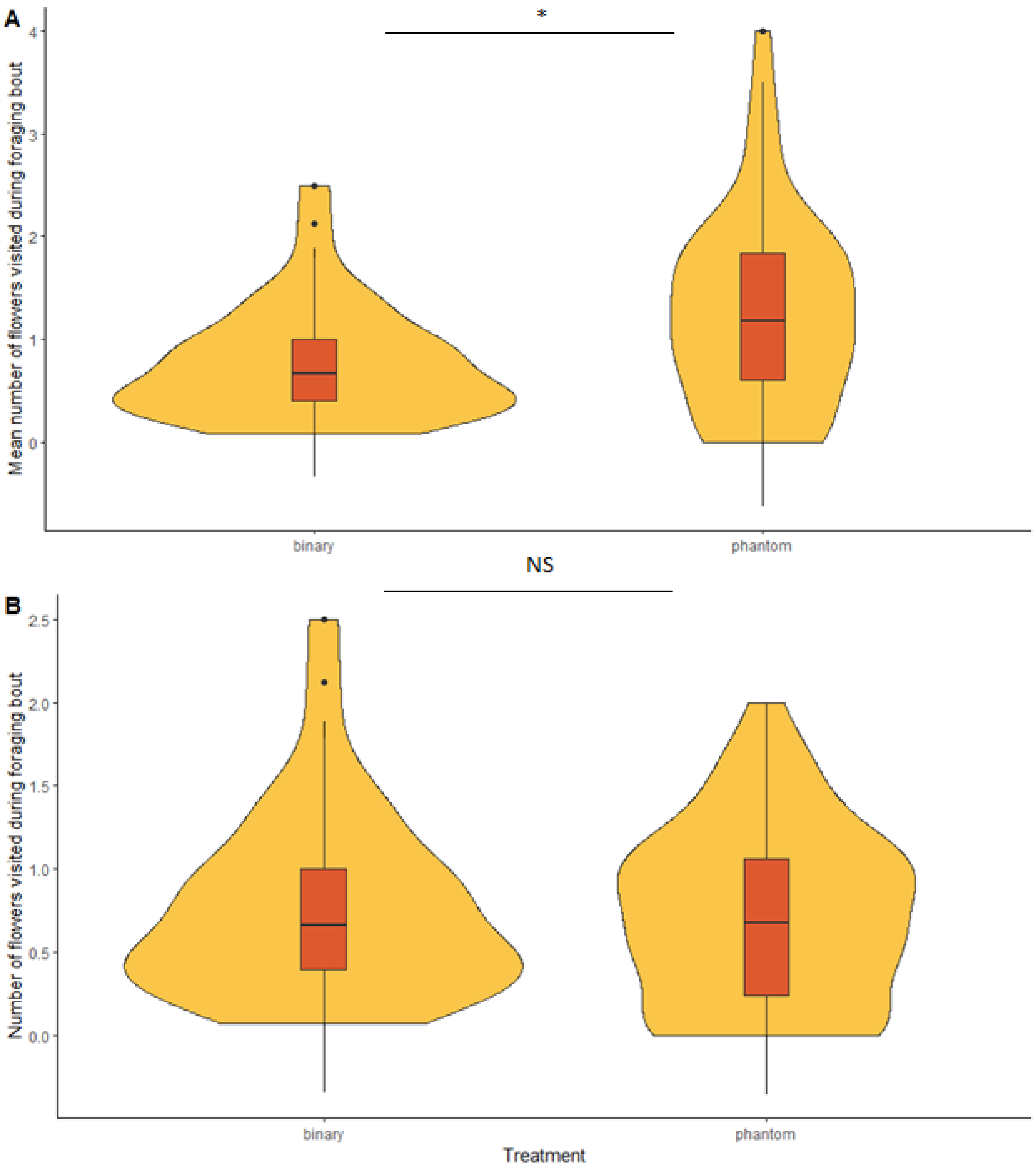
A
*
Mean number of flowers visited during foraging bout
0
1
2
3
4
binary
phantom
B
NS
Number of flowers visited during foraging bout
0.0
0.5
1.0
1.5
2.0
2.5
binary
phantom
Treatment

Figure 3.9: Median number of floral visits by individual honey bees in the presence and absence of an empty flower (A), Median number of floral visits by individual honey bees in the presence and absence of an empty flower, when visits to the empty flower were removed from the dataset (B). Note, that the first landing was not counted as a movement from a flower (n=45). Violin plot shows distribution of choices made by bees. 'NS' refers to non-significant results, and * refers to a significant ($p<0.05$) difference between treatment groups.

Table 3.3: GLMM outputs for the impact of the phantom decoy treatment on the number of floral visits per foraging bout. Asterisks represent significance at $\alpha < 0.05$

		First Floral Choice		
Predictors		*Odds Ratios*	*CI*	*p*
(Intercept)		0.80	0.56-1.16	0.238
Treatment [Empty flower]		1.45	1.03-2.04	**0.036**
Trained		0.86	0.60-1.24	0.430
Random effects				
σ^2	0.75			
^{T}00 bee identity	0.26			
ICC	0.26			
N bee identity	45			
Observations	705			
Marginal R^2/Conditional R^2	0.041/0.288			

Table 3.4 GLMM outputs for the impact of the phantom decoy treatment on the number of floral visits per foraging bout, when the empty flower was removed from the dataset

	First Floral Choice			
Predictors		*Odds Ratios*	*CI*	*p*
(Intercept)		0.77	0.54-1.09	0.137
Treatment [Empty flower]		0.88	0.63-1.23	0.444
Trained		0.92	0.65-1.31	0.649
Random effects				
σ^2	0.91			
^{T}00 bee identity	0.21			
ICC	0.19			
N bee identity	45			
Observations	705			
Marginal R^2/Conditional R^2	0.004/0.194			

Discussion

We tested if Western honey bees, *A. mellifera* were more likely to shift their preferences in the presence of an empty flower. Overall, we saw no change in preferences in the presence of an empty flower. Honey bees, however, were less likely to return to the floral patch in the presence of an empty flower.

Phantom Decoys usually result in the decision-maker picking options similar to the unavailable option. In Asian honey bees (Tan et al., 2015) the presence of decoys resulted in similarity effects. Our results showed that having an empty flower ‘phantom decoy’ that was not similar to either available option, in our case, nectar-filled flowers resulted in no change in preference.

Abandonment of patches containing empty flowers may occur because bees attempt to mitigate the risk of frequently visiting unprofitable patches. Encounters with empty flowers increase the probability that a bee will abandon a patch (Hodges, 1985; Cresswell, 1990; Kadmon and Shmida, 1992; Smithson and Gigord, 2003). While honey bees (*A. mellifera)* often return to unrewarding food sites if they’ve previously proven to be rewarding (Al Toufailia, 2013), multiple authors have reported risk-averse behaviour where bees are less likely to visit patches that contain a high number of empty flowers (Cartar and Dill, 1990; Shafir *et al.*, 1999; Biernaskie, Cartar and Hurly, 2002; Nakamura and Kudo, 2016). The level of risk-aversive behaviour is context-dependent, with bumble bees (*Bombus occidentalis)* partaking in riskier foraging behaviour if their colony food reserves are decreased (Cartar and Dill, 1990). In the context of foraging, empty flowers may play an important role in determining when a bee leaves a patch. The increased rate of patch abandonment in the presence of empty flowers could have an important impact on the reproductive success of neighbouring plants.

We found that movement between flowers occurred more frequently in the presence of an empty flower. Conversely, In *B. terrestris,* between flower movements decrease with increased rates of empty flowers (Smithson and Gigord, 2003). The impact of empty flowers on between-flower movements might depend on the probability that a flower will be empty. Keasar (2000) found that if there is a 50% chance that visited flowers will be empty the next time a bee (*B. terrestris*) visits, bees will move between flowers more than if flowers consistently had nectar (Keasar, 2000). Previously, experiments testing how often flowers were replenished involved testing flowers of the same reward quality. Given we tested the effects of unavailable, but also highly rewarding flowers, returning bees may have been anticipating the replenishment of a high-quality flower. It would be beneficial to test the impact of reward schedules of high-quality flowers on neighbouring flowers.

The increased movement we observed could be due to the presence of a third flower, rather than due to the presence of an empty flower. We did not include a treatment that included all three flowers (including a rewarding flower C) as we were only interested in the effects of empty flowers on foraging behaviour. Nevertheless, our results show that the presence of a previously rewarding flower can increase flower movements and patch abandonment rates compared to treatments where the empty flower is absent. Moreover, we have shown that an empty flower can impact movement rates even though it provides no reward. Future research could include a treatment where all three flowers are present; this would allow for direct comparisons between binary, phantom, and full trinary situations.

Our study aimed to test the effects of empty flowers on foraging choices in honey bees. We found no evidence that empty flowers influenced the preference relationships between neighbouring flowers. Instead, empty flowers appeared to increase between-flower movements and increased patch abandonment. Empty flowers have the potential to impact the reproductive success of neighbouring flowers, by impacting both movement and abandonment rates of floral visitors. Overall, our results suggest that the presence of empty flowers may not influence preference relationships between neighbouring plants. However, more research is necessary to determine if different attribute combinations or degrees of similarity can influence the impact of empty flowers on neighbouring flowers.

Acknowledgements

Bee image was created by Kamil S Jaron

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Chapter 4: The effects of empty flowers on foraging choice in the bumble bee *Bombus impatiens* in a social context

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Abstract

Plants offer rewards such as nectar and pollen to entice flower-visiting insects to visit. Sometimes, previously rewarding flowers become empty due to climatic conditions, time of day or previous floral visitation. When flowers become empty, flower visitors must decide whether or not to abandon the flower or the patch. If the forager decides to remain in the patch, it must decide which flowers to visit next. Previously, Asian honey bees and *Bombus terrestris* have shown evidence of similarity effects, where the bees were more likely to visit flowers more similar to the empty flower. Here we examine the response of bumble bees, *B. impatiens* when they encounter previously high-quality, now empty flowers. We predicted that the presence of an empty, but previously rewarding, flower changes bumble bee preferences for neighbouring flowers in the same patch. Since bumble bees are social foragers, we tested their flower preferences in the presence of nestmates. We found that for the most part empty flower did not affect bumble bees' preference relationships between the remaining flowers in a patch. Rather, their floral choice was influenced predominantly by social information, where bumble bees were more likely to forage on a flower where other bumble bees were physically present. We conclude that while high-quality yet unavailable options can affect the foraging preferences of individual bees, information from conspecifics is a more important driver of decisions in bumble bees.

Introduction

Optimal foraging theory assumes that animals make choices on where and when to feed by trading off between the likely losses and gains of each choice. Optimal foraging theory implies that animals assess each item's relevant attributes before assigning an absolute value to each item in a given set (Rapoport, 1998). A defining feature of absolute valuation strategies is adherence to the principle of transitivity of preferences. Transitivity requires a stable and internally coherent hierarchy of rankings for items within a choice set such that if item A is preferred over item B, and B is preferred over item C, then item A should be preferred over item C regardless of whether all items are available at any one time. Transitivity has been observed during foraging in pigeons (Siemann et al., 1996), chimpanzees (Boysen et al., 1993) and rats (Davis, 1992). Not all animals, however, show transitivity in all contexts. In contrast, animals may show intransitivity of preference, thereby indicating comparative decision-making, where the valuation of options can change due to outside factors. In such cases, animals will rank food sources differently according to which options in a set are available at any one time. For example, when faced with a choice between a short tube containing one raisin, and two raisins in a long tube, gray jays *Perisoreus canadensis* prefer the short tube containing one raisin. Birds then preferred two raisins over three in a medium-length tube. However, this preference reversed when birds were offered three raisins in a long tube versus one raisin in a medium-length tube, with birds always preferring three raisins over one raisin (Waite, 2001). The preference shift from one raisin to three raisins suggests that the birds used a comparative, rather than absolute, decision-making strategy such that the value assigned to the food choices depended on options in the choice set. Crickets (Gabel & Hennig, 2016) and honey bees (Shafir, 1994) have also been shown to have intransitive preferences, suggesting comparative decision-making strategies.

The second defining feature of an absolute valuation strategy is adherence to the principle of regularity. Regularity states that the addition of 'irrelevant' options should not influence the overall valuation of options in a choice set (Luce, 1959). Items can be irrelevant if they are of lower overall quality than the other items in the choice set, or are unavailable at the time of choice. Items that are unavailable at the time of choice are known as 'phantom decoys'. While few non-human animals have been tested for the effects of phantom decoys, cats, túngara frogs, and Asian honey bees *Apis cerana* have all been shown to shift preferences in the presence of a phantom decoy (Lea & Ryan, 2015; Scarpi, 2011; Tan et al., 2015). Typically, phantom decoys result in a similarity effect, where the option chosen is more similar in attribute space to the unavailable option (Tan et al., 2015).

Phantom decoys may exist in the real world, and hence, be relevant to natural decision-making. Flowers, for example, can act as phantom decoys when they are empty of nectar. Some flowers entice potential pollinators to visit by offering nectar as a reward. However, not all flowers produce nectar, and many flowers have peak times for nectar availability or may run out of nectar following frequent visitations throughout the day (Chalcoff et al., 2006). The presence of empty flowers can impact how bees choose to forage, resulting in reduced visitation to the plant (Smithson & Gigord, 2003). When empty flowers are abundant, bumble bees tend to avoid flowers of similar colour to the empty flower, even when those flowers belong to different species. (Smithson & Gigord, 2003).

Eusocial insects such as honey bees, bumble bees and stingless bees use social signals such as scent marks and visual cues to recruit nestmates to rewarding flowers. Except for ants (Edwards & Pratt, 2009), studies testing the effects of decoys on animals have typically been conducted on individuals in isolation, even when the animals are social species (e.g. honey bees). Yet the broader social context is an important influence on decision-making in many social and eusocial species. Public information refers to information that is available to individuals in an environment without prior individual knowledge (Cruz et al., 2019). Private information relies on the individual learning the information by direct means, such as the nectar quality of a flower. It can then be used to provide social information to a broader set of potential receivers. For example, scent marks can be detected by foraging bees, which may allow foragers to learn which flowers have been visited, and therefore empty (Goulson et al., 1998).

Social information can impact the effectiveness of decoys. *Temnothorax* ants when searching for new nests individuals choose nests most similar to decoys (Edwards & Pratt, 2009). However, while searching in groups, the effect is not seen. Tests on individual eusocial bees have shown that foraging is impacted by decoys (Shafir et al. 1994; Tan et al., 2015). In a real-world foraging context, it is likely, however, that individual bees will forage based on social information from nestmates and other foraging bees.

In this study, we determined whether or not bumble bees are susceptible to phantom decoy effects in the presence of an empty flower. Since we were interested in the potential ecological consequences of the phantom decoy effect, we tested bees in a realistic social environment where communication and feedback between individuals were possible. We also examined the impact of individual experience on the emergence of decoy effects. We predicted that individuals that had been foraging on all of the floral options multiple times would have more experience with the floral attributes than the more naïve bees. Experience of the high-quality flower while nectar was present as well as the other floral options, would likely result in experienced bees being more likely to be impacted by the decoy than bees that had only experienced a limited number of options due to reduced experience with all of the choices in the set. We predicted that :

(1) Experienced bumble bees would be more susceptible to the effects of empty flowers on choice in comparison to less experienced bumble bees, picking the flowers more similar to the empty flower, and

(2) Bumble bees would likely visit flowers containing more individuals.

Methods

Bumble bees, *Bombus impatiens*

We tested the foraging preferences of the Eastern bumble bee, *B. impatiens*. *B. impatiens* are a generalist commercially available pollinator, used across North America and Mexico. They are naturally distributed across the eastern side of Canada. Colonies typically contain up to 300 individuals but are often purchased in quantities of as few as 50 individuals.

Colony set-up

We conducted experiments using three commercially sourced, medium-sized colonies of *B. impatiens* (Natupol | Koppert Products, no date), each containing approximately 70 individuals. We fed bees with fed pollen from a glass container lined with pipe cleaners, which were sprinkled with crushed honey bee pollen. We provided a 15% (w/w) sucrose solution *ad libitum* via white-coloured artificial flowers. We carried out all experiments in a portable plastic greenhouse, with dimensions of 1480mm x 1460mm x 1950 mm (Rona) situated near open fields at McMaster University, Ontario, Canada. Colonies were kept at ambient temperatures outside the greenhouses. We placed a tube at the colony entrance and connected it to the greenhouse, to allow bumble bees to enter during trials. We tested bees between temperatures of 25 and 35°C, and under ambient daylight. We conducted experiments between the 19th- of July and the 20th of August 2019. All experiments occurred between 9 am to 5 pm EDT.

Artificial flowers

We followed the standard protocol for detecting phantom decoy effects used in previous studies, whereby we compared the relative preference for one of two options (A and B) when the decoy (C) is absent versus when it is present (Trueblood & Pettibone, 2017). If animals are using absolute valuation strategies, then the presence of a phantom decoy will not have an impact on their relative preferences for the two non-decoy options (Table 4.1).

We used artificial flowers to test if bumble bees showed a change in preference between two flowers when in a binary treatment, where a pair of flowers was present or in an empty flower 'phantom decoy' treatment, where three flowers were present, and one previously high-rewarding flower is available but empty of nectar. Artificial flowers consisted of a laminated daisy-shaped flower with a 5 ml specimen tube in the centre to hold sucrose solution (Figure 4.1). We glued specimen tubes to a dowel which was attached to a wooden block to hold flowers.

During experiments, we exposed bumble bees to a flower set consisting of three flowers, flowers A, B and C (Table 4.1), which varied in two attributes that were predicted to affect flower quality: sugar concentration and the accessibility of nectar. To allow bees to learn which flowers had these attributes, we assigned flowers a unique colour and pattern which was randomised each time a trial was run. We randomised colours and patterns to prevent biases due to the effects of innate preferences for particular patterns and colours on preferences made by bumble bees.

In pilot trials, we found that bumble bees preferred flowers with a cotton ball on top, likely because they made nectar more accessible. Based on pilot data, we designed flowers A and B to be similar in attractiveness to foraging bumble bees. Flower A contained a lower sugar concentration (40% w/w) but had a cotton ball to make the sucrose solution more accessible, while flower B contained higher (45%) concentrations of sucrose solution but had no cotton ball. Flower C, which was used as the empty flower, had both a high sugar concentration (55% sucrose solution) and a cotton ball present and thus was expected to be the most preferred flower type when all three flowers contained nectar (Table 4.1). We predicted that by removing the nectar from the highest quality flower (flower C), bumble bees would shift their preferences toward flower A as the accessibility of nectar (presence of a cotton ball) is the same as the high-quality, flower C, making it more visually similar to the empty flower (Table 4.1).

Briefly, we confirmed the discriminability of our six floral colours to bees by using spectrometry to record the reflectance of cardboard, before estimating their colour difference using the colour hexagon model of Chittka (1992), with the visual phenotype of honeybees (Maia et al. 2019). All three stimuli were separated by distances well above documented absolute discrimination thresholds (Supplementary Figure 2), and so should be readily separable by our focal bees.

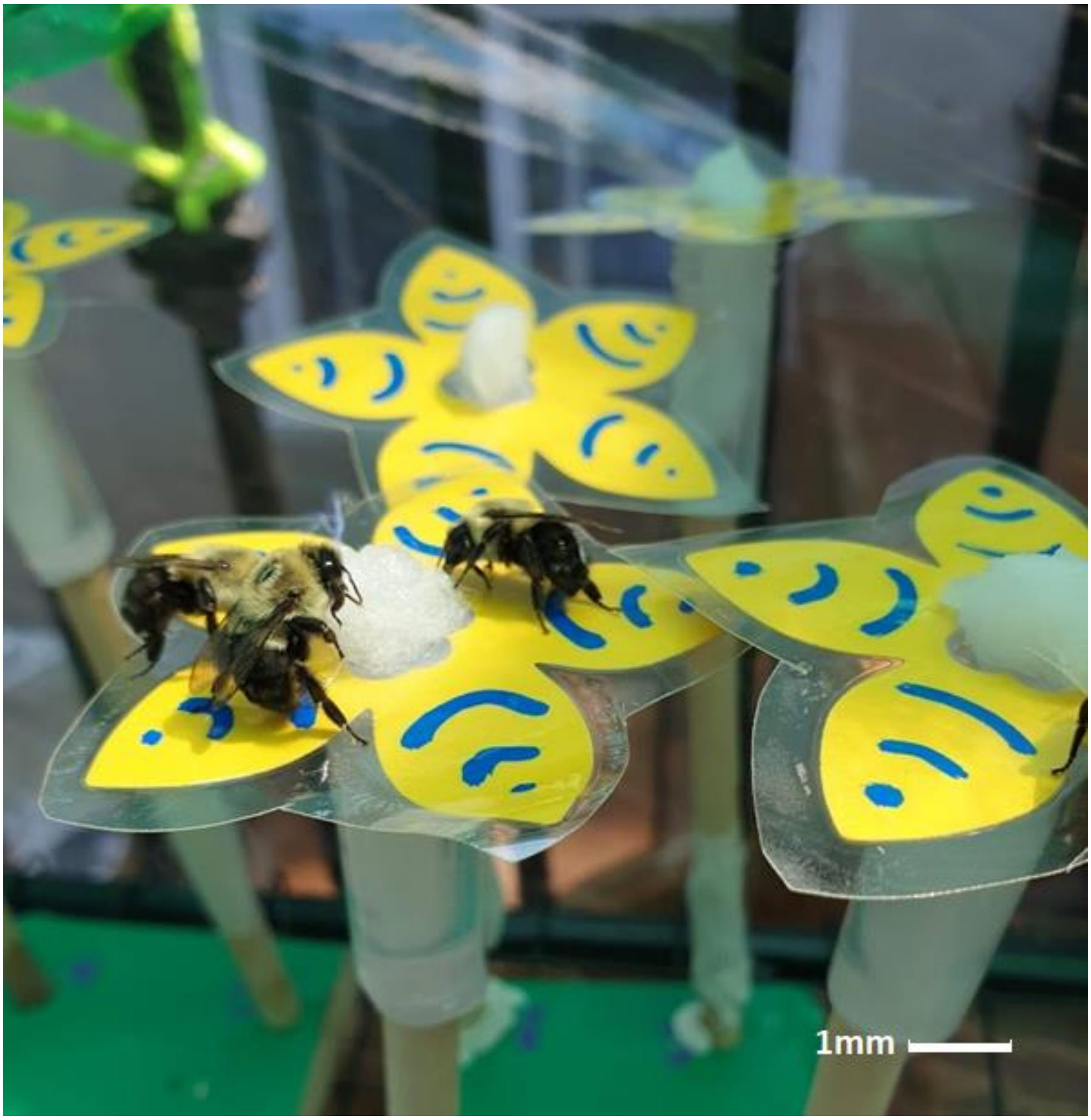


Figure 4.1: Bumble bees foraging at artificial flowers used for experiments. These flowers are an example of flower A.

Table 4.1: Flower attributes in different treatments used in the experiment

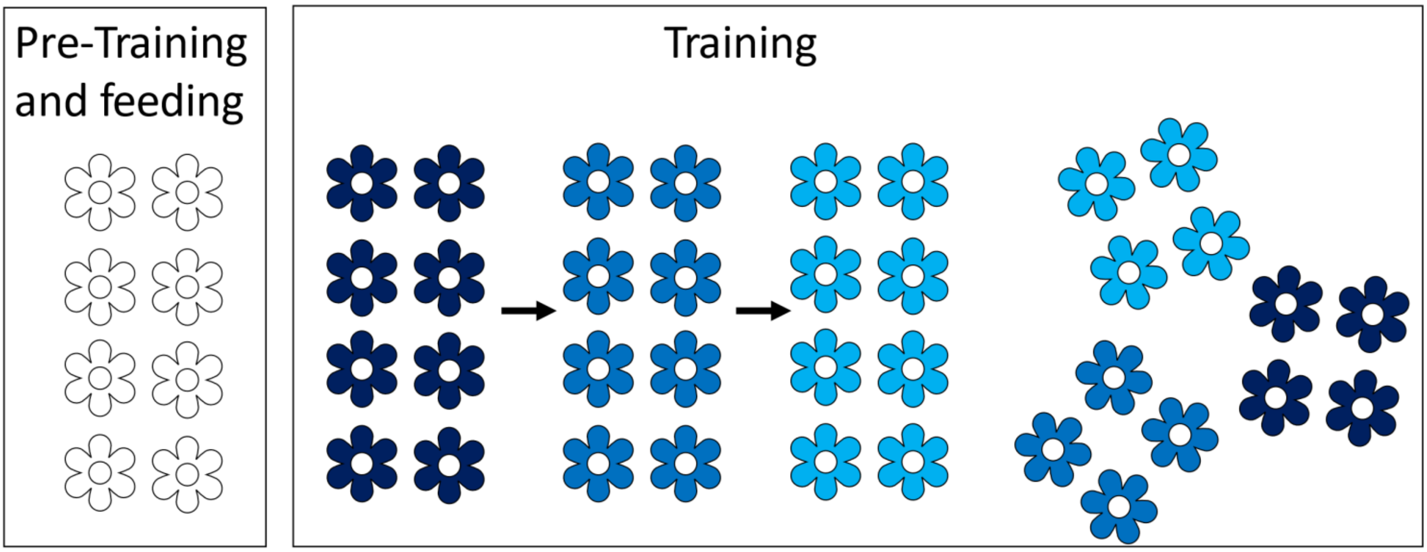
Treatment	Experimental phase	Flowers	Sugar Concentration	Cotton ball
Ternary	Training	A	40% (w/w)	Present
		B	45% (w/w)	Absent
		High-quality flower	55% (w/w)	Present
Binary	Testing	A	40% (w/w)	Present
		B	45% (w/w)	Absent
Phantom Decoy	Testing	A	40% (w/w)	Present
		B	45% (w/w)	Absent
		Empty flower	No sucrose solution	Present

Pre-training and training to flower types

We trained individuals to recognise artificial flowers and their associated attributes by placing flowers in a greenhouse approximately 1.5 m above the ground in patches of four. Each flower type (A, B or C) was associated with a different shade of either blue or yellow. To ensure colour preference did not skew preferences for flower types, a single trial consisted of a randomised selection of either three shades of yellow flowers, or three shades of blue flowers. We used reflectance spectrometry and visual modelling to ensure flowers could be discriminated against by bees. Briefly, this entailed quantifying the reflectance of blue and yellow papers using an OceanInsight JAZ reflectance spectrometer with pulsed PX-2 Xenon light, calibrated against a 99% diffuse white and 0% dark standard (Labsphere, New Hampshire). We then modelled the location of each sample in the hexagon colour space using the visual phenotype of the bumble bee *B. terrestris,* and selected shades that were separated by a Euclidean distance of at least 0.11; the absolute discrimination threshold for honey bees (Chittka, 1992; Maia et al., 2019). Bees can show constancy to colours before they learn about the qualities of other flowers in a choice set (Hill, et al., 1997). While constancy can be overridden by reward quality (Grüter et al., 2011), flowers A and B were designed to be of similar value to each other. Bees show less constancy when flowers are similar in colour (Dyer and Chittka, 2004).

We started a training session by placing a set of nine flowers of a single type into the greenhouse on a shelf approximately 1.5 metres from the ground. Bumble bees were then sequentially exposed to all three flower types (A, B, C) one at a time, plus a final training phase with all three flower types together (Figure 4.2). The order in which bumble bees experienced flower types was randomised. We recorded and individually marked all bees that landed on each flower with coloured paint pens, the colour combinations of markings were used to identify individuals. Bumble bees were allowed to forage freely from the flowers until at least 10 individuals had visited each flower at least 5 times. Some bees visited flowers more than five times in the process to train more individuals to flowers. It usually took approximately 45 minutes for a sufficient number of bees to visit each flower type at least five times and therefore be considered trained. It usually took 2.5 hours for bees to be trained to each individual flower type, and complete the 45-minute preference recording containing all three flowers. If training was not complete by 3 pm, training was resumed the next day. If experiments were conducted over two days, bees were given a ‘refresher’ at the beginning of the session where they were exposed to each flower type for 30 minutes. There were four occasions where a refresher was necessary.

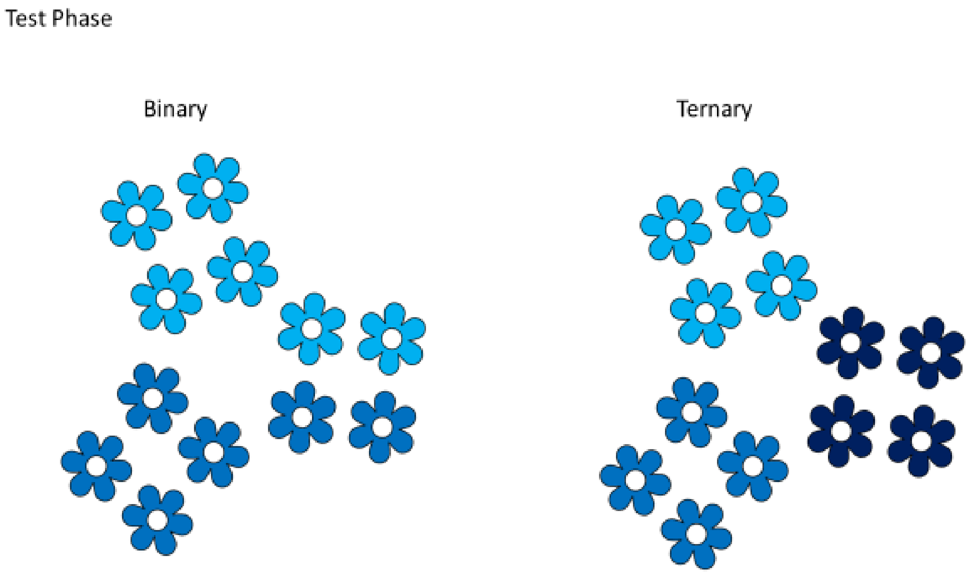
Figure 4.2: Experimental set-up for pre-training phase. Bees were originally fed on plain white flowers. During the training, bees were introduced to three different sets of flowers in a randomised order to train bees to visit a patch with the different flower types. Bees were considered trained after ten individuals had visited each flower type five times. Bees were then allowed to feed on all flowers together, offered in groups of four for half an hour. The number of visitors to each flower type was recorded. When experiments were conducted on a colony that had already been tested, we used a different colour and new pattern for the flower set.



Test phase: Choice trials

In the choice trials, we either presented bumble bees with a binary set containing flowers A and flower B (Table 4.1) or an empty flower set containing flowers A, B and C, with C being empty of nectar and so acting as the phantom decoy. We randomised the order in which bees received the two treatments. All choice sets (binary, training and empty flower treatments) consisted of the same total number of flowers (Figure 4.3).

We started trials by placing all three flower types approximately one metre away from the tube that allowed bumble bees to enter the greenhouse. The trial began once we had placed all flowers in the choice set. A visit was counted if a bumble bee landed and fed on the nectar available. When a bumble bee landed, we recorded its identity based on paint markings, the flower it landed on as well as the number of bees currently present on the flower at the time of visitation. Each trial lasted 1 hour. In total eleven trials were completed by 246 bees from three colonies. All bumble bees were allowed to forage throughout the experiment, as we intended on testing if social behaviour was important in foraging choice.



Test phase consisted of one hour of foraging on the binary choice set, and one hour on the ternary choice set in a randomised order.

Figure 4.3: Test Phase. Bees were introduced to 12 flowers which contained 2 groups of flowers in the binary treatment and 3 groups of all three flowers, with the empty flower (coloured in the darkest blue) flower empty of nectar during the empty flower treatment

Statistical analyses

Testing the attractiveness of the high-quality flower

We first confirmed that Flower C was the most preferred of the three flowers while it still contained nectar using Welch's ANOVA with the independent variable being the flower types (A, B or C), and the dependent variables being the number of visits to each flower by individual bees. A Welch's ANOVA was used as the data were not normally distributed. We used a Dunn's posthoc test to establish if the Flower C was most visited overall during the 30-minute training when all flowers were present and full of nectar.

Bees that foraged after the training period ended were not marked. Unmarked bees (2935 of 10109 visitation observations) were removed from the following analyses as we were unable to distinguish between individuals. There was no difference in the flower preference of bumble bees when unmarked bumble bees were excluded (data not shown).

The effect of empty flowers on foraging preferences of bumble bees

We determined if bumble bees changed their preference for flowers A or B in the presence or absence of an empty flower. For all analyses, we set flower choice as a binary choice, with flower A assigned as the target flower. Flower choice referred to the flower that an individual landed on during a foraging visit. To test the effect of the empty flower on floral choices by individual bumble bees we used a generalised linear mixed model (GLMM) with binomial error distribution with a link logit function (Table 2). We specified floral choice (either flower A or B) as a binary response variable. We included treatment (binary or empty flower), level of experience (experienced/naïve), and the number of bumble bees (explained below) present on the flower at the time of visitation as fixed effects, and individual bumble bee identity nested within the date as a random effect. The trial order for each colony was also included as a fixed effect to establish if retesting individuals within the colony resulted in changes in preference over time. GLMMs were created using the lme4 package (Bates et al., 2015) in R version 4.1.2 (R Core Team, 2021).

Experienced forager analysis

We were interested in testing if bees that were experienced with the flower types were more likely to change their preferences in the presence of an empty flower than those without foraging experience. Bumble bees considered experienced had to have visited all three flower types at least five times during the training sessions, and then needed to visit the patch of flowers at least ten times during the binary and empty flower treatments. We tested whether experienced bees were more or less likely to have significant preferences for Flower A or B between binary and empty flower treatments using a GLMM with a binomial distribution. We specified flower choice (A or B) as a binary response variable and included treatment (binary or empty flower treatment), trial number and the number of bumble bees present on the flower at the time of visitation as predictor variables. Bumble bee identification nested within the date of experiments was specified as a random effect.

We then tested if overall visits by each trained individual bee resulted in a preference for a flower type. To determine if individual bumble bees in the experienced subset significantly preferred flower A or B, we tested if the bumble bee visited flower A or B more than expected by chance using a binomial test, where the expected probability was 0.5. If the binomial test was significant, we classified the bumble bee as having a ‘preference’. Bees that showed no significant preference were classified as ‘indifferent’. We also corrected for multiple comparisons using Bonferroni corrections.

Results

We recorded visits by 246 individually marked bumble bees during test phases, with 27 considered ‘experienced’. Overall, 132 individual bees visited the empty flower during the test phase, including twenty-three of the twenty-nine experienced bumble bees.

Testing the attractiveness of the high-quality flower

Overall, bumble bees showed a significant preference for flower C (55% cotton ball; Welch’s test, $F_{2, 229} = 35.382$, $\alpha < 0.001$), thereby confirming a preference for the decoy when it contained nectar. Individuals had similar preferences for the remaining two flowers, A and B (Figure 4.4). Bumble bees were able to visit multiple flowers in a bout, which were also included in determining the overall preference by bumble bees.

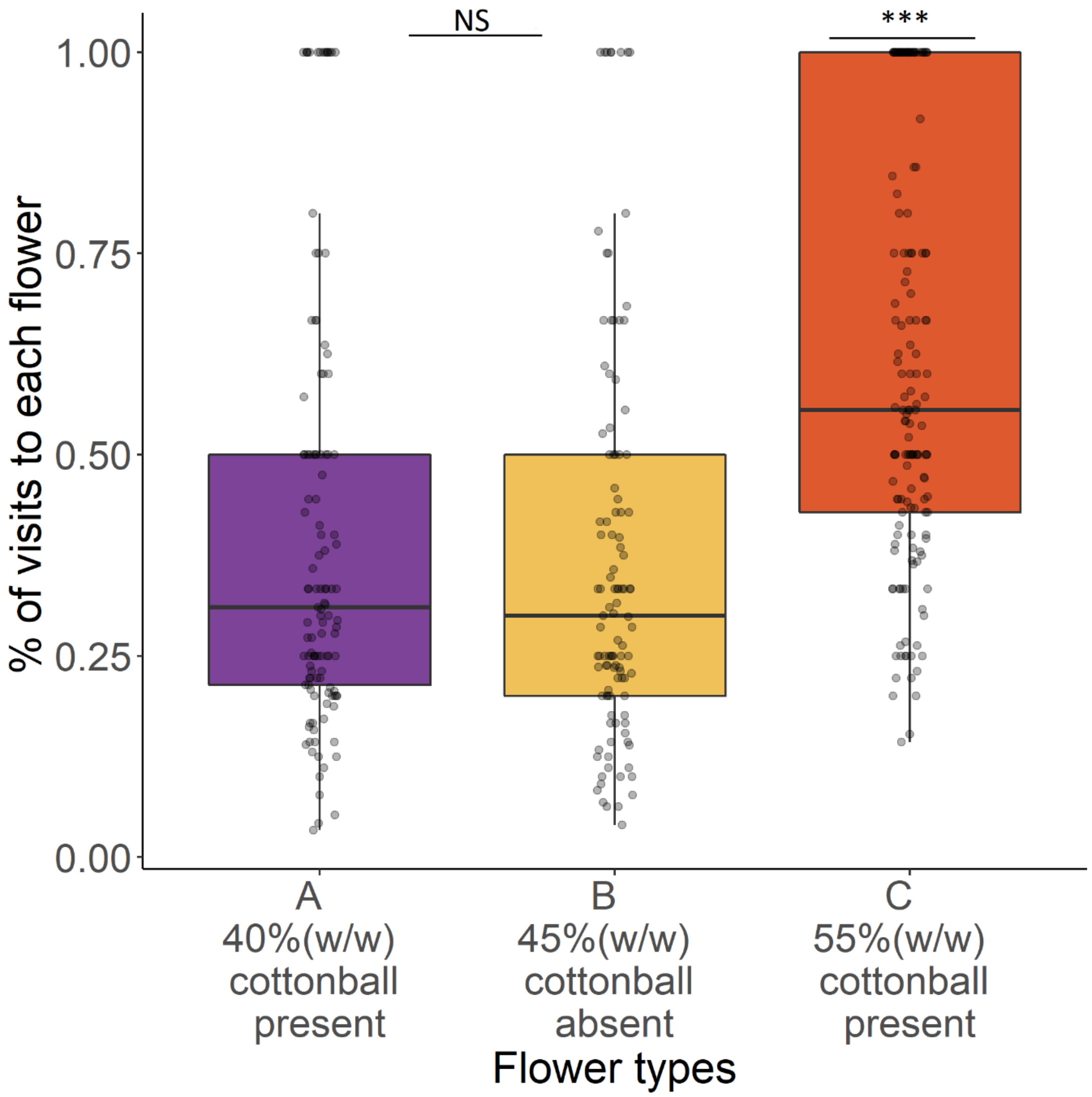


Figure 4.4: The proportion of visits to all flower types during the 30-minute training set (n = 183). Flower A (purple) refers to the flower with 40% sucrose and a cotton ball, flower B (yellow) refers to the flower with 45% sucrose and no cotton ball, flower C (orange) represents the flower with 55% sucrose and a cotton ball. Black lines refer to the median percent of visits. Error bars refer to standard error, with the median proportion of visits represented by horizontal lines in the boxplot. Asterisk refers to significant difference in the proportion of visits between flower types. 'NS' refers to non-significant effects.

Effect of empty flowers on foraging preferences in groups of bumble bees

The presence of an empty flower did not affect bumble bees' relative preference for flower A. Inexperienced bumble bees, however, were more likely to visit flowers that already had conspecifics feeding in comparison to experienced bumble bees (Table 4.2, figure 4.5). This did not hold for the experienced subset of bees whose choices were not influenced by the number of bees already foraging on flowers (Table 4.2).

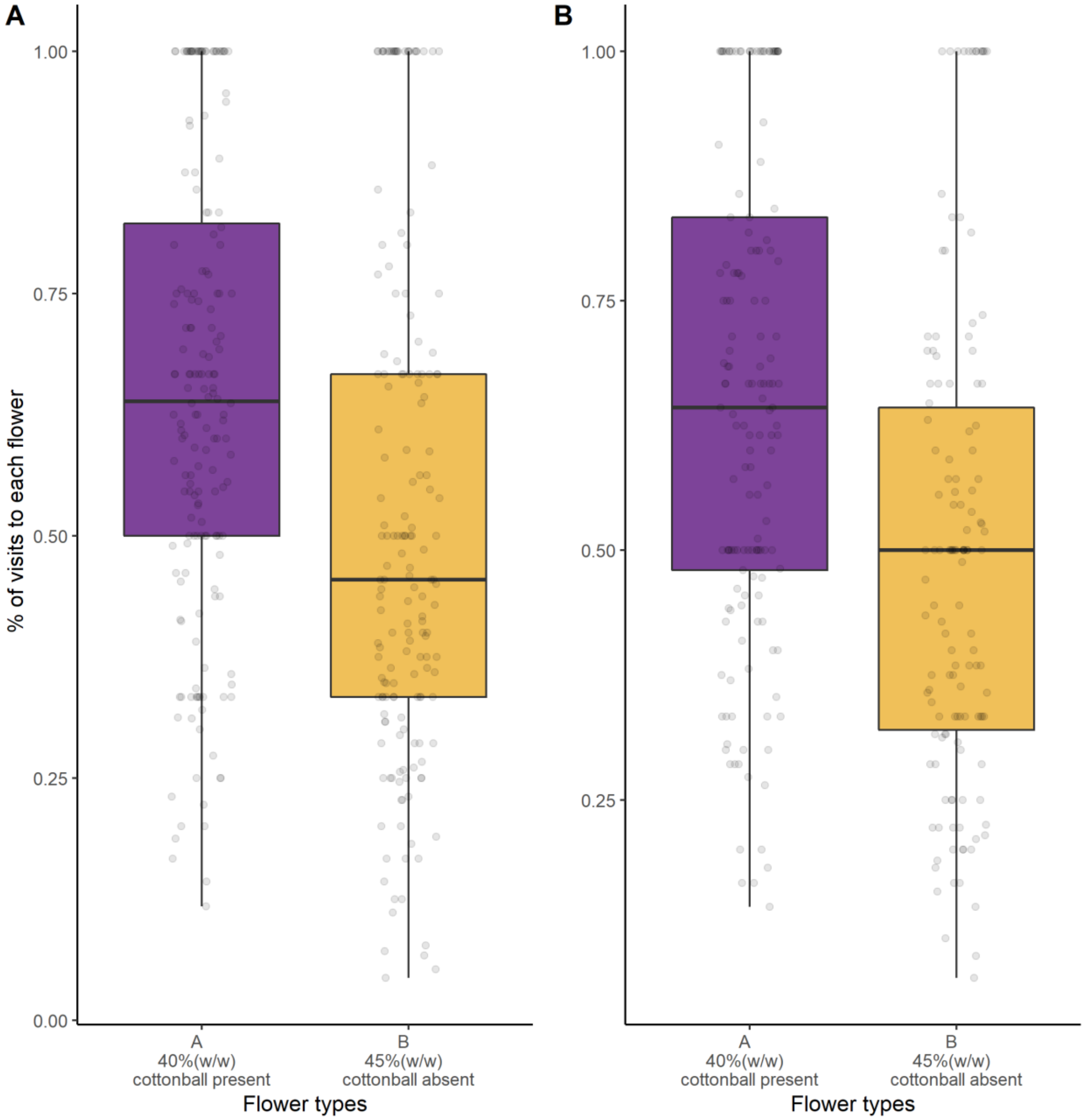


Figure 4.5: Proportion of visits to flowers in the binary (A), and empty flower treatment without (B) for *all* bees (N = 246). Flower A refers to the flower with 40% sucrose and a cotton ball, and Flower B refers to the flower with 45% sucrose and no cotton ball. Flower A (purple) refers to the flower with 40% sucrose and a cotton ball, and flower B (yellow) refers to the flower with 45% sucrose. Error bars refer to standard error, with median proportion of visits represented by horizontal lines in the boxplot. There was no significant flower choice made by bees in either treatment.

Table 4.2: GLMM output for the effect of treatment on flower choice by bees. Predictor variables are treatment, whether or not bees were experienced, the number of bees present on a flower when a new bee landed and the order in which bees were exposed to treatments. Asterisks represent significance at α < 0.05. Variance of random effects: Bee identity: 0.215, Date: 0.001.

Parameter	Estimate	Standard Error	Z	P
Intercept	0.168	0.241	0.696	0.486
Treatment	0.097	0.689	1.407	0.159

Experience	-0.078	0.121	-0.644	0.519
Number of bees present	0.168	0.050	3.361	0.000***
Treatment order	0.301	0.211	1.429	0.153
Trial Number	0.005	0.062	0.083	0.933

Experienced bee analysis

The presence of the empty flower did not affect preferences toward flower A (Figure 4.6; Table 4.3). The flower choice of experienced bumble bees was not impacted by the number of individuals on the flower before landing. Treatment order did impact the floral choices bees made. Bees were more likely to pick flower A if the empty flower treatment occurred before the binary treatment.

Of the 27 individual bumble bees that were considered experienced and had visited flowers in each treatment at least ten times, 12 initially showed a preference shift between binary and empty flower treatments. With Bonferroni corrections for multiple comparisons, this was reduced to five individuals (Supplementary Table 2). Of these five individuals, one moved from a preference for flower B in binary trials to no preference (in empty flower trials), one from no preference (binary trials) to flower B (empty flower trials) and three from no preference (binary trials) to flower A (empty flower decoy trials).

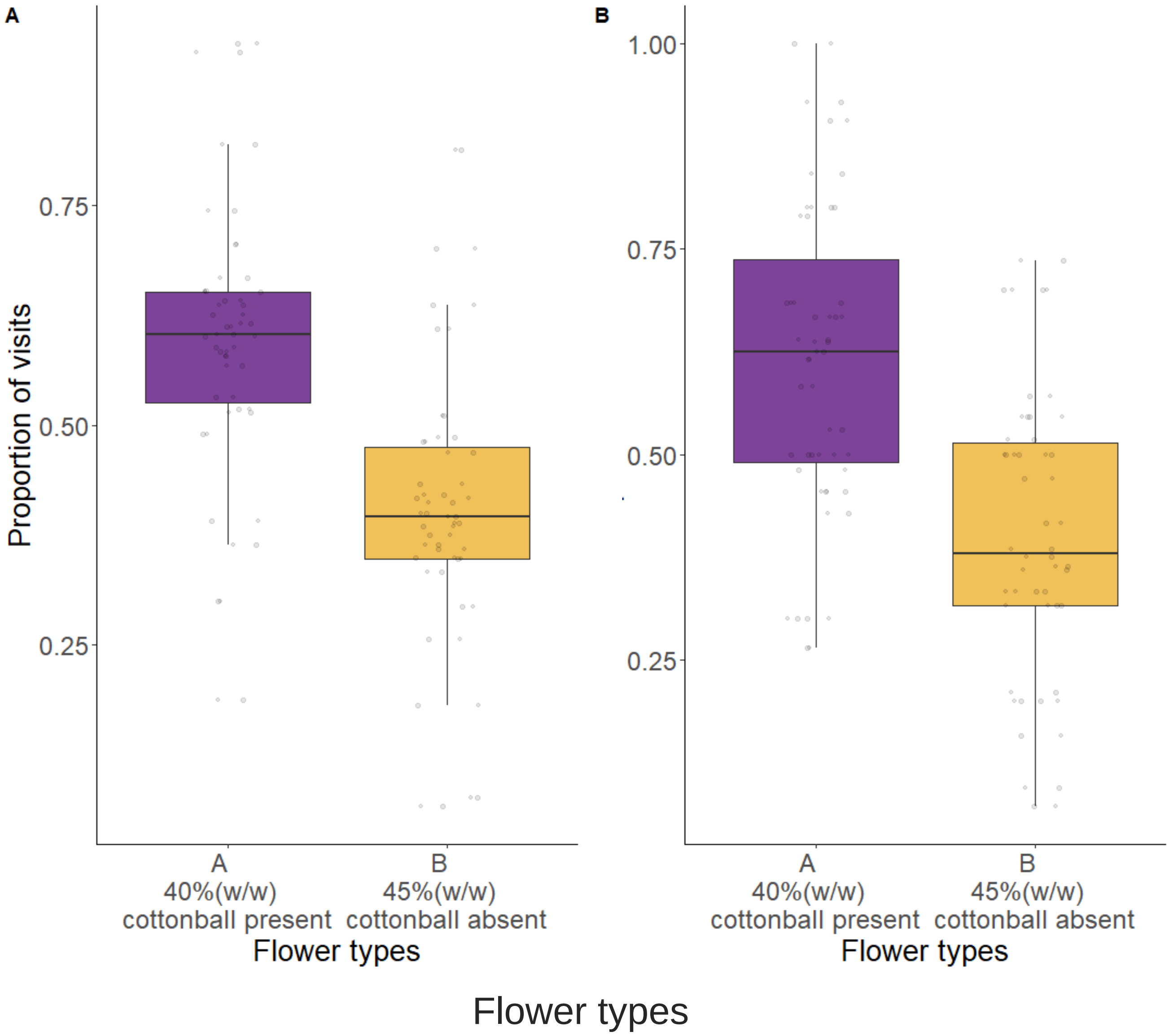


Figure 4.6: Proportion of visits by experienced subset to flowers in the empty flower (left) and binary treatments (N= 29). Flower A refers to the flower with 40% sucrose and a cotton ball (purple), and Flower B refers to the flower with 45% sucrose and no cotton ball (yellow). Error bars refer to standard error, with the median proportion of visits represented by horizontal lines in the boxplot. 'NS' refers to a non-significant difference

between visits to flowers. There was no significant flower choice made by bees in either treatment group.

Table 4.3:

GLMM summary for the effect of treatment on flower choice by the experienced subset of bees (n = 27). Predictor variables are treatment, the number of bees present on a flower when a new bee landed and the order in which bees were exposed to treatments. Asterisks represent significance at α < 0.05. (Conditional R^2: 0.092, Marginal R^2: 0.011).

Parameter	**Estimate**	**± Standard Error**	**Z Value**	**P-value**
Intercept	-0.425	0.224	-0.190	0.849
Treatment	0.079	0.114	0.694	0.487
Number of bees present	0.105	0.090	1.172	0.241

Treatment order	0.693	0.221	3.135	0.001**
Trial Number	0.046	0.061	0.751	0.452

Discussion

We tested the impact of empty flowers on foraging choice using bumble bees in a foraging task that provided an opportunity for social foraging. A small subset of experienced individuals changed their preferences in the presence of an empty flower, suggesting there is a small possibility phantom decoys could impact some individuals. We found that social context — the number of conspecifics foraging on a flower — was predictive of an inexperienced individual's choice. Social context no longer had an effect when considering only the subset of trained foragers, whose choices appeared unaffected by the presence of other foragers.

While there was no overall impact of phantom decoys at the population level, five of the twenty-seven individual bees in our study showed a significant switch in preference in the presence of the decoy. For example, one bee had no preferences in the binary treatment but preferred flower B in the empty flower treatment, while another bumble bee preferred flower A in the binary but had no preference in the empty flower treatment. Similar individual-level preference switches have been observed in *Apis cerana,* where the presence of an empty flower caused individual bees to pick flowers more similar in attribute space to the empty flower, there was some variation in individual preferences observed (Tan et al., 2015).

Similarly, hummingbirds showed individual-level variation in their direction of preference for artificial flowers when an empty third option was added, with 4 of the 11 birds changing their preference in the predicted direction, and 5 of 11 changed their preferences in the opposite direction (Bateson et al., 2003). Taken together, the literature (and our work) suggest that some individuals might be more susceptible to decoy effects than others. Individuals may have had different innate preferences, sensory biases or levels of experience with the foraging choice that could have impacted how they chose which flowers to forage on. Our results only show a small number of preference shifts (five out of twenty-seven individuals), so the impacts of decoys in this experimental context should be interpreted with caution. A larger study may be necessary to understand the variation in decoy susceptibility.

Bees, including bumble bees, also rely on visual local enhancement, where the presence of nestmates can impact visitation to flowers (D'Adamo et al., 2000; Slaa et al., 2003; Sommerlandt et al., 2014). Our results show that bumble bees used group foraging to make decisions as to which flower to visit. We did not separate individuals to understand their preferences in the absence of social information. To identify how foragers respond to empty flowers in the absence of social information, it would be necessary to test individuals one at a time. However, our experiment provided the opportunity to understand foraging responses to empty flowers in an ecologically-relevant social setting.

Our empty flower was a style of decoy where, the attributes of the flower were where its attributes were more similar to flower A, because it had a cotton ball present, as did flower A. Previously, animals have chosen items in choice sets in the presence of phantom decoy effects using similarity effects, where individuals are more likely to pick the more similar item to the unavailable one (Scarpi, 2011; Tan et al., 2015). However, the realisation that a preferred item is now unavailable can also result in more loss-averse individuals avoiding unavailable options, or picking options less related to the unavailable one (See (Highhouse,

1996) for example). Given a small subset of experienced bees shifted their preferences, it may be necessary to test the impact of phantom decoys using different attributes on individual bumble bees to untangle to decision-making strategies used by this species.

Individual bees may be influenced by foraging experiences from early on in their foraging ‘career’ which may impact individual decisions, and therefore susceptibility to the effects of empty flowers. There is evidence of individual honeybees having altered preferences based on foraging experiences from their earliest foraging experiences (Lajad et al., 2021). It may also be necessary to test the impact of empty flowers on bees of different ages.

In our experiment, less experienced individuals relied more on social information than experienced individuals. Overall, experienced foragers are generally more efficient foragers in terms of travel speed and nectar collection (Lihoreau et al., 2016), so there is less value in using social information once sufficient information on the foraging environment has been obtained. Our study provides further evidence for flexible information use in bumble bees, where individuals can preferentially use private information over social information when they are experienced In our experiment, we allowed bumble bees to forage in a group, with access to social cues.

Groups may be generally less susceptible to decoy effects, particularly in eusocial species. Edwards & Pratt (2009) found that while individual *Temnothorax* ants were susceptible to decoy effects, foraging groups were not. The communication systems of social insects can lead to high degrees of positive feedback which can cause a single option to be greatly preferred over the others (Sasaki & Pratt, 2011). Such amplification may lessen the impact of phantom decoys by preventing naïve individuals from ever encountering the empty flower. In our experiment, naïve bees were more likely to pick flowers based on the relative number of bees already foraging on that flower. Over time, it became increasingly likely for naïve individuals to never experience the empty flower. The collective nature of bumble bees foraging meant that some bees did not actually experience the complete choice set and were potentially 'unaware' of the presence of the empty flower.

Our experiment showed that empty flowers didn't directly impact foraging choice in bumble bees at the population level, suggesting that empty flowers may not affect the valuation of surrounding flowers. We did, however, find an impact of empty flowers on the behaviour of a small subset of experienced bumble bees although the direction of their preferences shifts was inconsistent. Further, we found that naïve bees were more likely to forage on flowers when conspecifics were present. Our study highlights the importance of including social behaviour in understanding the impacts of decoys on social species.

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Chapter 5: Covid impact statement: Flower choice by the stingless bee *Tetragonula carbonaria* is not influenced by the colour of nectar-depleted flowers in the same patch

The following chapter was written under the University of Sydney's COVID-19 impacted thesis protocol.

The following chapter was written with the intention of a sample size of approximately 20 colonies per treatment group. This experiment was conducted under COVID-19 conditions, where minimal on-campus work was permitted. The project was impacted significantly by:

COVID-19 Restrictions

- Restrictions to on-campus work at The University of Sydney
- Restrictions to access to Macquarie University
- Prohibition of fieldwork

Experimental work was not permitted, with only animal care listed as a reason to come on campus. Therefore, this experiment was conducted in the backyard of myself and F Mourmouurakis, using colonies that were rotated between houses. All colonies were housed at the University of Sydney and Ku-ring-gai Council. Access to colonies with the council was limited between November and December as interactions with the nursey were restricted, which reduced the number of colonies available.

Between the 23rd of December 2021 and the 7th of January 2022, fieldwork was not permitted at the University of Sydney. Due to significant rules in place that prevented fieldwork, as well as C. Forster becoming a close contact in December, the sample size was reduced.

The experimental design was also impacted by the COVID-19 pandemic. Under normal circumstances, this experiment would have been conducted in an isolated flight cage to prevent multiple colonies from entering the experiment. However, this was not possible as the cages were with a collaborator at Macquarie University and we were not permitted to go onto the Macquarie University campus.

Climatic impacts

Concurrently the delay in undertaking the experiment also corresponded with a La Nina event characterised by unseasonable high rainfall which limited *Tetragonula carbonaria* foraging activity.

Chapter 5: Flower choice by the stingless bee *Tetragonula carbonaria* is not influenced by colour-similarity to a higher-reward flower in the same patch

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Conflicts of Interest

None to declare

Ethics approval

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Consent to participate

Not applicable

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Keywords: stingless bee, pollination, artificial flower, colour

Abstract

Floral choice by bees is influenced by the bees' previous experience with flowers. For example, bees may learn to associate particular flower colours with rewards and prefer flowers of that colour in a given patch. In this study, we assessed whether floral choice by the stingless bee *Tetragonula carbonaria* was influenced by the colour of the most-rewarding flower in the patch. We trained *T. carbonaria* to visit highly rewarding artificial flowers (50% (v/v) honey solution) within a patch that also contained two types of less-rewarding artificial flowers (20% (v/v) honey solution): one of the same colour (though different pattern) as the high-quality flower and one a different colour (and pattern) to the other two flowers. Colonies were tested with blue and yellow colour sets, where either the blue flower was most rewarding and the yellow the least, or vice versa. We then compared preferences between the two equal-quality flowers in the patch under two conditions: (i) when nectar was available from the high-quality flower, and (ii) when the nectar was removed from the high-quality flower. We found that, when available, high-quality flowers were always visited more than low-quality flowers. Under this condition, adjacent lower-quality flowers in the patch received similar levels of visitation, regardless of their colour. When the reward was removed from the high-quality flower (simulating an emptied flower), foragers quickly switched to using the remaining two equal-quality flowers in the patch, but again showed no preference between them. Our results indicate that *T. carbonaria* are adaptable foragers capable of quickly learning and responding to floral reward changes in their foraging environment. At least under our experimental conditions, we found no evidence that *T. carbonaria* floral choice is influenced by colour similarity to a high-quality resource in the same foraging location.

Introduction

Nectar acts as a nutrient-rich reward to floral visitors and, by attracting floral visitors to flowers for nectar rewards, plants gain assistance in pollen transfer and therefore pollination benefits. However, the production of nectar can be costly (Pyke, 1991). Many plants, therefore, have strategies to tune their nectar production to floral visitor behaviour in ways that ensure adequate pollination benefits without an overproduction of nectar. One consequence of this is that not all flowers contain nectar at any given time. For example, climate, floral attractiveness, and time of day can all impact nectar availability in flowers (Langenberger & Davis, 2002; Waser & Price, 2016; Wright, 1988).

The presence of flowers empty of nectar ("empty flowers") in an environment can impact the foraging choices of floral visitors (Langenberger & Davis, 2002; Waser & Price, 2016; Wright, 1988). Over time, insects may learn to generalize floral traits associated with unrewarding flowers (Smithson & MacNair, 1997), resulting in decreased visitation to morphologically similar neighbouring flowers (Smithson & Gigord, 2003). For example, where unrewarding flowers are common in a patch, bumble bees *Bombus terrestris* will tend to avoid flowers similar in colour to the unrewarding variety and instead visit flowers of dissimilar colours (Smithson & Gigord, 2003; Internicola et al. 2009). In humans, such behaviour is known as a dissimilarity effect. Conversely, empty flowers could potentially increase visitation to similar flower types (a 'similarity effect'). That is, if a normally-rewarding flower is recently unavailable, bees may seek out morphologically similar flowers for rewards (Dyer & Murphy, 2009; Gigord et al., 2002, Internicola et al., 2009). Flowers that never contain nectar such as orchids have even evolved to mimic rewarding flowers to reap the benefits of colour generalisations made by bees towards rewarding flowers (Papadopulos et al., 2013).

Highly rewarding neighbouring plants can also impact the probability that a flower is visited by bees (Horna Lowell & Murphy, 2022). ‘Magnet plants’ are those whose flowers are highly desirable to floral visitors (Ghazoul, 2006; Gilpin et al., 2019a; Johnson et al., 2003). For example, lousewort (*Pedicularis sylvatica*) and lavender (*Lavandula spp.*) are often considered magnet plants for honey bees and bumble bees (Gilpin et al., 2019b; Laverty, 1992). By attracting floral visitors to the vicinity, magnet plants can increase pollination benefits to nearby plants due to ‘spillover effects’ (Ghazoul, 2006; Johnson et al., 2003). Such spillover effects are strongest where magnet plants are similarly coloured to neighbouring plants. Thus nectarless orchids of similar colour to adjacent magnet plants are more likely to gain pollination benefits than other nearby plants of distinct colours (Johnson et al., 2003; Peter & Johnson, 2008).

To date, most of our understanding of how bees respond to the colour similarity of flowers in a patch, whether they contain nectar or not (i.e. nectarless or nectar-depleted) has come from studies on two groups of social bees: bumble bees and honey bees. Stingless bees (Tribe Meliponini) are the other major clade of social bees, yet their foraging behaviour and floral choices are comparatively understudied. Stingless bees are abundant and important pollinators in tropical ecosystems across the globe, visiting upwards of 215 plant families (Bueno et al., 2021). They are also pollinators of a variety of tropical crops and are increasingly used as managed pollinators in agro-ecosystems (Grüter, 2020). In Australia, the stingless bee *T. carbonaria* is the species most widely propagated for commercial trade and is an effective pollinator of crops including macadamia, avocado, and blueberry (Heard, 1994; Kendall et al., 2020). *T. carbonaria* colonies comprise a single queen and typically 5000 -

10000 workers (Heard 1999). The species is common throughout both forests and disturbed environments in tropical and subtropical Eastern Australia.

In this study, we aimed to test if the colour of high-reward flowers in a patch influenced *T. carbonaria* foraging choice. We tested foragers visiting resources in their natural social context; that is, workers foraging in the presence of nestmates, rather than lone foragers absent of all social information. Specifically, we asked: (1) When a high-quality flower is present in a patch, do foragers visit nearby flowers of similar colours more than those of dissimilar colours?, and (2) When a high-quality flower is depleted of nectar, do foragers initially visit nearby flowers of similar colours more than those of dissimilar colours?

We predicted that *T. carbonaria* would generalise flower colour after learning the quality of flowers, as previously shown for the bumble bee *B. terrestris* (Dyer & Chittka, 2004; Gumbert, 2000; Rohde et al., 2013) and the honey bee *Apis mellifera* (Dyer & Murphy, 2009; Rohde et al., 2013). That is, we expected that *T. carbonaria* would preferentially visit flowers that were similar in colour to the most rewarding flower in the group. Following nectar depletion, we hypothesised that foragers would again be influenced by flower colour, but that their floral choices would differ in the short term and longer term after nectar removal. We predicted that when a highly rewarding flower is removed from a choice set, foragers would initially have a preference for similar coloured flowers to the high-quality flower. When a once highly rewarding flower had been empty for an extended period, we predicted that foragers would learn that this flower type was persistently unrewarding and begin to prefer the adjacent flowers that were least similar in colour.

Methods

Colonies

We used 14 colonies of *T. carbonaria* maintained by The University of Sydney and Kuringai Council Nursery, Sydney, Australia. Each colony was housed in a three-part Original Australian Trigona Hive (OATH) style wooden hive (Dollin 2002). To ensure that foragers were naive to the local natural flower locations (therefore encouraging foraging on our patch of artificial flowers), colonies were moved one at a time from their usual location to the yards of private houses in Lidcombe or Baulkham Hills, Sydney for the period of data collection. Experiments were conducted between December 2021 and March 2022.

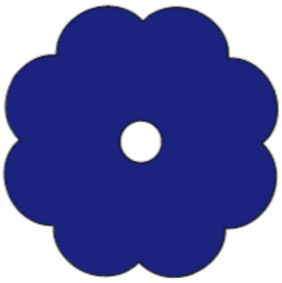
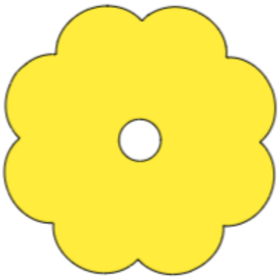
Artificial flowers

To assess the impact of high-reward flowers on other flowers in the patch, we created a patch of artificial flowers with three flowers that differed in colour and pattern (Table 5.1). Two flowers contained a reward of equal concentration (flowers A and B; 5 ml of 20% honey solution) and one which was of higher concentration (flower C, the “high-reward flower”; 5 ml of 50% honey solution). Flower A had a circular pattern, flower B had no pattern, and flower C (“high-reward”) was the same colour as A, but with a radiating pattern. Patterns were used as additional associative cues. We chose blue and yellow as flower colours since they are colours that can be readily discriminated by *T. carbonaria* (Spaethe et al., 2014). To control for any innate colour preferences, two colour sets were used: one set in which A and C were yellow and B was blue, and a second set in which A and C were blue while B was yellow (Table 5.1).

Artificial flowers consisted of the coloured paper flower underneath a 75 mm diameter clear Perspex disc containing radiating lines indented in the top to hold the honey solution. The honey solution was placed on a cotton ball that was put on top of the artificial flower, to allow the honey solution to seep into indentations on the artificial flowers.

Briefly, we confirmed the discriminability of our three floral colours to bees by using spectrometry to record the reflectance of cardboard, before estimating their colour-difference using the colour hexagon model of Chittka (1992), with the visual phenotype of honeybees (Maia et al. 2019). All three stimuli were separated by distances well above documented absolute discrimination thresholds (Supplementary Figure 3), and so should be readily separable by our focal bees.

Table 5.1: Sets of artificial flowers used for experiments. flower C is the "high-reward" flower, with 50% honey solution, while flowers A and B were lower concentrations (20% honey solution), with flower A sharing the same colour as C (and thus more colour-similar in appearance) while flower B was a different colour.

	Flower A (similar to C)	Flower B (dissimilar to C)	Flower C (high-reward)
Yellow set			
Blue set			

Reward (honey: water)	20%	20%	50%

Pretraining and training

For each colony, we first trained foragers to a gravity feeder to encourage them to forage at the test location, which was a green wooden board 1 m directly in front of the hive entrance (level with the colony). The gravity feeder consisted of a plastic plate covered by a single sheet of paper towel, onto which we positioned an upside-down cup containing a 50% (v/v) honey solution so that the solution progressively seeped onto the towel. The gravity feeder was initially placed right next to the hive, touching the colony entrance. Once 20 foragers were present on the feeder, we slowly shifted it to the test location. The training feeder was then removed and replaced by our three artificial flowers. Artificial flowers were placed edge-to-edge with each other during the 30-minute training phase. During this time, bees were able to freely forage on all three flower types. We could confirm foragers were coming from our trained colony by observing their flight to and from the hive entrance.

Test phase

During the test phase, the three artificial flowers were positioned in a triangular configuration, with flowers 10 cm apart from each other. The test phase had two parts (Figure 1). In the first 50 minutes of the experiment, all three flowers in the test set had a honey solution available. Just before the 60-minute observation interval, we removed the honey solution from flower C. Nectar removal was done by replacing the flower with a new clean feeder with a cotton ball containing no nectar on top. All flowers were also replaced with new clean Perspex top-feeders at this stage. During the second part of the test phase (60 - 150 minutes), all three flowers were available for the bees to visit, but only Flowers A and B offered any food. We performed forager counts on artificial flowers at ten-minute intervals throughout the test phase (i.e. five counts before removing the reward from flower C, and ten counts after removal). We counted every stingless bee that landed and fed on a flower for one minute. One minute was chosen to prevent pseudoreplication, as it was a short enough period of time to prevent bees from returning to the feeder after previously feeding. Between each count, foragers were allowed to continue accessing the flowers. However, before each new count, flowers were replaced with new flowers. All flowers were cleaned with 70% ethanol before being used again and rotated in the triangular configuration to reduce any effects of their location and remove any scent marks.

To confirm that any responses to nectar removal from flower C were due to the removal, and not just the passage of time, we performed both our experimental treatment (flower C reward was removed during the test phase; N = 12 trials**)** and a control treatment (no reward removal during the test phase; N = 10 trials). In both treatments, two colonies were only tested until 100 minutes rather than the full 150 minutes. In the experimental treatment, eleven colonies were used across all trials, with three colonies used twice with different colour sets.

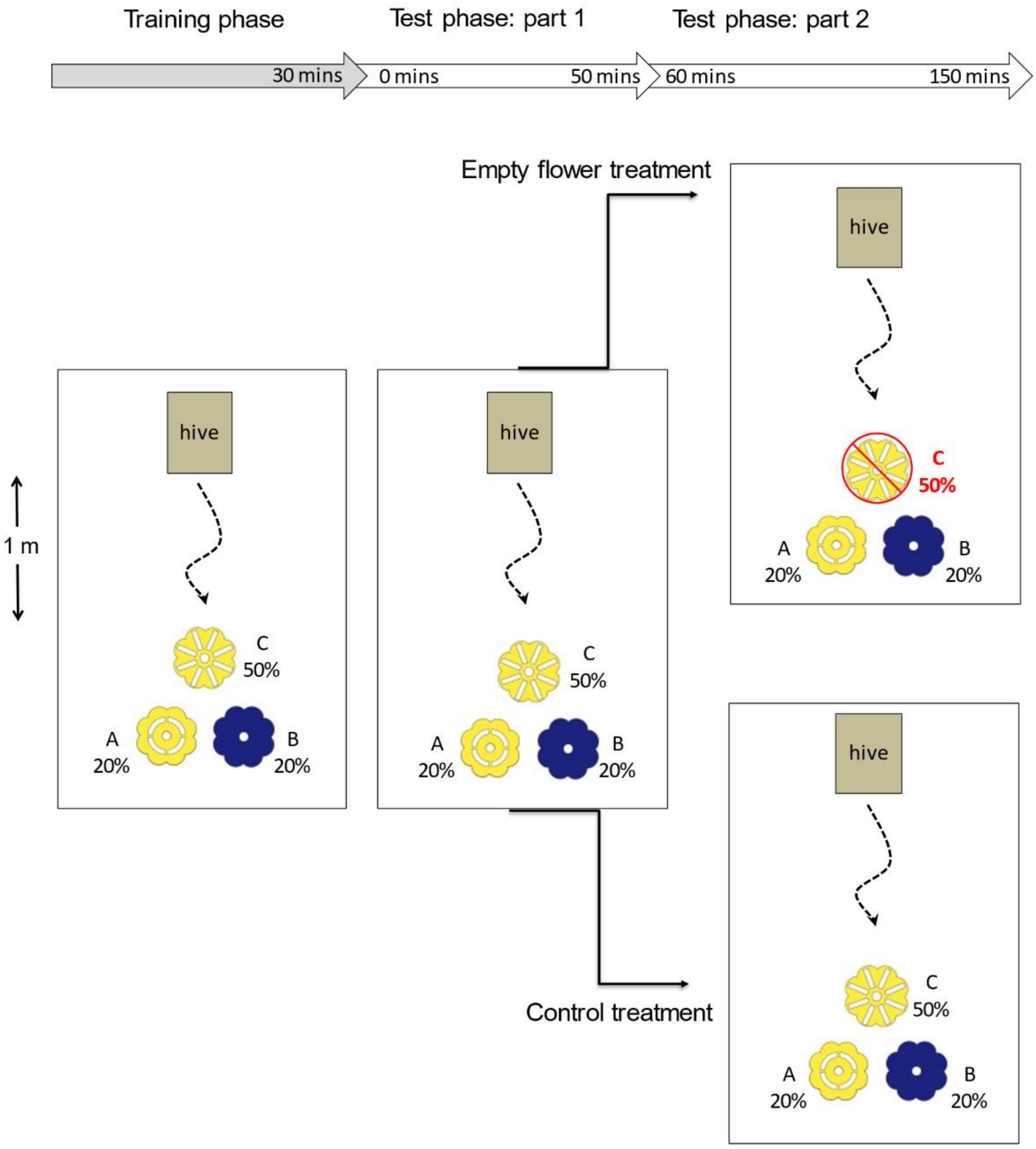


Figure 1: Overview of the experimental design used to assess if *T. carbonaria* foragers are influenced by the presence of high-reward flowers (either full or emptied) in a patch (flower C). Percentages indicate the percentage of honey used in each solution (v/v). Note,

that the layout of the three flowers was randomised during each time interval where data was recorded.

Data analysis

All analyses were conducted in R version 4.1.2 (R Core Team, 2021). To establish if the high-quality flower was the most preferred artificial flower, we performed a Kruskal-Wallis test considering all visits to flowers before the 60-minute observation interval. The Kruskal-Wallis test was used due to a lack of normality in data (McKnight and Najab, 2010). We then used posthoc pairwise tests to compare forager numbers on flowers C vs A and B (Dunn test), with p-values adjusted for multiple testing via the Benjamini Hochberg Method (Benjamini & Hochberg, 1995).

We used ANOVAs to compare the number of foragers visiting each of the neighbouring flowers (Flowers A and B) to a high-reward flower (flower C) at three key time points: one directly before nectar removal from flower C (after 50 minutes, i.e. count 5), one directly after nectar removal (70 minutes; count 7) and one after 100 minutes (i.e. after foragers had time to learn that flower C was now consistently unrewarding). Significant differences in forager counts between Flowers A and B at any of these time points would indicate a preference for the option that was either similarly coloured or differently coloured to flower C.

Finally, we assessed whether the removal of reward from flower C caused changes in the number of total foragers using the patch after the nectar was removed in the empty flower treatment, using a Kruskal-Wallis test. That is, we assessed whether foragers abandoned the patch once the best-quality flower was no longer available.

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Results

When a high-reward artificial flower was available in the patch, foragers showed a strong preference for this flower (i.e. flower C), with around twice as many foragers visiting flower C than each of Flowers A or B at the end of part 1 test phase in both experimental and control treatments (Table 5.2, Figure 2; $\chi 2(2) = 30.391$, $p < 0.0001$, where posthoc pairwise tests for Flowers A vs. C and B vs. C both $p < 0.0001$). There was no difference in visitation rates between Flowers A and B at this time ($F(1) = 0.866$, $p = 0.729$); that is, while some foragers did use the lower-reward flowers adjacent to a high-quality flower, there was no preference among these for flowers similarly-coloured to the high-reward option.

Once the nectar was removed from the high-reward flower, visitation to this flower decreased rapidly within 10 minutes, indicating foragers quickly learnt it was now empty (Table 5.2; Figure 2). Foragers shifted at this time to use the remaining rewarding flowers (A and B) with similar numbers on each ($F(1) = 0.25$, $p = 0.621$; Table 5.2, Figure 2). Foragers continued to show no preference between Flowers A and B after a longer interval post-nectar removal from the high-reward flower (i.e. once they had time to learn that flower C was now consistently unrewarding; $F(1) = 0.942$, $p = 0.338$; Table 5.2). Overall visitation to the patch by *T. carbonaria* decreased in the presence of a previously rewarding but now empty flower (experimental treatment vs control treatment: $\chi 2(1) = 61.687$, $p = < 0.001$; Figure 2; Table 2).

Table 5.2. Mean numbers (+/- S.E.) of *T. carbonaria* foragers visiting artificial flowers in a patch during three-time points in the test phase (50, 70 and 100 minutes) for experimental and control treatments. Flower C was high-reward while Flowers A and B were equal, lower-reward flowers. Flowers C and A were the same colour, while flower B was a different colour. In the experimental treatment, the feeding solution ('nectar') was removed from flower C at the end of part 1 of the test phase (i.e. after 60 minutes) and the flower remained empty for the rest of the test phase. Images in this table show the "yellow set" flower colours.

Timepoint		50 minutes test phase part 1 (bees/min)	70 minutes test phase part 2 (bees/min)	100 minutes test phase part 2 (bees/min)
Experimental treatment (N = 12)		High-reward flower (C) available	High-reward flower (C) recently depleted	High-reward flower (C) depleted for >30 mins
	 Flower A	2.91 (±0.83)	4.00 (±1.16)	2.81 (±0.65)
	 Flower B	2.13 (±0.74)	3.57 (±0.92)	3.45 (±1.01)
	 Flower C	4.47 (±1.02)	2.53 (±0.49)	2.67 (±0.50)
		High-reward flower (C) available		
	 Flower A	2.22 (±0.22)	3.88 (±0.83)	4.43 (±0.68)

Control treatment (N = 10)	Flower B	2.00 (±0.37)	2.00 (±0.21)	3.40 (±0.54)
	Flower C	4.70 (±0.68)	7.60 (±1.44)	7.80 (±1.28)

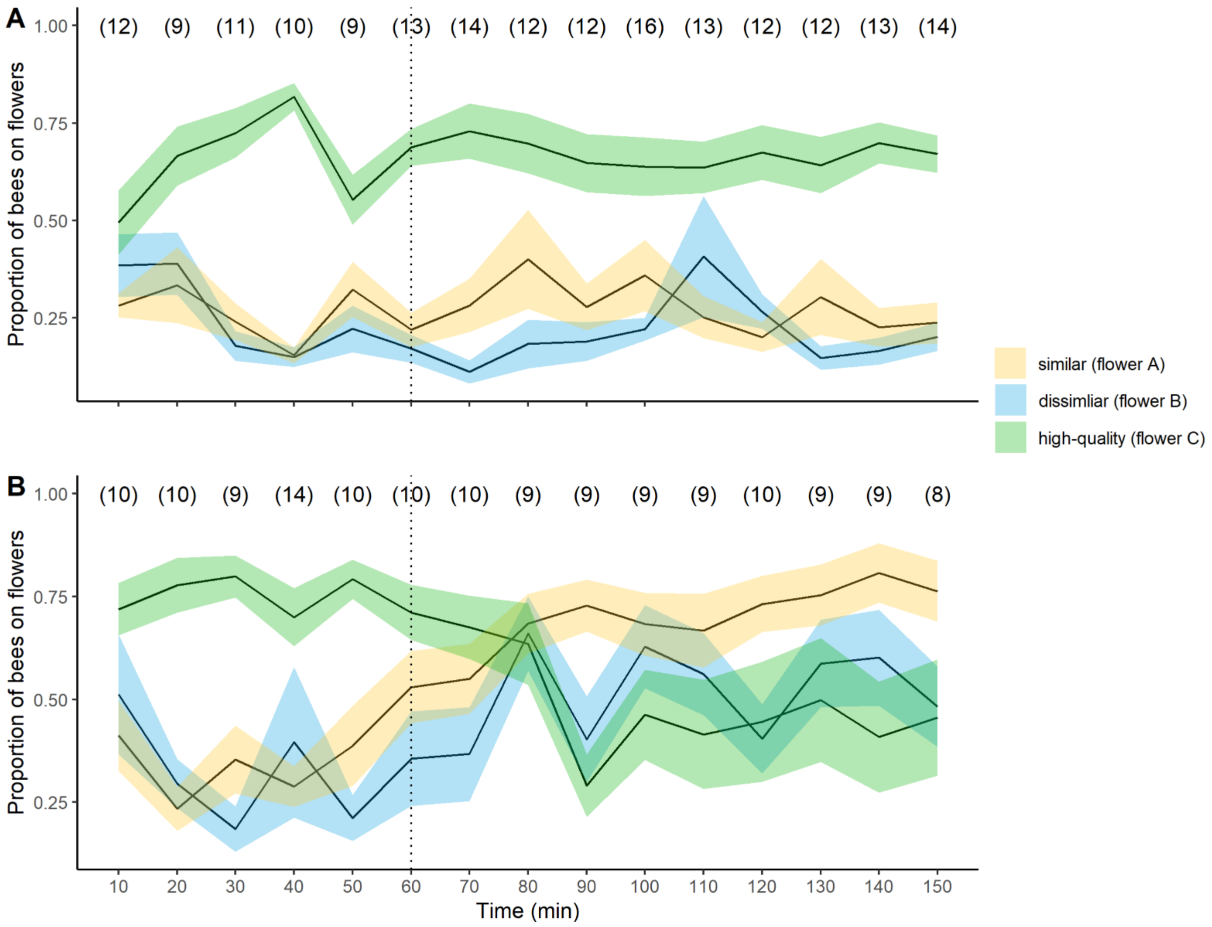


Figure 2: The mean proportion (+/- standard error) of *T. carbonaria* foragers feeding on artificial flowers in our patch throughout the test phase (150 minutes) for A control treatments (N = 10 colonies) and B, experimental treatments (N = 12 colonies). Counts were made for 1 minute at 10-minute intervals. Flower C was a high-quality flower (green plot). flower A was similar in colour to flower C but lower reward (yellow plot) and flower B was dissimilar in colour to flower C and also lower reward (blue plot). The dotted line indicates the time at which nectar was removed from the high-quality flower in the experimental treatment. Numbers in brackets at the top of each plot indicate the mean forager numbers for each count.

Discussion

We investigated whether foragers of the stingless bee *T. carbonaria* were influenced by the colour of a high-quality flower when choosing between other flowers in a patch, including after the preferred flower became nectar-depleted. We found that while *T. carbonaria* readily foraged on less-rewarding artificial flowers adjacent to the high-quality flower, they were no more likely to choose those flowers if they colour-matched the high-reward resource than if they did not. Nor were foragers' flower choices between neighbouring nectar-containing flowers influenced by the presence of an empty, previously high-quality flower in the patch. That is, in all, *T. carbonaria* foragers showed a consistent lack of preference between two flowers of equal-reward quality but different colours, regardless of the presence and availability of a neighbour flower.

In general, bees can quickly learn to make generalisations about flowers based on colour across a range of foraging contexts (Dyer & Chittka, 2004; Dyer & Murphy, 2009; Giurfa, 1991; Gumbert, 2000; Rohde et al., 2013). In bumble bees, colour generalization extends to a preference for flowers that are similar in colour to those that that have been previously rewarding (Gigord et al., 2002; Johnson et al., 2003). Indeed, this habit in bumble bees is so reliable that some orchids have evolved to exploit it, by mimicking the colour of high-quality flowers yet offering no nectar (Gigord et al., 2002). Why was there an absence of colour generalisations in our experimental treatment with *T. carbonaria*? One possibility is that most foragers in our experiment were simply too experienced with all three artificial flower types in a set to need to make generalizations about flower colour. Some visitation of the low-quality flowers persisted throughout the pre-removal stage, though we cannot confirm whether this was the result of a few foragers repeatedly visiting these flowers, or many different foragers visiting them infrequently. If enough foragers learnt from direct experience with both lower-quality flowers, then they would presumably not be at risk of mistaking them for higher-quality ones, despite one being colour-matched to the more rewarding flower. In this case, it remains possible that *T. carbonaria* does sometimes respond to empty flowers by selecting similar colour choices (e.g. by moving to novel flowers in new patches), but our experimental scenario failed to stimulate these responses.

Alternatively, *T. carbonaria* may not have responded with colour-generalization to empty flowers in our experiment owing to fundamental differences in foraging ecology between this and other studied pollinators. The foraging landscape for Australian stingless bees differs considerably from that of bumble bees and other commercially available pollinators. In Australia, many naturally occurring mass flowering plants are trees, such as those in the family Myrtaceae, for example, eucalypts (Somerville, 2019). Therefore stingless bees in Australia often forage in trees (Bueno et al., 2021; Grüter, 2020) that are less heterogenous than the foraging environments such as meadows, where there are likely multiple flower species growing next to each other, in comparison to the landscape of a tree containing a single flower species. Future efforts to understand the responses of stingless bees to empty flowers may depend on a better understanding of how they experience foraging spaces with both high and low flower heterogeneity. For example, *T. carbonaria* and other stingless bees may regularly need to consider empty flowers' effects at the level of inflorescences on a single plant, rather than patches of different flowers.

It is also possible that the Australian floral communities haven’t evolved to have many similar coloured flowers, so generalisations are a less effective strategy. Prior studies involving colour generalisations have been seen with *B. terrestris* and *A. mellifera* (Dyer & Murphy 2009, Internicola et al. 2009), where floral communities may have different colour structures. Evidence in Australia so far, suggests that woodlands have a community of distinctly coloured flowers (Shrestha et al. 2019).

Social behaviour could have been an important factor in foraging choice in stingless bees. We assessed *T. carbonaria* foraging choices in a social environment rather than in isolation, where foragers could see and smell nestmates using the same patch. *T. carbonaria* uses scent marks at food sources to recruit nestmates to profitable resources (Bartareau 1996; Gloag et al 2021), as do other stingless bee species (Bartareau, 1996; Roselino et al., 2016; Schmidt et al., 2003; Schorkopf et al., 2007; Sommerlandt et al., 2014). Bees, including stingless bees, also rely on visual local enhancement, where the presence of a conspecific can impact the visitation of rewarding flowers (D'Adamo et al., 2000; Slaa et al., 2003; Sommerlandt et al., 2014). Social cues combined with individual bees' own learn cues surrounding flower colour and patterns were likely an important contributor to foragers' ability to rapidly associate flower types with nectar rewards and also to quickly learn to abandon the empty flower. Because individuals continually visited the low-quality flowers, the combination of scent marks and local enhancement could have allowed for quick uptake in individuals feeding on lower-quality flowers that were already being utilised by other individuals. To identify how foragers respond to empty flowers in the absence of social information, it would be necessary to test individuals one at a time. However, by allowing interactions with nestmates, our experiment provided the opportunity to understand foraging responses to empty flowers in an ecologically relevant social setting.

Bees may gain some benefits by visiting flowers other than the flowers known to be the most profitable. For example, bumble bees (*B. terrestris)* that are more likely to visit less profitable flowers in a patch are also more likely to visit novel flowers (Evans & Raine, 2014), suggesting that these more error-prone individuals might ultimately be most likely to locate new (and potentially better) flowers when they are newly available. Among *A. mellifera*, there is evidence that individual bees have different 'personalities' of foraging search strategies (Dyer et al., 2014; Smithson & Gigord, 2003). Some foragers are fast to learn the value of new resources, while others are slow to change preferences once established. The efficiency of these different strategies vary depending on how often flower quality changes in a patch (Dyer et al., 2014). In the case of social bees, individuals may also vary in their reliance on social information to make foraging choices, ensuring an optimal balance between benefiting from group knowledge and finding new resources. (Gigord et al., 2002; Johnson et al., 2003). Consistent with this, our data suggest the T. *carbonaria* foragers from the same colony are variable in their response to flowers of different quality, with all three flowers being used by at least some foragers.

High-quality flowers can sometimes facilitate increased visitation to the lower-quality flowers in the same patch (a 'magnet plant' effect). Throughout our experiment, *T. carbonaria* foragers continually visited the lower-quality flowers adjacent to the high-quality flower in our patch, which is consistent with a possible magnet effect for flower C. Whether this was occurring in our patch of artificial flowers is unclear because we did not compare visitation in experimental patches to patches where high-reward plants were never present. A study in Australia, focussing on the honey bee *A. mellifera*, suggested that magnet plants were not as effective in the southern hemisphere to attract honey bees as studies in the northern hemisphere had previously found (Gilpin et al., 2019a). Our study suggests that there may be implications for using magnet plants for native species of bees. In these conditions, some plants might benefit from producing less nectar if there are high-quality flowers in the vicinity that ensure they receive sufficient pollinator visits.

Magnet plant effects are also important in the context of crop pollination because they can be used to entice pollinators to crop blooms which might otherwise be an unpreferred floral resource (Montero-Castaño et al., 2016). Such magnet effects are more successful if there are multiple magnet plants in the area (Horna Lowell & Murphy, 2022; Johnson et al., 2003). Given the promising prospects of *T. carbonaria* as a pollinator of some Australian crops, the susceptibility of this species to magnet plant effects should be further investigated. For example, testing the effects of decoys on a patch level would allow for an increased understanding of the effects of empty flowers on stingless bees' foraging movement between patches.

In all, our experiment demonstrates that *T. carbonaria* are versatile social foragers, capable of quickly learning to abandon previously rewarding but unavailable resources when foraging alongside nestmates. Their floral choice in a patch was not influenced by colour-similarity to high-quality flowers, and some foragers visited lower-quality resources in a patch even when high-quality options were present. We suggest that *T. carbonaria's* ability to adapt quickly to new resources, and willingness to forage on resources irrespective of their quality, may make them suitable targets for magnet plants in a crop pollination context, and warrants further investigation. If and when their foraging choices are influenced by floral colour generalizations, and whether such behaviour impacts pollination networks in Australia, also requires further study.

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Chapter 6: General discussion

This thesis (i) reviewed the literature on artificial flowers used for experiments involving floral visitors, and (ii) tested the foraging choice of three species of social bee, *Apis mellifera, Bombus impatiens* and *Tetragonula carbonaria,* in the presence of empty flowers. My review of artificial flowers in research highlighted how to effectively make and use artificial flowers for experimental settings, and made the case that simple, mass-produced designs can be deployed in a range of scenarios to ask important questions about floral visitor behaviour. My experiments then made use of some artificial flowers to show that, at least in the experimental scenarios I tested, neighbouring empty flowers can impact bee movements between flowers and the rates at which patches are abandoned. Empty flowers generally did not affect the relative preferences of bees for neighbouring flowers. By understanding the impacts of empty flowers on bee pollinators, we may be able to better utilize extrafloral resources in crops to benefit both fruit set and insect biodiversity. Ideally, such management decisions would be based on a deep understanding of bee foraging ecology and bees' responses to floral decoys. The work in this thesis is a step towards that goal.

This thesis also contributed insight into the cognition of social bees, and their capacity to learn floral attributes and forage based on prior information. In particular, it provided evidence of the impact of social cues when foraging and thus highlighted the importance of considering social behaviour when testing the impacts of floral choice. Our understanding of empty flowers and choice in bees may also have future relevance to how we manage wildflowers strips for efficient pollination, though a much richer understanding of the impacts of floral decoys would be necessary to make management decisions.

Summary of thesis

In **Chapter 2**, I reviewed the literature on experiments using artificial flowers to establish how artificial flowers have been used in pollination ecology and behaviour. I found 160 studies, with a range of artificial flower types used to test different aspects of behaviour and pollination ecology on many different species. I established that most of these experiments are done on three species of bees, *A. mellifera, B. impatiens* and *B. terrestris,* and suggest it's important to consider using artificial flowers to test floral choice by other non-bee floral visitors. I also showed that very few studies involving artificial flowers were conducted outside of controlled laboratory-style environments or in-flight cages. I discussed the benefits of artificial flowers for research and provided the traits of flowers that are important for researchers to consider, particularly when conducting experiments using free-flying species.

In **Chapter 3,** I tested the effects of empty flowers on foraging choice and behaviour in honey bees using phantom decoys. Honey bees, *Apis mellifera,* are commercially available pollinators that are widely used across the globe for pollination purposes. They are also a popular model species for studying bee behaviour and cognition. *Apis mellifera* has previously been shown to be susceptible to decoys, using unattractive decoys (Shafir et al., 2002). Overall I showed that honey bee flower choice was not impacted by empty flowers if the empty flower was not similar to the flowers containing nectar, rather, honey bees were more likely to move between more flower types and abandon patches in the presence of empty flowers.

In **Chapter 4,** I tested the impact of empty flowers on *B. impatiens.* In North America, the eastern bumble bee *B. impatiens* is a common commercial pollinator that has frequently been used to model bee behaviour. I investigated foraging choices in bumble bees in the presence of empty flowers. Overall, *B. impatiens* showed minimal effects of empty flowers on a group level, though a small number of individuals changed their preference for flowers in the presence of an empty flower. Individuals who were more experienced with the patch were less likely to join conspecific groups on flowers in comparison to more naïve individuals.

Honey bees and bumble bees have been consistently studied as models for the behaviour and cognition of eusocial bees. However, the third clade of social bees - the stingless bees – has received less research attention. In **Chapter 5** I tested if the stingless bee *Tetragonula carbonaria* made colour generalisations in the presence of a high-quality flower, and if these generalisations occurred when the high-quality flower was empty. Over 20 minutes, the bees quickly learned to shift their foraging choice from the highly rewarding flower to either of the two other nectar-containing options. Overall, no evidence of colour generalisations was observed in this experiment. I found evidence, however, that stingless bees are likely to visit flowers surrounding highly attractive plants while foraging.

Artificial flowers as a tool for studying floral visitor behaviour and ecology

Artificial flowers offer the opportunity to manipulate ‘flowers’ to answer a range of questions about the behaviour and ecology of wild species of floral visitors in a controlled manner. These flowers are useful for certain types of experiments, however, I found that there are considerable barriers to using artificial flowers on free-flying species, particularly regarding attracting hymenopteran groups. For example, I trialled the use of artificial flowers in mid-summer on campus and found that artificial flowers struggled to compete with natural resources and so received little attention from wild bees. This may indicate that more work is needed for flower designs that can be effective in attracting species that already have access to floral resources. Research currently underway testing different styles of artificial flowers suggests that multi-attribute flowers that incorporate reward and scent can increase how attractive artificial flowers appear to a range of floral visitors (Chapman et al. in prep). If further experiments can untangle what floral traits are necessary to make attractive artificial flowers, they may become a useful tool for answering a range of questions about pollinator behaviour and ecology in species beyond honey bees and bumblebees.

Using artificial flowers, we could investigate why co-flowering plants have evolved certain traits, like being morphologically similar to their neighbours, whereas other traits, like scent, are sometimes different. One hypothesis is that differences in scent profiles allow for the fine-scale discrimination of flowers, while morphological traits such as colour function as longer-range attractants and thus similarity improves detectability. With artificial flowers, it would be possible to test the floral preferences of bees when foraging on flowers of different combinations of similar/dissimilar colour and scent profiles (Figure 6.1).

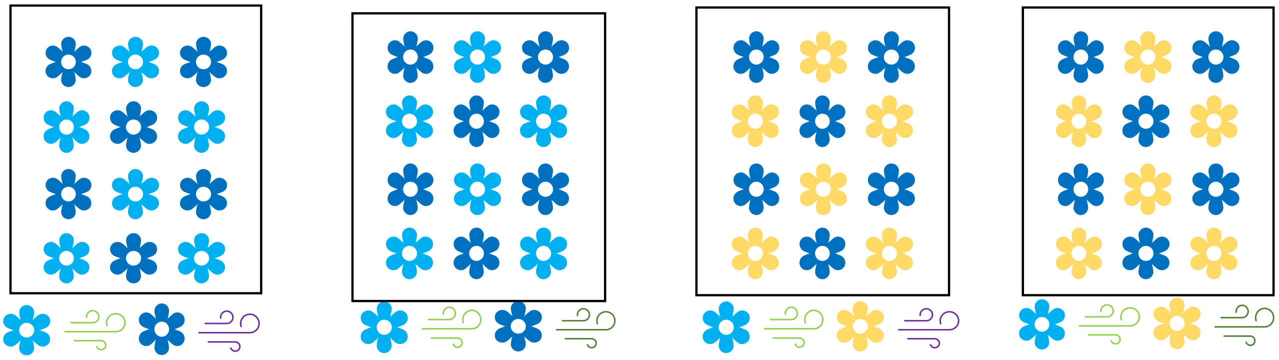

Figure 6.1: Floral preference trials looking at preferences between flowers of similar morphology (represented by similarly colours flowers), and similar scent (represented by wind colour in the legend below flower sets)

The work in this thesis shows that the effects of co-flowering plants can be studied using artificial flowers. My experiments utilized artificial flowers that could be visually and morphologically manipulated in a simple manner. My experiments also consisted of flowers created using a paper cutting machine, which allowed for the mass production of flowers. One limitation of my work for understanding the effects of empty flowers, however, was that I focused on single flower effects and not the impact of multiple empty flowers on foraging choices in bees. A future experiment could test the effects of not only empty flowers but the replenishment of magnet flowers and their effect on forager movement between flowers in a patch containing many flowers. This would help to understand not only the patch-level effect of empty flowers and magnet plants on choice but also allow for a deeper understanding of why I saw an increased rate of movement and abandonment in the presence of empty flowers. Such follow-up experiments could be easily achieved using artificial flowers.

One important factor I established when using artificial flowers in my thesis, was the importance of seasonality when attracting wild visitors. Currently, it is difficult to attract sufficient floral visitors to artificial flowers in spring, which I found through tests of patches for phantom decoys (Figure 6.2), but also when training honey bees outside of a flight cage. The likely issue is that the abundant floral resources available to floral visitors in spring outcompeted my artificial flowers. While it is easier to run artificial flower experiments in autumn and winter (when there is less natural forage), there are also fewer available foragers in the environment during such times due to the lack of most native bees. To run experiments in spring, scent and a large floral display may be needed. Tests on the effectiveness of artificial flowers and floral displays could be achieved by laying out arrays of flowers in the field and testing rates of discovery and visitation by floral visitors.

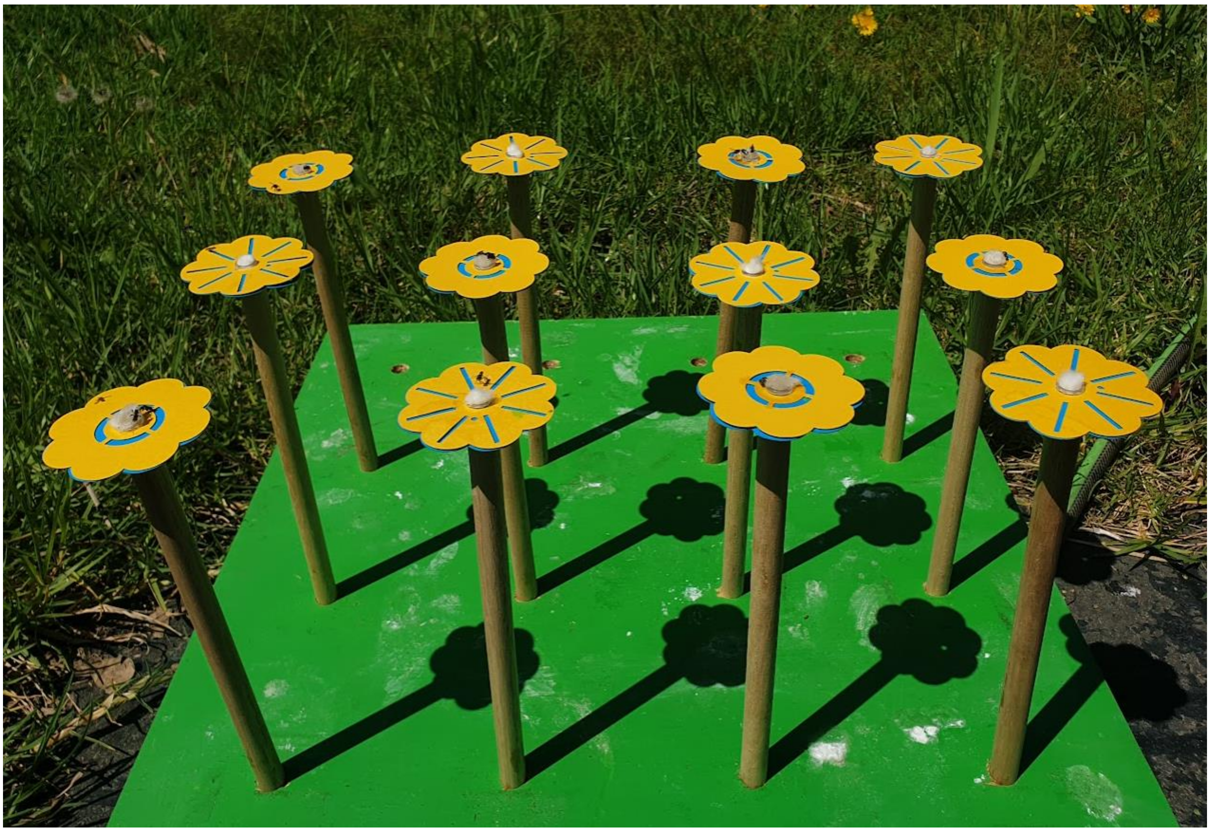

Figure 6.2: Patches of artificial flowers can be used to answer questions on the movement meant of bees in the presence of empty flowers, as well as questions regarding patch-level foraging choice

My thesis took a global approach to test the behavioural ecology of three bee species. While the methodology of my experiments varied across species, many questions would benefit from multi-species approaches regarding pollinator ecology, preference and behaviour. My thesis has provided sources of technology, and supplied resources to create effective mass-produced paper and 3D printed flowers. My artificial flower review also explained necessary traits that need to be reported to ensure reproducible flowers. Mass-produced artificial flowers can create cross-continental pollinator studies and encourage collaboration between groups studying different bee taxa.

Lastly, except for a few small studies that used silk flowers or moulds to mimic real flowers (Mainali & Lim, 2008; Muth et al., 2015; Policha et al., 2016), most artificial flowers used for experiments used round disk shapes or daisy-shaped flowers. However, there is evidence that floral visitors visit a range of flower shapes. It is important to expand the scope of artificial flowers in experiments, particularly when testing their efficacy. Previous studies have suggested that floral visitors have an innate preference for images of flowers of different shapes associated with their pollinator guild, so this should be considered when using artificial flowers. For example, in Australia, the use of flowers that mimic the distinctive appearance of eucalypt blossoms might increase the range of species that visit artificial flowers (Figure 6.3). To test the importance of flower shape, it might be valuable to test the innate preference of a range of flower types in floral visitors. It may also be possible to mimic other key plant parts by incorporating leaves and stems (Rivest et al., 2017), or even use inflorescences as opposed to single flowers (Ishii et al., 2008).

Figure 6.3: Example of artificial flower I designed to mimic a Eucalypt flower

Empty flowers and their impacts on co-flowering plant pollination

This work in this thesis suggests that the impacts of empty flowers on foraging choices in social bees will at least sometimes be of small effect, which gives insight into the implications of empty flowers for the pollination of crop plants. The use of extrafloral resources in crops for pollination can lead to competition among pollinators (Landry, 2013; Levin & Anderson, 1970), or facilitation (Johnson et al., 2003; Liao et al., 2011; Ruttan, 2017; Yang et al., 2013). However, all three of my experimental chapters showed that high-quality flowers, with or without (I.e. temporarily unavailable) nectar, result in bees foraging on flowers surrounding the high-quality and empty flowers. Given that spill-over of visitors between flowers occurred irrespective of floral traits or colours, it may be sufficient to plant high-quality plants to increase pollination efficiency, as opposed to attempting to plant morphologically (for example) similar flowers to the target plant for pollination (Ha et al., 2021).

The presence of empty or minimally rewarding flowers is predicted to cause floral visitors to reduce their visitation to a patch (Biernaskie et al., 2002; Smithson & Gigord, 2003). My experiments saw similar results with honey bees, but similar rates of abandonment were not seen using stingless bees. It would be beneficial to test the impact of empty flowers on a patch level to determine if patch departure rules are species-specific, and how empty flowers impact movement patterns between floral species in a patch. Previously, patch departure has been studied in bumble bee species where encountering empty flowers can result in both abandonment of patches, and movement to new flowers in the area (Biernaskie et al., 2002; Cresswell, 1990; Smithson & Gigord, 2003). Testing the patch departure rules would be possible across multiple species by creating arrays of artificial flowers with varied numbers of empty flowers and determining how the number of empty flowers influences the probability a forager will leave the patch. By placing flowers of different colours within the patch it would also be possible to test how many empty flowers it takes for bees to move to different flowers in the patch in the presence of empty flowers.

In my experiments, bees visited lower-quality flowers even though a higher-quality flower contained nectar, a behaviour that was particularly evident in the stingless bee T. carbonaria. There is some benefit to having individuals that don't always visit the best flower in the patch. The exploration-exploitation trade-off (Mehlhorn et al., 2015), is the trade-off between sticking to a known good option and exploring to find a potentially better option. High-quality flowers might be worth visiting, but if they are frequently empty, there may be some disadvantages to continually foraging on them. In Western honey bees, it has been shown that there are individual foragers that will consistently visit rewards other than the best quality one (Dyer et al., 2014), which allows these individuals to be more adaptable to change if a high-quality resource becomes available. In the context of social insects, these individuals can be particularly important, as they can recruit members to new food sources if profitable ones become unavailable. It would be beneficial in future to test if there was any consistency in which individual stingless bees visited lower-quality floral resources in a patch.

When a high-quality flower was removed from a patch, stingless bees continued foraging on lower-quality flowers. This finding suggests that *T. carbonaria* respond to magnet plant effects (at least in the context of my experiments). The only study on magnet species in cropping systems in Australia has been focused on pollination by *A. mellifera* due to its high abundance in the study (Gilpin et al., 2019a). The rising popularity of *T. carbonaria* as a commercial pollinator highlights the benefits of understanding how magnet plants may attract stingless bees to crops. My experimental design was a highly controlled test of magnet effects, so we have only established that nectar quality is important for creating flowers that attract high numbers of stingless bees. Understanding the identity and traits of plants that act as magnet plants to the target species for pollination is also important. My thesis provides the scaffold for magnet plants to be relevant in an Australian context, but it is still necessary to find the correct plants and begin trials within crops and other complex environments

Finally, it is important to note that while this thesis focuses heavily on empty flowers, many flowers are only temporarily empty, particularly in the context of popular flowers. It is important to test the effects of nectar replenishment. Previous studies have created flowers that can automatically refill nectar to specific columns after a floral visitor has fed (Keasar, 2000; Nachev et al., 2017). Given the capacity to change rates of refill, or quickly refill all flowers in the patch after visitation, it would be useful to see how quickly social bees return to a patch if high-quality flowers temporarily disappear and reappear.

Using behavioural economics to understand foraging choice

In my experimental work, I found little effect of phantom decoys on foraging choice in social bees. Why might this be the case? Either the species I tested are not influenced by all types of decoys when making foraging choices, or they are influenced only under certain conditions (which my experimental designs failed to mimic). It would be beneficial to understand if particular attribute configurations influence susceptibility to phantom decoy effects in bees. Currently, we know that Western honey bees *A. mellifera* are susceptible to asymmetrically dominated decoys in experiments that varied nectar volume and corolla tube length (Shafir et al., 2002). The closely related Asian honey bee *Apis cerana* is susceptible to the effects of phantom decoys when the attributes of nectar concentration and nectar temperature were considered (Tan et al., 2015). My experiments showed that all three species of bees did not change their foraging choices in the presence of an empty flower that contained differed in nectar concentration and access to nectar. While it may appear from this thesis that decoys are not very common in bees, effects are likely sensitive to particular attribute configurations. Artificial flowers allow for the opportunity to test a range of attribute configurations; indeed, this was an initial aim of my thesis that needed to be adjusted due to Covid19 impacts. In future, I suggest testing different attribute combinations on a single bee species to establish what attributes are likely to cause decoy effects. For example, attributes such as nectar volume, flower handling difficulty (Krishna & Keasar, 2021), the temperature of flowers and reward, pollen quality and quantity (Ruedenauer et al., 2016), could be considered for decoy experiments.

To test which attributes result in decoy effects, it may be beneficial to start by testing attribute configurations on *A. mellifera*, both in the presence and absence of social behaviour. It is possible to train individuals to forage within an experimental box, void of social behaviour, making them a good species for individual foraging tasks. Decoy attribute conditions could be tested on individually foraging honey bees, as well as honey bees in the presence of conspecifics. While I was unable to test the effects of empty flowers on dance behaviour, future studies should also incorporate this method into experiments on honey bees involving decoys. By incorporating dance behaviour, we will be able to understand the impact of empty flowers on the overall ‘rating’ of a patch. It would also allow us to understand if social behaviour impacts the individual bees' willingness to return to a patch containing empty flowers, which could be tested by looking at the effects of decoys on honey bees when the dance was disrupted, or allowed to occur (Nürnberger et al., 2017).

Decoys have been tested on diverse taxa and they are important in the decision-making of many animals. Organisms including cats (Scarpi, 2011), bees (Shafir et al., 2002; Tan et al., 2015), birds (Bateson et al., 2003; Shafir et al., 2002) primates (Parrish et al.,

2015) and even slime moulds (Latty & Beekman, 2011) are susceptible to the effects of unattractive decoys. In contrast, species such as mice (Rivalan et al., 2017) and bats (Hemingway et al., 2017) are not impacted by unattractive decoys. Ants in groups also appear to be less impacted by unattractive decoys than are individuals (Edwards & Pratt, 2009; Sasaki & Pratt, 2011). In contrast to most previous studies, my thesis has shown that decoys do not have a strong effect on the foraging preferences of bees. It is possible that my experimental choice sets involved attributes that didn't lead to decoy effects, or that other variables (e.g. animal state, environmental conditions, details of the feeders) prevented the emergence of decoy effects. The lack of null result studies to compare to could simply be a desk drawer problem (Rosenthal, 1979), where null decoy effects have been observed, but not published. Future research, using a wider variety of choice sets would be needed to rule out the possibility that my focal taxa are susceptible to decoy effects.

Studies from humans suggest that phantom decoys can impact decision-maker behaviour beyond shifting preference relationships. Unavailable or sold-out items can cause an increased sense of urgency where people will be more likely to make quick, inefficient decisions in the presence of unavailable options; this is known as the "immediacy effect" (Ge et al., 2009). Our understanding of speed-accuracy trade-offs in bumble bees, *B. terrestris* suggest that some individual bees prioritise either speed or accuracy when choosing flowers (Chittka et al., 2003). Individuals prioritising speed over accuracy may be less likely to wait for high-quality food to return, and feed on lower-quality food sources in the meantime. Bumble bees, *B. impatiens* can also learn reward schedules and can time their foraging to reward availability (Boisvert et al., 2007; Boisvert & Sherry, 2006). The impact of reward intervals on foraging choice could be tested experimentally by training bees to forage on high-quality flowers that are only refilled at set intervals. Foraging choice could then be tracked during intervals of empty and replenished flowers. This would be compared to foraging intervals by bees trained to the original intervals on flowers that had constant nectar available.

A promising next step in this research would be to investigate bee behaviours in the context of real flowers. However, using real flowers has its challenges. Real flowers are also much harder to control with variation in attributes such as the number of flowers on the plant, number of inflorescences, temporal changes in nectar availability and blooming time occurring between plants of the same species. For example, during the early stages of work for this thesis, I trialled an experiment in which I placed sets of co-flowering plants in community gardens (Figure 6.4). Many plants stopped flowering early or had vast variations in the number of inflorescences. This experiment did, however, gather foraging data from a small number of mostly solitary bees from the Megachilidae family. From an ecological point of view, real flowers offer a more realistic foraging experience to the floral visitors. However, from a logistical standpoint, the cost to purchase and upkeep plants is much higher and there are significant time windows that need to be considered to have multiple species that bloom at the same time.

Figure 6.4: Testing the effects of phantom decoys in real flowers is possible by creating small patches of flowers, where the most popular flower becomes inaccessible, as seen here by the *Salvia* plants covered in transparent plastic.

Expanding our understanding of foraging choice beyond model species

The study of foraging choice, cognition and behaviour in bees is strongly biased toward honey bees and bumble bees. In the artificial flower chapter, the majority of studies focussed on *A. mellifera*, *B. impatiens* and *B. terrestris.* These three species are capable of complex tasks, including string-pulling (Alem et al., 2016), proto-counting (Chittka &

Geiger, 1995), numerical discrimination (Howard et al., 2018) addition and substraction (Howard et al., 2019) and assessing flwer quality (Cnaani et al., 2006; Muth et al., 2016). Cognition in bees is often tested using foraging experiments and artificial flowers (Çakmak & Wells, 2001; Smithson & Gigord, 2003; Wells et al., 1986). In Australia, there are over 2000 species of bees, with only 11 coming from eusocial groups (none of which are bumble bees or honey bees). There is a great capacity to learn about learning and cognition beyond social bees in Australia alone.

Social species only make up a small percentage of the foraging bees across the world. While many plants are often highly visited by social species like honey bees (Gilpin et al., 2019b; Landry, 2013; Ojija et al., 2019), the majority of bees are solitary, and therefore there is a large gap in our understanding of foraging behaviour of bees. In Australia, *Lassioglossum spp* has shown promise as a solitary bee species that can be used for cognition and learning experiments (Howard, 2021). In North America, *Megachile rotundata* or *Osmia spp.* (Bosch & Kemp, 2002; Leonard & Harmon-Threatt, 2019) are commercially available solitary species and may be a good experimental species for understanding solitary bees' behavioural responses to empty flowers.

Even some social bee species have been largely overlooked to date in many studies of foraging choice. This thesis provided a springboard into future behavioural studies on stingless bees in Australia. Currently, there are 11 species of stingless bees in Australia, with limited information on their foraging behaviour, including decision-making and social foraging. As a whole, minimal research has been conducted in controlled environments testing the preferences and social behaviour of stingless bees in Australia. Given the limited available information for Australian stingless bees, we should consider behavioural experiments that aim to understand their innate preferences for floral shapes, size colour and scent. I have shown that it is possible to train stingless bees to artificial flowers, making them good candidates for manipulative choice experiments (Figure 6.5). By learning more about the floral preferences of stingless bees, particularly regarding floral trait preferences, we will better understand how they can be effectively used as pollinators.

Figure 6.5: It is possible to train *T. carbonaria* to forage on artificial flowers, making them a great option for future studies on bee behaviour and cognition.

Concluding remarks

By studying empty flowers in a floral neighbourhood through the lens of the phantom decoy effect, I advanced our understanding of how empty flowers influence bee foraging behaviour and therefore pollination. Taking a cross-continental and multi-species approach to understanding the impacts of empty flowers, I was able to show that species showed variation in their choice to forage on flowers when empty flowers were present and that social species utilise information from their nestmates greatly to make decisions. My results can serve as a springboard for future experiments where artificial flowers can be used to understand floral visitor behaviour. My thesis also offers an important addition to the understanding of cognition and floral choice in three bee species.

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Appendices

Supplementary Table 1: Experiments used for artificial flowers review. Keywords refer to keywords associated with study. Flower type is defined as flowers with a simple or complex shape, containing a reward, or no reward. Single species focus refers to if only one species was studied in isolation.

Citation	Keywords	Flower Type	Reward	Attributes considered	Study Taxa Species	Single Species Focus	Colour Used	Colour Reproducibility	Scents Used	trained to use flowers	Environment
(Cembrowski et al., 2014)	ant pollinator conflict, pollination, competition, *Bombus impatiens*, myrmica rubra	simple reward	both	scent, male/female fitness	*Bombus impatiens*	Yes	blue	colour listed	ant pheromones	trained for 4 days	flight cage
(Thomson et al., 2012)	bumble bee, food dye, male fitness, pollen analogue, pollinator visitation, reproductive success	simple reward	both	colour, nectar concentration	*Bombus impatiens*	Yes	blue	colour listed, paint brand provided	none	trained for 2-3 days	flight cage
(Makino, 2008)	bumble bee, foraging rate, inclination of the ground, pollination, sloping habitats	simple reward	nectar	flower angle	*Bombus ignitus*	Yes	yellow	colour listed	none	trained for unknown time period	flight cage
(Real, 1981)	*Bombus* sandersoni, bumble bee, foraging behaviour, gene flow, nectar, optimisation, pollination, uncertainty, vespula vulgaris, wasps	simple reward	nectar	colour, nectar volume	*Bombus sandersoni, Vespula vulgaris*	Yes (but multiple species studied separately)	blue, yellow	colour listed	none	trained for a few hours	flight cage
(Smithson & Macnair, 1996)	frequency dependent selection, assortative mating, pollination, colour preferences, learning	simple reward	nectar	colour, colour frequency, nectar concentration	*Bombus terrestris*	Yes	blue, yellow	spectrophotometer used but info not provided	none	trained for unknown time period	flight cage
(Hartling & Plowright, 2011)	na	simple reward	nectar	nectar presence/absence	*Bombus atratus*	Yes	white	colour listed	honey	trained for unknown time period	flight cage

(Dukas, 2001)	bees, flowers, pollination, predation, spiders	simple reward	nectar	predator presence/absence	*Apis mellifera*	Yes	blue	colour listed	anis	Trained using von Frisch for unknown time period	near apiary
(null Keasar, 2000)	na	simple reward	nectar	flower spatial arrangement, colour, nectar presence/absence	*Bombus terrestris*	Yes	white, green, blue, yellow	cite previous spectrophoto meter details	none	na	flight cage
(Muchhala, 2007)	burmeistera, disruptive selection, chiropterophily, fitness trade off, floral evolution, ornithophily	realistic reward	both	corolla flare, male/female flowers	*Anoura geoffroyi, Adelomyia melanogenys*	Yes (but multiple species studied separately)	na	na	honey	trained for 3 hours	flight cage
(Møller & Sorci, 1998)	developmental stability, fluctuating asymmentry, plant sexual selection, pollinator rewards	simple rewardless	none	floral symmetry	*Range of Insects*	No	yellow, red	colour listed	none	na	wild
(von Helversen et al., 2000)	bat pollination, olfactory orientation, floral scents, sulphur compounds, dimethyl disulphide	simple rewardless	none	scent	*Glossophaga soricina*	Yes	na	na	20 scent compounds	na	flight cage
(Alm et al., 1990)	amino acids, apis *mellifera*, pieris rapae, nectars pollinators	simple reward	nectar	colour, amino acid presence/absence	*Pieris rapae, Apis mellifera*	Yes (but multiple species studied separately)	blue, purple, white	colour listed	none	trained for 3 days	flight cage, near apiary
(Gegear & Laverty, 1998)	na	simple reward	nectar	colour, flower handling	*Bombus impatiens*	Yes	blue	colour listed	none	na	flight cage
(Ballantyne & Willmer, 2012)	ant nectar thieves, associative learning, foraging strategies, pollinator behaviour, scent marks	simple reward	nectar	scent	*Bombus terrestris*	Yes	na	na	ant pheromones	na	flight cage

(Russell et al., 2017)	division of labor, interindividual variation, flower constancy, bee, hymenoptera, preferences	simple reward	nectar and pollen separately	pollen and nectar	*Bombus impatiens*	Yes	white	colour listed, brand of lid provided	none	trained for unknown time period	flight cage
(Russell & Papaj, n.d.)	artificial flowers, behavioural assay, bumble bee, pollen collection, learning, pollinator behaviour	simple reward	pollen	colour, feeder type	*Bombus impatiens*	Yes	blue, yellow	colour listed	none	na	flight cage
(Giger & Srinivasan, 1995)	vision, template, orientation analysis, honey bee, apis *mellifera*	simple reward	nectar	pattern, nectar presence/absence	*Apis mellifera*	Yes	black, white	colour listed	none	trained for unknown time period	near apiary
(Gumbert, 2000)	colour vision, colour learning, generalisation, innate preferences, bumble bee	simple reward	nectar	colour, nectar presence/absence	*Bombus terrestris*	Yes	violet, dark blue, light blue, blue-green, blue, dark green, light green, orange, red	brands listed	none	trained for unknown time period	flight cage
(Chittka & Thomson, 1997)	*Bombus impatiens*, flower constancy, memory, motor learning, specialisation	simple reward	nectar	colour, nectar presence/absence	*Bombus impatiens*	Yes	blue, yellow	colour listed	none	trained for unknown time period	flight cage
(Gegear, 2005)	odour, flower visit, floral trait, floral diversity, pollinator behaviour	simple reward	nectar	size, scent	*Bombus impatiens*	Yes	blue	colour listed	clove, peppermint oil	trained for 3 hours	flight cage
(Muth et al., 2016)	associative learning, *Bombus impatiense*, bumble bee, colour preference, memory, pollen	simple reward	pollen	colour, pollen presence/absence	*Bombus impatiens*	Yes	white, blue, yellow	spectrophotometer	none	trained for unknown time period	flight cage
(Konzmann & Lunau, 2014)	na	simple reward	both	colour, pollen quality, nectar quality	*Bombus terrestris*	Yes	blue, green-orange	brands listed	none	na	flight cage

(Nicholls & de Ibarra, 2014)	pollen, learning, preferences, evaluation, bumble bees	simple reward	pollen	colour, pollen quality	*Bombus terrestris*	Yes	blue, green-orange, white	spectrophoto meter	none	trained for unknown time period	flight cage
(Lunau, 2011)	na	simple reward	none	pollen types, scent	*Bombus terrestris, Bombus lucorum*	Yes (but multiple species studied separately)	blue	spectral purity measured	n-Hexane, pollen scents	na	flight cage
(Roy & Raguso, 1997)	halictid bees, diptera, floral fragrance, pollination ecology, rust fungi	simple rewardless	none	scent	*Range of Insects*	No	white, yellow	colour listed	floral scent	na	wild
(Raguso & Willis, 2005)	na	simple reward	nectar	scent	*Manduca sexta*	Yes	white	spectrophoto meter	floral scent	na	wild
(Burger et al., 2010)	anchusa, echium, floral scent and colour, foraging behaviour, foraging naïve and experienced, hoplitis adunca, multimodal stimuli, specialised solitairy bee	simple rewardless	none	colour, scent	*Hoplitis adunca*	Yes	blue, yellow	spectrophoto meter	floral scent	na	flight cage
(Giurfa & Núñez, 1992)	honey bee, scent mark, movement pattern, foraging behaviour, foraging efficiency	simple reward	nectar	scent	*Apis mellifera*	Yes	yellow	colour listed	scent marks	trained for unknown time period	near apiary
(Leonard et al., 2013)	flowers, bees, foraging, flowering plants, pollen, sucrose, honey bees, pollination	simple reward	nectar	nectar guide presence/absence	*Bombus impatiens*	Yes	blue	brand listed	none	trained for multiple days	flight cage
(Shafir et al., 2002)	asymmetric dominance, context dependent preferences, foraging, rationality, regularity	simple reward	nectar	corolla length, nectar volume	*Apis mellifera, Perisoreus canadensis*	Yes (but multiple species studied separately)	blue, yellow, white	colour listed	none	trained for unknown time period	flight cage, wild

(Rivest et al., 2017)	colour preference, pollination syndrome, environmental complexity, flower colour, *Bombus impatiens*, artificial flowers	simple reward	nectar	colour, background colour	*Bombus impatiens*	Yes	red, blue	spectrophoto meter	none	trained for unknown time period	flight cage
(Kawaguchi et al., 2006)	*Bombus terrestris*, foraging behaviour, information transfer, local enhancement, plant animal interactions	simple reward	nectar	conspecific presence/absence	*Bombus terrestris*	Yes	yellow	colour listed	honey	na	flight cage
(Witjes & Eltz, 2007)	flower discrimination, reppellant scent marks, chemical cue, signal, bumble bees, *Bombus*	simple reward	nectar	scent	*Bombus terrestris*	Yes	yellow	colour listed	none	na	flight cage
(Slaa et al., 1998)	flower constancy, foraging strategies, stingless bees, meliponinae, trigona fulviventris, trigona fuscipennis, trigona nigra	simple reward	nectar	colour, scent	*Trigona fuscipennis, Trigona fulviventris, Trigona nigra*	No	blue, yellow	brand listed	peppermint oil, rosewood oil	trained for unknown time period	wild
(null Hill et al., 1997)	na	simple reward	nectar	colour, nectar presence/absence	*Apis mellifera*	Yes	blue, yellow, white	spectrophoto meter, brand listed	none	trained for unknown time period	near apiary
(Wells & Rathore, 1994)	apis cerana, apis *mellifera*, honey bees, foraging, sugars	simple reward	nectar	colour, nectar composition, nectar concentration	*Apis cerana*	Yes	blue, yellow, white	brand listed	cinnamon oil	trained for unknown time period	wild
(Church & Plowright, 2006)	bumble bees, spatial memory, egocentric information, landmark	simple reward	nectar	colour, nectar presence/absence	*Bombus impatiens*	Yes	yellow	colour listed	none	trained for unknown time period	flight cage

(Spaethe et al., 2001)	vision, detection, *Bombus* terrastris, ultraviolet, neuronal channel	simple reward	nectar	colour, size	*Bombus terrestris*	Yes	yellow, white, blue, turquoise, red, lemon	spectrophoto meter	none	trained for unknown time period	flight cage
(Kelber, 1997)	macroglossum stellatarum, hawkmoth, sphingidae, Lepidoptera, spontaneous choices, innate behaviour, colour vision, pattern vision	simple reward	none	colour, size, background contrast, pattern	*Macroglossum stellatarum*	Yes	violet, blue, blue-green, yellow-green, yellow, orange, red, white	spectrophoto meter, brand listed	none	na	flight cage
(Gegear & Laverty, 2005)	na	simple reward	nectar	colour, size, scent, handling	*Bombus impatiens*	Yes	blue, orange, yellow, purple		peppermint oil, clove oil	trained for two days	flight cage
(Cnaani et al., 2006)	na	simple reward	nectar	nectar volume, nectar concentration	*Bombus impatiens*	Yes	yellow	colour listed	none	na	flight cage
(Mainali & Lim, 2011)	frankliniella occidentalis, sticky card, flower model, olfactometer, anisaldehyde, ethyl nicotinate	simple rewardless	none	colour, shape, scent, symmetry	*Frankliniella occidentalis*	Yes	yellow, white	spectrophoto meter	p-Anisaldehyde, ethyl nicotinate, floral scents		flight cage
(Sanderson et al., 2006)	na	simple reward	nectar	colour, nectar volume, corolla length	*Apis mellifera*	Yes	blue, yellow, white	spectrophoto meter, brand listed	none	na	near apiary
(Blarer et al., 2002)	na	simple reward	nectar	colour, size	*Bombus terrestris*	Yes	blue, yellow	colour listed	none	na	flight cage
(Lunau et al., 2006)	colour pattern, flight path, colour contrast, colour patch, spectral purity	simple reward	nectar	nectar guides	*Bombus terrestris*	Yes	violet, yellow, light yellow, blue	brand listed	none	na	flight cage
(Karahan et al., 2015)	apis *mellifera*, foraging behaviour, neonicitinoids	simple reward	nectar	pesticide dose, colour, nectar concentration	*Apis mellifera*	Yes	blue, white	spectrophoto meter, brand listed	none	trained for unknown time period	near apiary

(Yoshioka et al., 2007)	bumble bee, computer graphics, elliptic fourier descriptiors, fower corolla shape, primula sieboldii, principal component, visual cue	realistic reward	nectar	shape	*Bombus ignitus*	Yes	pink	colour listed	none	trained for 2 days	flight cage
(Goyret & Raguso, 2006)	pollination, lepidoptera, sensory, multimodal, sphingidae	realistic reward	nectar	corolla texture	*Manduca sexta*	Yes	grey	brand listed	bergamot oil	na	flight cage
(Gegear & Thomson, 2004)	na	simple reward	nectar	colour, nectar volume, distance between flowers	*Bombus impatiens*	Yes	blue, yellow	spectrophoto meter	none	trained for 4 hours	flight cage
(Good et al., 2014)	na	realistic reward	nectar	bacteria presence	*Apis mellifera*	Yes	yellow	colour listed	none	na	near apiary
(Harrap et al., 2017)	na	simple reward	nectar	floral temperature signature	*Bombus terrestris*	Yes	white, yellow	colour listed	none	trained for unknown time period	flight cage
(P. S. M. Hill et al., 2001)	na	simple reward	nectar	nectar volume, colour	*Apis mellifera*	Yes	blue, yellow, white	spectrophoto meter, brand listed	none	trained for unknown time period	near apiary
(Sprayberry & Daniel, 2007)	hawkmoth, flower tracking, feeding rate	realistic reward	nectar	floral movement	*Manduca sexta*	Yes	white	na	none	na	flight cage
(İ. Çakmak & Wells, 2001)	apis *mellifera* races, honey bees, foraging, prey predator relationship, artificial flowers	simple reward	nectar	colour, nectar presence/absence	*Apis mellifera*	Yes	blue, yellow, white	spectrophoto meter	clove oil	trained for unknown time period	near apiary
(Lunau & Wacht, 1997)	eristalis, hoverfly, flower visitation, colour preference, pollen, floral guide	simple reward	nectar	colour	*Eristalis tenax*	Yes	blue, yellow, UV	spectrophoto meter	none	na	flight cage

(Temeles et al., 2009)	coevolution, eulampis jugularis, foraging trade offs, heliconia, hummingbird, mutualism, purple throated carib, specialisation, species interactions, West Indies	realistic reward	nectar	handling	*Eulampis jugularis*	Yes	red	colour listed	none	trained for unknown time period	flight cage
(Ishii et al., 2008)	artificial, flower, *Bombus*, nectar, pollinator	simple reward	nectar	inflorescence traits	*Bombus hypocrita*	Yes	yellow	colour listed	none	trained for unknown time period	flight cage
(Smith et al., 1996)	pollination, archilochus colubris, monanda, feeding, nectar guides	simple reward	nectar	shape, size	*Archilochus colubris*	Yes	na	na	none	trained for 1 day	flight cage
(Ômura & Honda, 2005)	flower, selection, attractive, colour, scent, preference, hierarchy, taraxacum officinale, cirsium japonicum	simple rewardless	none	colour, scent	*Vanessa indica*	Yes	yellow, red, orange, purple, green, blue	spectrophoto meter	none	na	flight cage
(T. Keasar et al., 1996)	bumble bees, foraging, movement rules, innate behaviour	simple reward	nectar	colour, nectar presence/absence	*Bombus terrestris*	Yes	blue, green, white	colour listed	none	na	flight cage
(T. Keasar et al., 1997)	na	simple reward	nectar	colour	*Bombus terrestris*	Yes	blue, green, white	spectrophoto meter	none	trained for unknown time period	flight cage
(West & Laverty, 2011)	na	simple reward	nectar	shape, symmetry, nectar guides	*Bombus impatiens*	Yes	blue	colour listed	honey	trained for unknown time period	flight cage
(Gegear et al., 2005)	na	simple reward	nectar	handling	*Bombus impatiens*	Yes	blue	colour listed	none	na	flight cage

(Otterstatter et al., 2005)	behaviour, *Bombus*, crithidia, bombi, foraging, locustacarus buchneri, parasites	simple reward	nectar	colour	*Bombus impatiens*	Yes	blue, yellow	colour listed	none	trained for unknown time period	flight cage
(Kandori & OhSaki, 1998)	associative learning, innate response, foragine on flowers	simple reward	nectar	colour, nectar guide	*Pieris rapae*	Yes	blue, red, yellow	spectrophotometer	none	na	flight cage
(Mainali & Lim, 2008b)	frankliniella occidentalis, sticky card, flower model, olfactometer, anisaldehyde, ethyl nicotinate	realistic rewardless	none	shape	*Frankliniella occidentalis, Frankliniella intonsa*	Yes (but multiple species studied separately)	yellow	brand listed	none	na	flight cage
(Mainali & Lim, 2008a)	frankliniella occidentalis, sticky card, flower model, olfactometer, anisaldehyde, ethyl nicotinate	realistic rewardless	none	shape	*Trialeurodes vaporariorum*	Yes (but multiple species studied separately)	yellow	brand listed	none	na	flight cage
(Gegear & Laverty, 2011)	na	simple reward	nectar	colour	*Bombus impatiens, Apis mellifera*	Yes (but multiple species studied separately)	blue, yellow	spectrophotometer	none	trained for up to 5 days	flight cage
(Slaa et al., 2003)	flower constancy, foraging conditions, stingless bee	simple reward	nectar	scent, colour	*Trigona dorsalis, Oxytrigona mellicolor*	Yes (but multiple species studied separately)	blue, yellow	colour listed	aniseed oil, rosewood oil	trained for unknown time period	near nest
(Waller et al., 1973)	na	simple reward	nectar	scent, nectar concentration	*Apis mellifera*	Yes	na	na	citral, geraniol, ocimene, myrcene, limonene, linalool	na	flight cage
(Ladurner et al., 2005)	oral toxicity test, artificial flower, osmia lignaria, apis *mellifera*, megachile rotundata	simple reward	nectar	scent	*Osmia lignaria, Megachile rotundata, Apis mellifera*	Yes (but multiple species studied separately)	blue	colour listed	none	training was treatment	flight cage

(Wells et al., 1986)	na	simple reward	nectar	colour, flower frequency	*Apis mellifera*	Yes	blue, yellow	brand listed	none	na	near apiary
(Cisarovsky & Schmid-Hempel, 2014)	crithidia bombi, *Bombus* terrastris, trypanosomatidae, hymenoptera, apidae, kinetoplastea, transmission pathway, social insect, pollinator, artificial flower	simple reward	nectar		*Bombus terrestris*	Yes	blue	colour listed	none	trained for 3 days	flight cage
(Jones et al., 2015)	*Bombus terrestris*, decision making, flower choice, foraging, innate colour, bias, social learning	simple reward	nectar	colour, nectar concentration, social information	*Bombus terrestris*	Yes	blue, yellow	colour listed	none	na	flight cage
(Wiegmann et al., 2003)	foraging, negative incentive contrast, flower constancy, choice behaviour	simple reward	nectar	colour, nectar concentration	*Bombus impatiens*	Yes	blue	colour listed	none	na	flight cage
(Tang et al., 2013)	behavioural tests, colour selection, EAG responses, fruit feeding, foraging adults, volatile compounds	simple reward	nectar	scent	*Kallima inachus*	Yes	red, purple, white, yellow	colour listed	fermented juice, ethanol	na	flight cage
(Weiss & Papaj, 2003)	na	simple reward	nectar	colour, nectar presence/absence	*Battus philenor*	Yes	yellow, green, blue	spectrophoto meter	none	na	flight cage
(Satoh et al., 2016)	behaviour, colour vision, nocturnal moth	simple reward	nectar	colour, nectar presence/absence	*Helicoverpa armigera*	Yes	blue, green, yellow, red, grey	spectrophoto meter	none	na	flight cage
(Kandori et al., 2009)	associative learning, forewing length, innate colour preference, lepidoptera, lifespan	simple reward	nectar	colour, nectar presence/absence	*Idea leuconoe, Argyreus hyperbius, Pieris rapae, Lycaena phlaeas*	Yes (but multiple species studied separately)	red, red-purple, blue, green, yellow, yellow-green, orange, brown, light	colour listed	none	na	flight cage

							blue, white, pink, purple				
(Rodrigues & Weiss, 2012)	na	simple reward	nectar	colour, nectar presence/absence	*Danaus plexippus*	Yes	blue, yellow, red	spectrophoto meter	none	trained for four days	flight cage
(Kandori & Yamaki, 2012)	positive associative learning, appetitive learning, negative associative learning, aversive learning, aversion learning habituation	simple reward	nectar	colour, nectar presence/absence	*Byasa alcinous*	Yes	red, red-purple, purple, blue, green, yellow-green, yellow, orange, brown, light blue, white, pink	spectrophoto meter	none	na	flight cage
(Rodrigues & Weiss, 2012)	na	simple reward	nectar	colour, nectar volume, nectar presence/absence	*Danaus plexippus*	Yes	purple, yellow, blue, green	spectrophoto meter	none	na	flight cage
(Kinoshita & Arikawa, 2014)	foraging behaviour, insect colour, brightness, polarisation	simple reward	nectar	colour, contrast, brightness, polarised light	*Papilio xuthus*	Yes	blue, green, yellow, red	spectrophoto meter	none	na	flight cage
(Kinoshita et al., 2012)	vision, insect, compound eye, neuroethology	simple reward	nectar	colour, brightness	*Papilio xuthus*	Yes	red, orange, purple	spectrophoto meter	none	trained for unknown time period	flight cage
(Yoshida et al., 2015)	colour vision, olfaction, innate preference, sexual dimorphism, lepidoptera, foraging	simple rewardless	none	colour, scent	*Papilio xuthus*	Yes	blue, green, yellow, red	spectrophoto meter	lavender oil, bitter orange oil, floral scents, synthetic scent mimics	na	flight cage
(Hannah et al., 2019)	chromatic signal, colour model, floral colour, fly pollination, plant pollinator, vision	simple reward	both	colour, nectar presence/absence	*Eristalis tenax*	Yes	blue, yellow	spectrophoto meter	honey, pollen	trained for 3 days	flight cage
(Blackiston et al., 2011)	monarch, lepidoptera, learning, vision,	simple reward	nectar	colour, nectar presence/absence	*Danaus plexippus*	Yes	red, orange, yellow, blue, purple, green	spectrophoto meter	none	trained for unknown time period	flight cage

	innate colour preference										
(Orbán & Plowright, 2013)	visual properties, bumble bees, visual stimuli, flowers	simple rewardless	none	flower pattern	*Bombus impatiens*	Yes	blue	RGB values	none	na	flight cage
(Wiegmann et al., 2000)	discrimination, foraging, relational learning, transposition	simple reward	nectar	colour, flower height, nectar presence/absence	*Bombus impatiens*	Yes	blue, yellow	colour listed	none	trained for unknown time period	flight cage
(T. Keasar et al., 2013)	artificial flower, *Bombus*, exploitation exploration trade off, foraging, handling time, learning, pollination, travel time	simple reward	nectar	colour, nectar refilling probability, nectar presence/absence	*Bombus terrestris*	Yes	white, green, blue, yellow	colour listed	none	trained for unknown time period	Enclosed
(Tofilski, 2000)	senescence, learning, foraging, honey bee, apis *mellifera*	simple reward	nectar	colour, nectar presence/absence	*Apis mellifera*	Yes	na	na	none	na	Enclosed
(I. Çakmak et al., 2010)	foraging, behaviour, honey bee, subspecies, apis *mellifera*	simple reward	nectar	colour, nectar volume	*Apis mellifera*	Yes	blue, yellow, white	spectrophotometer, brand listed	none	trained for unknown time period	near apiary
(Petrikin & Wells, 1995)	honey bees, foraging, insect behaviour, apiculture, biological pigments, bumble bees, nectar, entomology	simple reward	nectar	patterns, nectar composition	*Apis mellifera*	Yes	white	spectrophotometer, brand listed	clove oil	trained for unknown time period	near apiary
(Decourtye et al., 2004)	apis *mellifera*, imidacloprid, deltamethrin, sublethal effect, associative	simple reward	nectar	pesticide dose, nectar concentration, scent	*Apis mellifera*	Yes	na	na	linalool	trained for unknown time period	flight cage

	learning, olfactory conditioning										
(Patt et al., 1997)	searching behaviour, biocontrol, parasitoids, edovum puttleri, pediobius foveolatus, intercropping, floral architecture, antheum graveolens, foeiculum vulgare, coriandrum satvium	simple reward	nectar	nectar composition, flower handling	*Pediobius foveolatus, Edovum puttleri*	Yes	yellow	colour listed	honey	na	flight cage
(Johnson et al., 2006)	aloe vryheidensis, bird pollination, flower models, honest signal, mutualism, nectar colour, nectar palatability, pollination syndrome, South Africa, specialisation, sun bird	simple reward	nectar	colour	*Pycnonotus tricolour, Zosterops pallidus, Chalcomitra amethystina*	Yes (but multiple species studied separately)	yellow	colour listed	none	na	flight cage
(Johnson & Dafni, 1998)	behaviour, bombyliidae, insect vision, linum pubescens, mimicry, pollination	simple rewardless	none	colour, size, shape, pattern	*Usia bicolour*	Yes	orange, pink, yellow, blue, white, red	spectrophoto meter	none	na	wild
(Frey & Bukoski, 2014)	experimental array, flowers, geranium robertianum, pollen production, pollination, symmetry	realistic reward	nectar	size, symmetry	*Toxomerus sp., Papillio sp.*	No	pink	colour listed	none	na	wild
(Jürgens et al., 2015)	carnivorous plants, drosera, flower trap distance,	realistic rewardless	none	colour	*Range of Insects*	No	green, red, white	spectrophoto meter	none	na	wild

	pollinator prey conflict, trap colour										
(Johnson & Midgley, 2001)	foraging behaviour, hopliinae, insect vision, mimicry, plant pollinator interactions	simple reward	none	colour	*Monkey beetles*	No	red, orange, yellow, blue	upon request	none	na	wild
(Ne'eman & Kevan, 2001)	honey bee, vision, floral shape, parameters, detection, distance	simple reward	nectar	size	*Apis mellifera*	Yes	blue	spectrophotometer, brand listed	none	trained for unknown time period	enclosed
(Dyer & Murphy, 2009)	batesian, mimicry, psychophysics, decision, model	simple reward	nectar	colour, nectar presence/absence	*Apis mellifera*	Yes	blue	spectrophotometer	none	trained for unknown time period	near apiary
(Dyer & Chittka, 2004)	ultraviolet, vision, foraging efficiency, greenhouse, bumble bee, *Bombus terrestris*	simple reward	nectar	colour	*Bombus terrestris*	Yes	yellow	spectrophotometer	none	na	flight cage
(Sutherland et al., 1999)	episyrphus balteatus, syrphidae, foraging, flowers, preferences	simple reward	both	colour, pollen volume, nectar concentration	*Episyrphus balteatus*	Yes	yellow, green, white, blue, cream, yellow-green	spectrophotometer	honey	na	flight cage
(Van Kleunen et al., 2007)	beetle marks, beetle pollination syndrome, cantharophily, Greater Cape Floral Region, convergent evolution, iridaceae, monkey beetles, pollinator attraction	simple rewardless	none	colour, pattern	*Monkey beetles*	No	yellow, orange, red	colour listed	none	na	wild
(Dafni & Potts, 2004)	amphicoma, beetles, depth perception, floral character, flower choice, vision	simple rewardless	none	corolla length, colour, flower orientation	*Amphicoma*	No	red	No	none	na	wild

(Zhang et al., 2012)	5-hydroxy flavium, anthocyanin, bird pollinator, dark purple nectar, foraging signal, Himalayas, leucosceptrum canum	simple reward	nectar	nectar colour	*Minla cyanouroptera, Zosterops palpebrosus, Apis mellifera*	Yes (but multiple species studied separately)	white	Yes	floral nectar	na	flight cage
(Free, 1970)	nectar, circle, honey bees, bumble bees, odours, insect behaviour, petals, aerial locomotion, foraging	simple reward	nectar	shape, pattern	*Apis mellifera*	Yes	blue, yellow	No	methyl heptenone	trained for unknown time period	near apiary
(de Jager et al., 2017)	*Bombus*, deception, insect behaviour, learning , nectar guide, plant pollinator interaction	simple reward	nectar	pattern, nectar guide	*Bombus terrestris*	Yes	orange	No	none	trained for unknown time period	flight cage
(Goyret, 2010)	flower handling, lepidoptera, nectar guides, pollinator, mechanoreception, sensory ecology	simple reward	nectar	colour, pattern, tactile	*Manduca sexta*	Yes	blue, white, black	No	none	trained for 3 minutes	flight cage
(Peixoto et al., 2012)	habitat selection, visual signals, chemical signals, optimal foraging theory, crab spiders, Brazil	realistic rewardless	none	colour, scent, morphology	*Epicadus heterogaster*	Yes	green, yellow, white, pink	No	prey extraction solution, honey	na	Enclosed
(Cameron, 1981)	alcohol, ethyl pentane, recent, past, ethyl alcohol	simple reward	nectar	scent, nectar presence/absence	*Bombus vosnesenskii*	Yes	blue	No	honey	trained for two days	flight cage
(Kunze & Gumbert, 2001)	na	simple reward	nectar	colour, scent	*Bombus terrestris*	Yes	blue, green, violet, orange	spectrophoto meter, brand listed	rose and clove oil	trained for unknown time period	flight cage
(Newman et al., 2012)	batesian mimicry, local adaptation,	simple reward	none	colour	*Aeropetes tulbaghia*	Yes	orange, red	spectrophoto meter	none	na	wild

	ecological divergence, disa ferruginea, pollinator selection, geographical colour variation										
(Hempel de Ibarra et al., 2001)	honey bee, colour vision, pattern, vision detection, compound eye	simple reward	nectar	colour, pattern	*Apis mellifera*	Yes	cyan, blue, orange, yellow, brown, violet	spectrophoto meter	none	na	flight cage
(Hempel De Ibarra et al., 2000)	honey bee, apis *mellifera*, colour vision, behaviour	simple reward	nectar	colour, brightness	*Apis mellifera*	Yes	white, blue, green, grey	spectrophoto meter	none	trained for unknown time period	flight cage
(Jersáková et al., 2012)	batesian floral mimicry, colour, disa, fly pollination, scent	realistic rewardless	none	colour, shape, nectar guides	*Philoliche aethiopica*	Yes	white, dark blue, blue, orange, yellow, dark pink, pink, red	spectrophoto meter	none	na	free flying
(Spaethe et al., 2006)	attention, visual cognition, colour vision, search asymmetries, foraging	simple reward	nectar	colour	*Apis mellifera*	Yes	white, light blue, yellow, orage, red, violet, blue	spectrophoto meter	none	trained for unknown time period	flight cage
(Wertlen et al., 2008)	na	simple reward	nectar	visual angle	*Apis mellifera, Bombus terrestris*	Yes (but multiple species studied separately)	violet, yellow	spectrophoto meter, brand listed	none	trained for unknown time period	flight cage
(Kipp, 2011)	na	simple reward	nectar	nectar	*Apis mellifera*	Yes	blue	colour listed	none	trained for unknown time period	near apiary
(Souza & Martins, 2004)	body size distribution, Brazilian savanna, guilds, habitat structure, inflorescence, fauna, patch choice, plant dwelling spider	realistic rewardless	none	flower presence	*Range of Spiders*	No	white, purple	colour listed	none	na	wild

(Chen et al., 2015)	amorphophallus, deceit, pollination, livor mortis, pigments, sapromyophily, spectral reflectance	simple rewardless	none	colour, scent	*Range of Flies*	No	red, white	spectrophoto meter	floral scent	na	wild
(Ishii, 2005)	bumble bee, constant flight, flower constancy, short term memory, visitation sequence	simple rewardless	none	colour	*Bombus hypocrita*	Yes	blue, yellow	colour listed	none	na	flight cage
(Jager & Ellis, 2012)	behaviour, divergent selection, floral diversification, mating signals, pollen export, sexual deception	simple rewardless	none	colour, texture, pattern, scent	*Megapalpus capensis*	Yes	orange	spectrophoto meter	receptive female extract, hexane, floral scent	na	flight cage
(Dafni et al., 1990)	na	simple rewardless	none	colour	*Amphicoma*	No	violet, blue, blue green, green, yellow, red	spectrophoto meter	none	na	wild
(Whitehead et al., 2019)	batesian mimicry, colour vision, conditioning flower, colour, learning, pollination, vision	realistic rewardless	none	colour	*Prosoeca ganglbaueri*	Yes	pink, white	spectrophoto meter	none	na	wild
(Essenberg et al., 2019)	floral rewards, foraging behaviour, geitonogamy, honest signals, patch departure pollination	simple reward	nectar	size, nectar presence/absence	*Bombus impatiens*	Yes	orange	colour listed	none	na	flight cage
(Nachev et al., 2017)	na	simple reward	nectar	nectar concentration	*Glossophaga commissarisi*	Yes	na	na	none	na	wild
Muth et al., 2016	pollen, *Bombus*, pollination, taste	simple reward	pollen	colour	*Bombus impatiens*	Yes	blue, yellow	spectrophoto meter	none	trained for unknown time period	flight cage

(Heuschen et al., 2005)	colour pattern, floral guide, pollen, bumble bee, *Bombus terrestris*, innate behaviour, mimicry system	simple reward	pollen	colour, netar guides, patterns	*Bombus terrestris*	Yes	violet, blue green	spectrophotometer, brand listed	none	na	flight cage
(Farina et al., 1994)	hawk moth, hovering flight, distance regulation, speed compensation	simple reward	nectar	patterns	*Macroglossum stellatarum*	Yes	blue	spectrophotometer	honey	na	flight cage
(Dinkel & Lunau, 2001)	floral guide, eristalis, flower recognition, proboscis extension, innate reaction	simple reward	nectar	colour, nectar guide	*Eristalis tenax*	Yes	yellow, white	spectrophotometer	none	na	flight cage
(Josens & Farina, 2001)	macroglossum stellatarum, hawk moths, nectar feeding, intake rate, nectar viscosity	simple reward	nectar	nectar concentration	*Macroglossum stellatarum*	Yes	blue	colour listed	none	na	flight cage
(Farina & Josens, 1994)	food source, visual stimulus, compensatory response, source profitability, food source profitability	simple reward	nectar	nectar concentration	*Macroglossum stellatarum*	Yes	blue	colour listed	none	na	flight cage
(Nuzhnova & Vasilevskaya, 2013)	pieris napi, ethological experiment, colour preferences, foraging behaviour	simple reward	nectar	colour	*Pieris napi*	Yes	blue, red, yellow	spectrophotometer, brand listed	none	na	flight cage
(Muchhala & Thomson, 2009)	Darwin's Race, chiropterophily, anoura fistulata, bat pollination, centropogon nigricans, coevolution	simple reward	nectar	corolla length	*Anoura fistulata*	Yes	na	na	none	na	flight cage

(Dunlap et al., 2016)	na	simple reward	nectar	colour, nectar concentration	*Bombus impatiens*	Yes	orange, yellow	spectrophoto meter	none	trained for unknown time period	flight cage
(Fenster et al., 2015)	correlational selection, floral evolution, pollination syndrome, trait interaction	realistic rewardless	nectar	flower height, flower orientation	*Archilochus colubris*	Yes	red, white, pink	spectrophoto meter	none	na	wild
(Schemske & Agren, 1995)	begonia involucrata, deceit pollination, floral evolution, mimicry, pollination, natural selection, trade offs	realistic rewardless	none	size	*Range of Insects*	No	white	colour listed	none	na	wild
(Ohashi et al., 2010)	artificial flowers, *Bombus*, foraging, LED sensors, renewing resources, RFIDs, spatial use	simple reward	nectar		*Bombus impatiens*	Yes	blue	colour listed	none	na	flight cage
(Policha et al., 2016)	cloud forest, dracula lafleurii, drosophilidae, GC-MS, neotropical, orchidaceae, pollination	realistic rewardless	none	shape, scent, colour	*Range of Flies*	No	white	spectrophoto meter	floral scent	na	wild
(du Plessis et al., 2018)	carrion flower, functional trait, house fly, orbea variegata, oviposition site mimicry, sapromyophily	realistic rewardless	none	colour, scent pattern	*Range of Flies*	No	yellow, brown	spectrophoto meter	floral scent	na	free flying
(Pohl et al., 2008)	learning, floral colour pattern, dichogamy, floral guide, bumble bee, colour preference, mimicry	simple reward	nectar	colour, nectar concentration	*Bombus terrestris*	Yes	blue	colour listed	none	trained for unknown time period	flight cage

(Tello-Ramos et al., 2015)	daily timing, ordinal timing, rufous hummingbirds, time-place learning, traplining	simple reward	nectar	flower frequency, nectar concentration, nectar volume	*Selasphorus rufus*	Yes	orange	No	none	trained for unknown time period	free flying
(Howard et al., 2019)	angiosperm, apis *mellifera* (Western honey bee), bird pollinated, flower, insect pollinated, pollinator	simple reward	none	shape	*Apis mellifera*	Yes	range	spectrophoto meter	none	trained for unknown time period	near apiary
(Thompson & Plowright, 2014)	picture–object correspondence, generalisation, bumble bee, *Bombus*	realistic reward	nectar	shape, coloure, nectar presence/absence	*Bombus impatiens*	Yes	yellow, red	colour listed	none	trained for unknown time period	flight cage
(Rohde et al., 2013)	*Bombus terrestris*, apis *mellifera*, colour preference, flower colour, spectral purity	simple reward	nectar	colour, spectral purity	*Apis mellifera, Bombus terrestris*	Yes	blue, cyan, purple	spectrophoto meter, HSB provided	apiinvert	trained for unknown time period	flight cage
(Vergara et al., 2011)	flower fragrance, Corolla visual display, larcenists, night-blooming plants	simple rewardless	none	scent, visual cues	*Blatta orientalis*	Yes	white	Yes	floral scent	na	wild
(Kulahci et al., 2008)	plant pollinator interactions, bumble bees, *Bombus*, multimodal signals, decision making, speed accuracy trade off	simple reward	nectar	colour, spectral purity	*Bombus impatiens*	Yes	yellow	spectrophoto meter	peppermint and clove oil	na	flight cage
(Riffell & Alarcón, 2013)	flowers, moths, butterflies, visual signals, learning, foraging, odourants, sensory cues, Corolla	simple reward	nectar	scent, visual cues	*Manduca sexta*	Yes	white	brand listed	floral scent	na	flight cage

(Hempel de Ibarra et al., 2002)	colour vision, compound eye, honey bee, pattern vision	simple rewardless	none	colour, visual angle	*Apis mellifera*	Yes	blue, yellow, cyan, orange	spectrophoto meter	none	trained for unknown time period	flight cage
(Muth et al., 2015)	*Bombus impatiens*, bumble bee, complex, forage, handling time, individual variation, skill learning, specialisation	realistic reward	nectar	flower morphology, handling time, nctar presence/absence	*Bombus impatiens*	Yes	purple, pink	spectrophoto meter	none	trained for unknown time period	flight cage
(Papiorek et al., 2016)	bee pollination, bird pollination, colour vision, flower colour, nectar guides, stingless bees, UV pattern	realistic reward	nectar	patterns	*Apis mellifera, Bombus terrestris, Melipona quadrifasciata*	Yes (but multiple species studied separately)	yellow	spectrophoto meter	none	trained for unknown time period	flight cage, near nest
(Giurfa, 2004)	discriminative stimulus, gray background, conditioning procedure, colour discrimination	simple rewardless	nectar	colour	*Apis mellifera*	Yes	violet, blue, yellow	spectrophoto meter	none	trained for unknown time period	enclosed
(Lunau et al., 2009)	honey bee, bumble bee, spatial resolution, floral guide, colour pattern	simple reward	nectar	patterns, nectar guides	*Apis mellifera, Bombus terrestris*	Yes	blue	brands listed	apiinvert	trained for unknown time period	flight cage, near apiary
(Maglianesi et al., 2015)	artificial feeders, biotic interactions, Costa Rica, floral morphology, floral traits, foraging preferences, hummingbirds, interaction niche, morphological constraints, resource partitioning	simple reward	nectar	corolla length	*Eupherusa nigriventris, Phaethornis guy, Lampornis calolaemus*	No	red	colour listed	none	na	free flying

(Nordström et al., 2017)	multimodal factors, categorisation, syndrome, hoverfly, multivariate	realistic rewardless	none	colour, scent	*Range of Insects*	No	range	spectrophoto meter	range	na	free flying
(Midgley & Johnson, 1998)	flowers, pollinators, symmetry	simple rewardless	none	patterns, symmetry	*Hopliini beetles*	No	orange	colour listed	none	na	free flying
(Wells et al., 1981)	na	simple reward	nectar	colour, nectar volume	*Apis mellifera*	Yes	blue, white	brands listed	clove oil	trained for unknown time period	near apiary
(Grüter et al., 2011)	na	simple reward	nectar	colour, nectar concentration, nectar volueme	*Apis mellifera*	Yes	white, blue, yellow	colour listed	none	trained for unknown time period	near apiary

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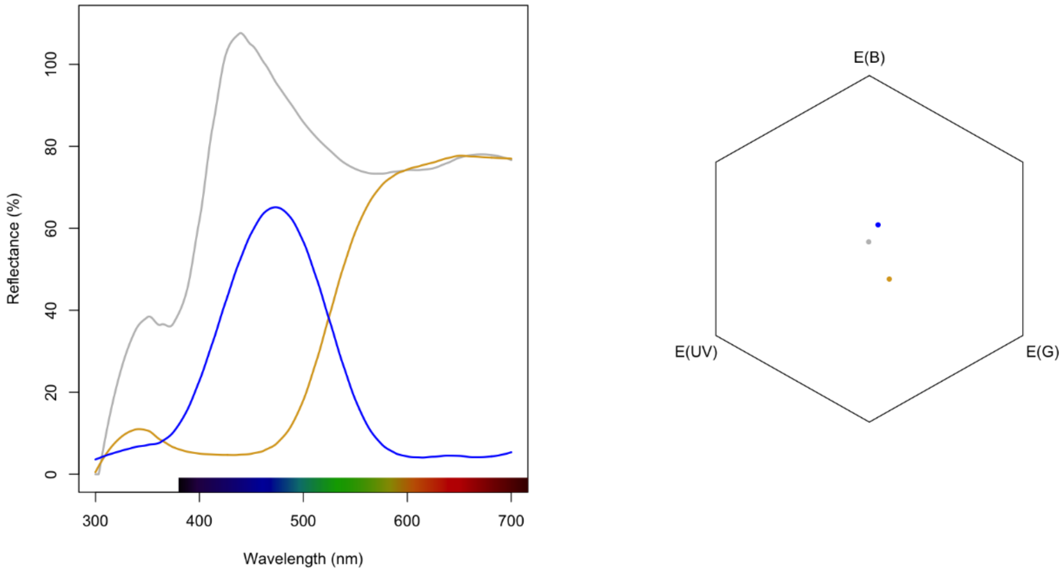
Supplementary Table 2. Summary of choices made by trained subset of bumblebees (Chapter 4) bees including results of binomial tests, with Bonferroni sequential correction to account for multiple comparisons. Confidence intervals 95%.

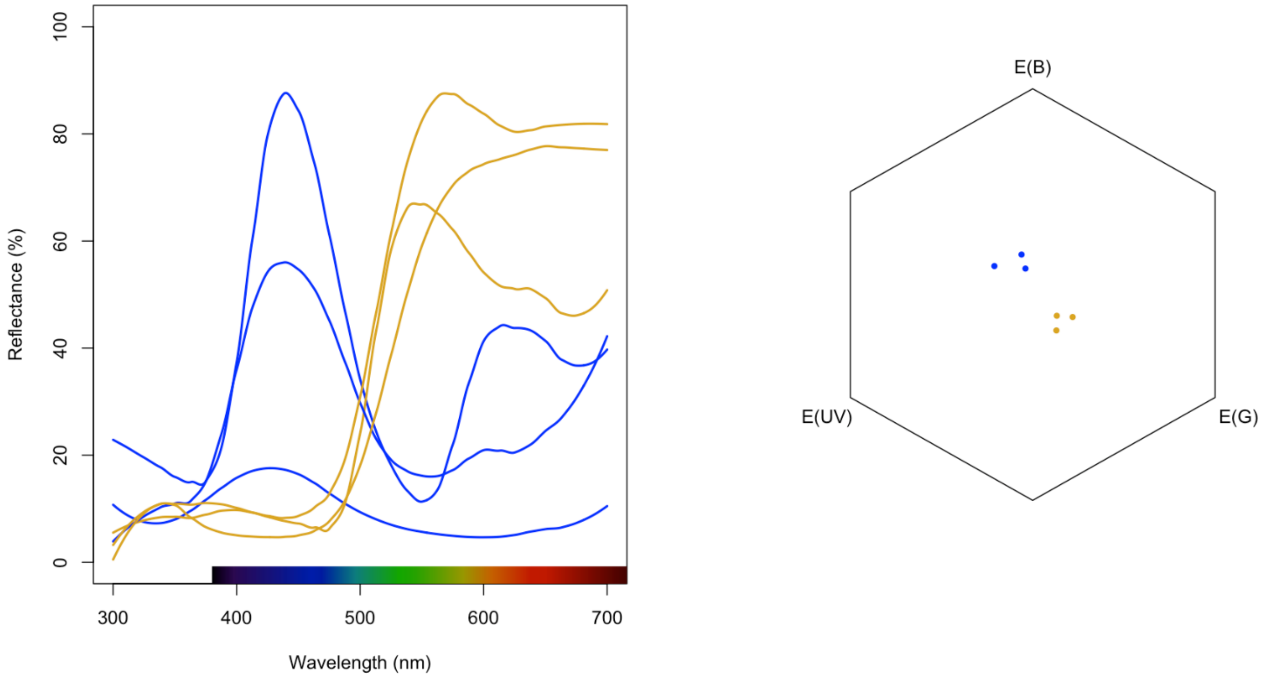
Bee ID	Treatment	Number of visits to flower A	Total visits	p-value	lower CI	Upper CI	Preference	Bonferonni corrected p value
1	Binary	30	51	0.2621	0.442	0.724	None	1
	Phantom	33	66	1	0.374	0.626	None	1
2	Binary	18	30	0.362	0.406	0.773	None	1
	Phantom	12	18	0.238	0.410	0.867	None	1
3	Binary	25	64	0.103	0.271	0.521	None	1
	Phantom	10	15	0.302	0.384	0.882	None	1
4	Binary	6	32	0.001*	0.072	0.364	B	0.029*
	Phantom	7	14	1	0.230	0.770	None	1
5	Binary	28	43	0.066*	0.491	0.790	None	1
	Phantom	3	10	0.344	0.067	0.652	None	1
6	Binary	21	37	0.511	0.395	0.729	None	1
	Phantom	23	87	0.000***	0.176	0.370	B	0.001*
7	Binary	24	34	0.024*	0.525	0.849	A	1
	Phantom	9	18	1	0.260	0.740	None	1
8	Binary	35	58	0.148	0.466	0.730	None	1
	Phantom	13	19	0.167	0.434	0.874	None	1

9	Binary	11	20	0.824	0.315	0.769	None	1
	Phantom	15	35	0.500	0.5	0.263	None	1
10	Binary	3	10	0.344	0.067	0.652	None	1
	Phantom	7	11	0.549	0.308	0.891	None	1
11	Binary	29	39	0.003*	0.579	0.879	A	1
	Phantom	14	24	0.541	0.366	0.779	None	1
12	Binary	14	27	1	0.319	0.713	None	0.182
	Phantom	5	11	1	0.617	0.766	None	1
13	Binary	18	35	1	0.340	0.686	None	1
	Phantom	13	14	0.002*	0.661	0.998	A	1
14	Binary	25	39	0.108	0.472	0.788	None	1
	Phantom	5	11	1	0.167	0.766	None	0.099
15	Binary	17	32	0.860	0.347	0.709	None	1
	Phantom	29	32	0.000***	0.750	0.980	A	1
16	Binary	23	47	1	0.341	0.639	None	1
	Phantom	16	26	0.327	0.406	0.798	None	1
17	Binary	15	24	0.307	0.406	0.812	None	1
	Phantom	40	50	0.000***	0.663	0.900	A	0.001*
18	Binary	12	13	0.003*	0.640	0.998	A	0.185
	Phantom	13	19	0.167	0.434	0.874	None	1
19	Binary	11	18	0.481	0.357	0.827	None	1

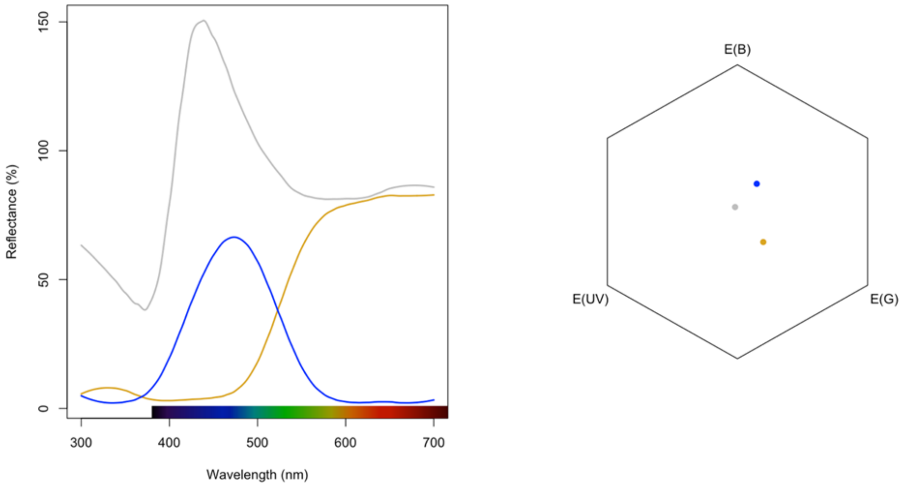
	Phantom	13	27	1.00	0.287	0.681	None	1
20	Binary	20	55	0.058	0.238	0.504	None	1
	Phantom	15	19	0.019*	0.544	0.939	A	1
21	Binary	7	12	0.775	0.277	0.848	None	1
	Phantom	16	16	0.454	0.354	0.848	None	1
22	Binary	14	15	0.001*	0.681	0.998	A	0.053
	Phantom	16	25	0.230	0.425	0.820	None	1
23	Binary	12	18	0.238	0.410	0.867	None	1
	Phantom	18	34	0.864	0.351	0.702	None	1
24	Binary	16	26	0.327	0.406	0.789	None	1
	Phantom	24	24	0	0.858	1	A	0.000***
25	Binary	15	23	0.210	0.427	0.836	None	1
	Phantom	3	10	0.344	0.067	0.652	None	1
26	Binary	9	11	0.065	0.482	0.977	None	1
	Phantom	8	10	0.109	0.444	0.975	None	1
27	Binary	14	22	0.286	0.407	0.828	None	1
	Phantom	32	38	0.000***	0.687	0.940	A	0.001*



Supplementary Figure 1: Reflectance spectra (left) of the yellow, white and blue cardboard used to construct artificial flowers in our behavioural assays, and their location in the hexagon model of hymenopteran colour vision (right). As points are separated by Euclidean distances well above empirically validated absolute discrimination thresholds, they should be readily discriminated by bees under our experimental conditions.



Supplementary Figure 2: Reflectance spectra (left) of the yellow and blue cardboard used to construct artificial flowers in our behavioural assays, and their location in the hexagon model of hymenopteran colour vision (right). As points are separated by Euclidean distances well above empirically validated absolute discrimination thresholds, they should be readily discriminated by bees under our experimental conditions.



Supplementary Figure 3: Reflectance spectra (left) of the yellow, blue, and white cardboard used to construct artificial flowers in our behavioural assays, and their location in the hexagon model of hymenopteran colour vision (right). As points are separated by Euclidean distances well above empirically validated absolute discrimination thresholds, they should be readily discriminated by bees under our experimental conditions.