

**The Impact of Colour and Olfactory Features of Artificial
Flowers on Their Attraction to Bumble Bees**

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Abstract

This study investigated the influence of colour and scent on the attraction of bees to artificial flowers, focusing on their preference for floral blends compared to singular compounds, and the effect of scent concentration on bee behaviour. Experiments were conducted to assess how variations in these sensory cues affect bee attraction and foraging patterns, specifically differences between the sexes. These findings indicate that bees exhibit a stronger preference for floral blends over single compounds, with scent concentrations significantly influencing their behaviour. These results have potential applications in enhancing crop yields and promoting biodiversity by enabling targeted introduction of plant species that align with the sensory preferences of local bee populations. This study highlights the importance of considering the sensory systems and ecological roles of bees when selecting plants for pollination, demonstrating the need for a multimodal approach to support bee health and ecosystem stability.

Introduction

In the UK, the only species of bumblebee currently available for commercial use is *Bombus terrestris*, owing to its effective pollination of many common agricultural crops, such as tomatoes and strawberries (Hjort et al., 2022). However, this reliance on a single species raises concerns regarding biodiversity, potential competition with native bee species, and broader impacts on crop production (Vesterlund et al., 2019). To address these concerns, it is essential to explore the behaviour and plant preferences of different bee species. By identifying the specific plants that attract these species, it may be possible to enhance local bee populations by increasing the availability of the preferred plant species. This study aimed

to determine whether bees exhibit preferences for particular floral attributes, including colour and scent, and the extent to which these preferences influence their foraging behaviour.

The study hypothesised **that bees are more attracted to floral blends than to singular scent compounds and that the concentration of these scents, as well as the colour of the flowers, play a significant role in their attraction to artificial flowers**. Additionally, this study considered other factors, such as pollen content, seasonality, and multimodal sensory experiences of bees.

This study used a series of experiments involving artificial flowers with varying properties to compare bee responses to these artificial models against their interactions with natural flowers. The primary objective was to analyse how altering one or more attributes of a flower, specifically scent and colour, affects bee behaviour. Understanding these interactions can provide valuable insights for selecting plant species that cater to the sensory preferences of specific bee species.

The findings of this study have implications for conservation and agriculture. By strategically introducing plant species based on their chemical composition and visual appeal, it might be possible to attract and support specific bee populations, thereby enhancing pollination and biodiversity. This is because of the need for entomophilous plants that rely on insect pollination for reproduction (Mahramov and Bayramov, 2024), which is responsible for ½ of the global crop yield (Kaya et al., 2023). Ultimately, this study contributes to increasing crop yields and promoting ecological stability by facilitating the targeted introduction of plant species that align with the sensory systems of bees already present in local areas.

Literature Review

In *Bombus* populations, high sensitivity to molecular volatiles is essential, because these chemicals are crucial for finding nutrients and communicating with other bees in the colony through pheromones (Fonta & Masson, 1987). Variations in floral scents among plants enable bees to identify and select flowers that offer nectar. Differences in these rewards can influence bee responses to flowers, helping them distinguish honest signalling flowers that provide nectar (Wright & Schiestl, 2009). Floral volatiles also add specificity to plant-pollinator interactions, differentiating them from competitors that rely solely on visual cues, such as colour or nectar content. Certain scented compounds, such as limonene and pinene, are particularly attractive to bees and are found in a wide range of flowers (Giuliana et al., 2020).

Pore plate sensilla are key anatomical features of bees and are characterised by an elongated and porous structure that extends above the antennal surface (Ren et al., 2023). Sensilla are heavily innervated by sensory neurones and have branched dendrites along their longitudinal axis to enhance sensitivity to chemicals (Barlin & Vinson, 1981). They play a critical role in detecting odour molecules and differ between sexes, thereby affecting the decision-making processes (Erickson, 1982). Bees with a higher number of sensilla tended to be specialised foragers, exhibiting greater sensitivity to environmental changes. These bees are more adept at collecting pollen and water than those primarily collecting nectar (Andre et al. 2010).

There is also notable sexual dimorphism in bee morphology and sensory systems. Males have longer antennae with 11 segments than females with 10 segments, enhancing their spatial abilities during the breeding season and making them more efficient in associating visual-colour stimuli with sugar rewards (Wolf & Chittka, 2016). Conversely, female workers and queens possess denser sensilla, enabling them to detect molecular volatiles better (Fonta &

Masson, 1987). These sensory differences have evolved because of the distinct roles played by each sex within the hive, such as foraging, food selection, and mating, which affect their perception and interaction with floral volatiles (Belsky et al., 2020).

The foraging patterns of male and female bees also differ, and are influenced by their sensory systems and roles in the hive. Male bees primarily focus on reproduction, whereas female bees are responsible for building and maintaining nests. Female bees collect both pollen, which is essential for larval development, and nectar, whereas male bees only collect nectar (Rasheed & Harder, 2003). Consequently, females are expected to forage at higher rates to meet the nutritional needs of their colonies (Roswell et al., 2019). This difference in foraging behaviour is linked to plant choice, with females being more likely to target species with higher rewards in terms of pollen quantity and quality (Rasheed & Harder, 2003).

Studies have shown that Bees also exhibit preferences for plants based on their colour. The trichromatic visual systems of bees allow them to perceive violet, blue, and green hues, with green often used as a contrasting background (Dyer et al., 2008). Additionally, as their visual systems are unable to detect red wavelengths, they have struggled to locate red flowers in previous experiments, especially within a natural green background (Rivest et al., 2017). Bumble bees are also highly sensitive to colour hue and saturation, and can detect subtle differences between plant species, possibly contributing to the memory and preference of plant species (Lunau, 2020). This has been extensively studied in behavioural experiments using artificial flowers, demonstrating that *Bombus* spp. can associate specific colours with nutritional rewards (Gumbert, 2000).

In addition to visual and olfactory cues, bees rely on less-obvious sensory experiences to select flowers. These include humidity, temperature, electrical fields, and surface textures, which are challenging to replicate in an experimental setting (Rands et al., 2023). These

multimodal sensory experiences enable bees to make more specific choices between plant species and to discern which flowers will provide an adequate reward (Kulahci, Dornhaus, & Papaj, 2008).

This study builds on previous investigations of bee behaviour in laboratory settings by exploring bee behaviour in natural environments. While most studies have focused on the common bee species *B. terrestris*, this study aimed to expand our knowledge by examining a diverse range of bee species found in Durham and surrounding areas, and by identifying how their behaviours differ across species.

Methodology

Foraging Experiments

A wide range of plants was investigated, with a range of flower colours and areas across the city of Durham. Varying locations were chosen across the city because of the range of available flowers, including wildflower meadows and artificial plant beds. A minimum of three samples from each plant were taken to allow repeated measurements; however, the lack of visitation or number of plants in some locations was challenging. Both native and artificially planted flowers were measured, with a tendency for bees to prefer native flowers, with the exception of *L. angustifolia*.

In the first aspect of the research, bees and flowers were collected using a simple method. The bees were caught in plastic vials, labelled, and then placed in a cooler to allow for ethical death. The plants that the bee landed on were then covered with a plastic bag to allow the plant volatiles to diffuse into it, and the air was then removed using a pump with a small filter to collect the volatiles. For larger flowers, the bag was left for an hour, whereas for smaller flowers, the bag was left for 4 hours to allow for the release of an adequate amount of

volatiles. For each plant species, at least three bees were collected to observe the variations in species.



Figure 1 Bombus caught from flowers using plastic tubes and then cooled and frozen to allow identification of sex, followed by further experimentation of their species.

Once the plant samples were left to release their chemicals, the bag was punctured with a small hole, a filter attached to a pump was then placed within the bag, and air was sucked out for a total of 10 min. The plant samples were sent off for analysis by an external chemical spectroscopy lab to identify the chemicals that were released by the plants, along with any potential contamination. These chemical samples were then labelled as good, bad, or weak depending on the concentration of chemicals identified, as well as the amount of contamination in the samples.

The bees were then analysed microscopically to identify their sex and assign them a species based on their morphology. To further investigate the species, they were genotyped using DNA samples extracted from their legs, and then PCR was performed on the separated

DNA bands. DNA was extracted from the bands on these gels and sent for external analysis to formally identify the genotype of the bee.



Figure 2 Bombus are placed under a microscope to be sexed by identifying the presence of a stinger in females and the different numbers of antennae segments.

Negative controls were also taken on plants where bees were not observed to have landed so that any patterns in chemicals contained in these plants could be identified and added to the knowledge of chemicals that bees found attractive. Additionally, ambient measurements were performed each day to identify any compounds which were abundant in the surrounding air.

Behavioural Experiments

Our methodology used in behavioural experiments aimed to use novel techniques to explore the behaviour of bees and was carried out in areas surrounding the Durham University campus. Previously, laboratory collected samples of molecular volatiles from a range of plant species that bees are known to visit, and the strongest signalling plants have been selected

and the most common compounds chosen to create a floral blend and be used as single samples.

Artificial flowers were created to hold molecular samples, replicate natural flowers, and provide a stable landing platform for bees. These were initially formed using plastic disks as the base of the flower along with a straw for the stem which was covered with white paper to prevent the bees from being distracted by colour. In subsequent experiments, paper flowers were used to allow scents to be pipetted onto their surfaces for absorption and diffusion. These scents, with known attractive compounds and either a single or floral blend, were used and were formed from a series of dilutions of d-limonene, alpha-pinene, or methyl-salicylate, using mineral oil as a solvent. The results were based on how much time the bees spent on each flower, how many times each flower was visited, and any observable physiological changes, such as extension of the antennae or tongue, as well as the active avoidance of certain compounds.

In the first experiment, three bees of different species were captured and released into an arena formed by a mesh tent. A control vial on a weighing tray containing no compounds was also placed in the arena to observe bee reactions to this foreign object alongside a vial containing 100 μm of d-limonene dissolved in hexane. These trays were then placed around natural flowers to observe their behaviour.

The method was then adapted to create artificial flowers that replicated the shape of natural flower petals using both white and blue trays which are more attractive colours for bees. These flowers contained vials of alpha-pinene and limonene at concentrations of 0.71M, 0.071M and 7.1mM. A blank vial was used as control. For this experiment, new artificial flowers were placed on stems amongst natural flowers and observed for 20 min to determine how bees reacted to them. This method was then repeated using mineral oil to

replace hexane, as this is odourless and would allow the scents to be tested without masking by the solvent. These compounds were further dissolved to 0.0071 (alpha-pinene) and 0.0072(limonene), and the experiment was repeated. Some of these dilutions were also pipetted into natural flowers to observe how the bees behaved when only scent was added.

This method was then used to test the reactions of bees to floral blends of commercially available lavender essential oils and Mediterranean orange essential oils. A few drops of these were diluted to 1% of the original concentration and placed in artificial flowers made from either white filter paper or blue, yellow, or purple coloured paper, and a sugar reward was placed within the centre. These were placed in an arrangement around the flowers which the bees had visited. The bees were then observed for 30 min to compare the visitation patterns of the artificial flowers with those of the natural flowers.

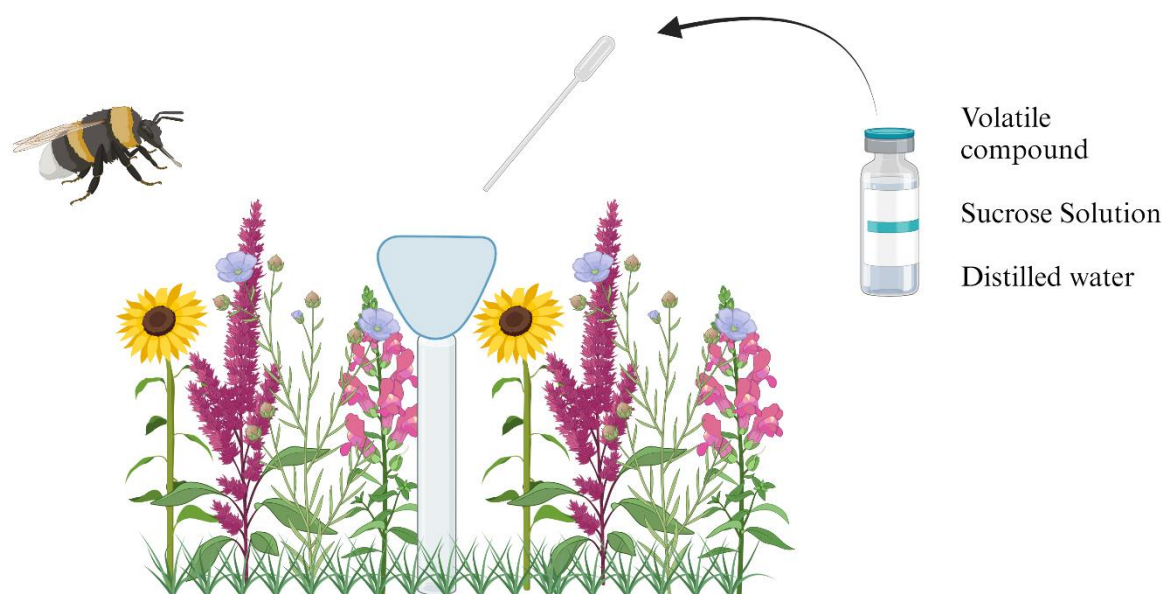


Figure 3 Molecular volatile samples were diluted and pipetted into paper flowers among the real flowers. To enhance attraction and comparison with real flowers, a sucrose solution was added to a central vial so that the bees would receive a reward for visitation. Variations in colours were used including white, purple, blue and yellow. image created using Biorender



Figure 4 Image of the white paper artificial plants in an arrangement, they contain drops of varying scents and include blank samples



Figure 5 Example of artificial flowers made using white and blue plastic trays and then placed in a floral arrangement

This methodology has limitations, as unlike in the laboratory environment, it is impossible to maintain certain environmental controls, such as temperature and weather patterns. To maintain the control of the artificial flowers, some were added without any scented compounds to observe how the bees reacted to an unfamiliar object. In many similar experiments, bees are also used within a laboratory and are commercially sourced, whereas ours are caught in the natural environment. This prevented us from obtaining information on bees' previous experiences with flowers and rewards.

Results

Foraging Experiments

From the observations of foraging bees, the bee sample results were segregated and analysed using bee sex and species. Samples were also taken from 19 different plant species, including the bees that visited the plant, and a sample of the molecular volatiles that were released over an hour during the day.

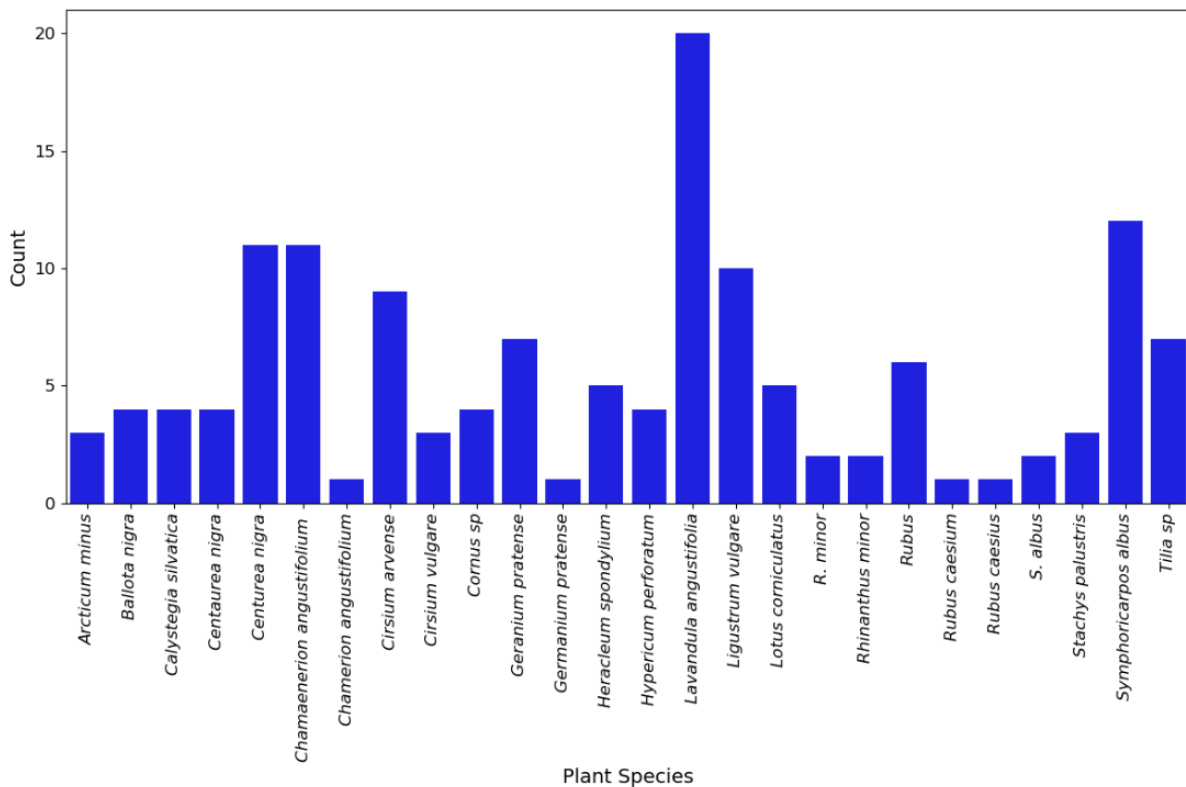


Figure 6. The total number of plants of each species was collected over a six-week period with a minimum of three samples for each plant species. The x-axis represents the plant species, whereas the y-axis indicates the total number of samples collected. The variation in sample counts among species reflected the relative abundance or detectability of each species in the study area during the sampling period.

Different patterns depending on sex

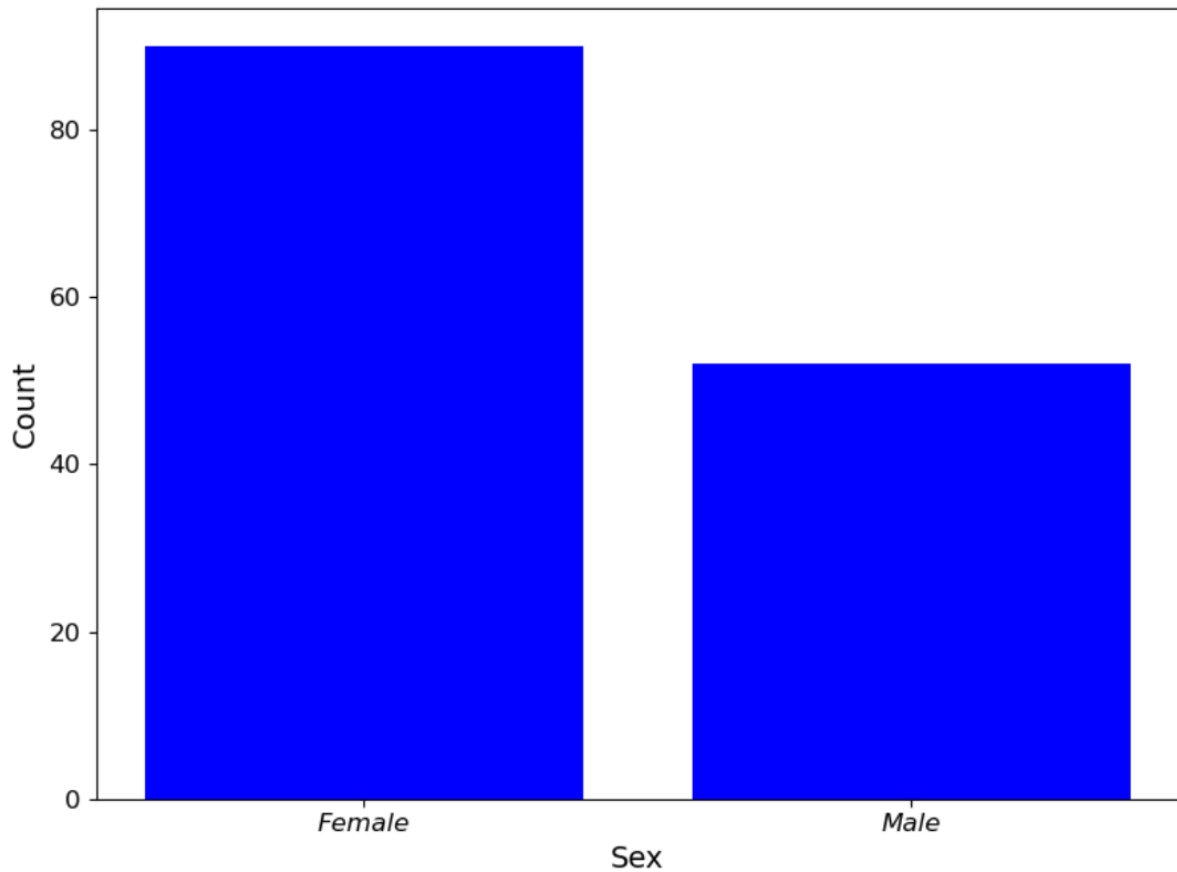


Figure 6 Graph showing the difference in number of bees caught separated by sex with n of females = 90 and n of males = 52 with a ratio of 1:0.58 female:male. This difference could be attributed to the different roles within the hives of each sex of bee and the preferences of each bee within the locations from which we sampled.

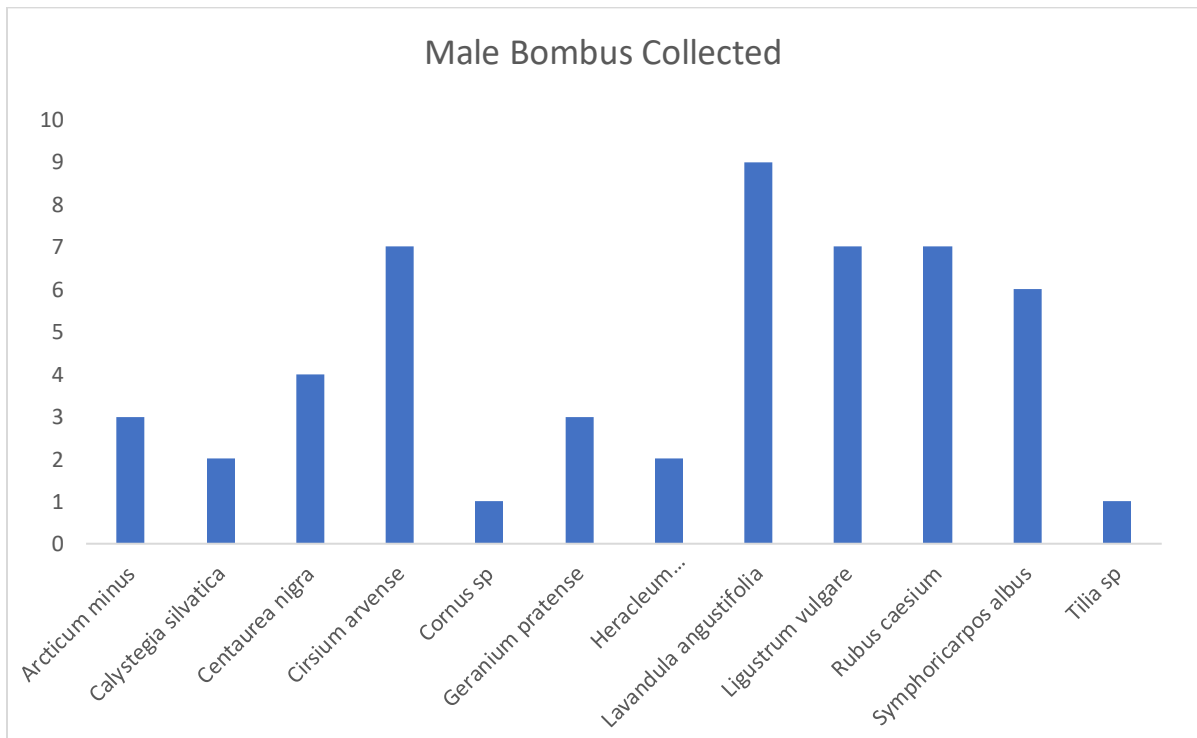
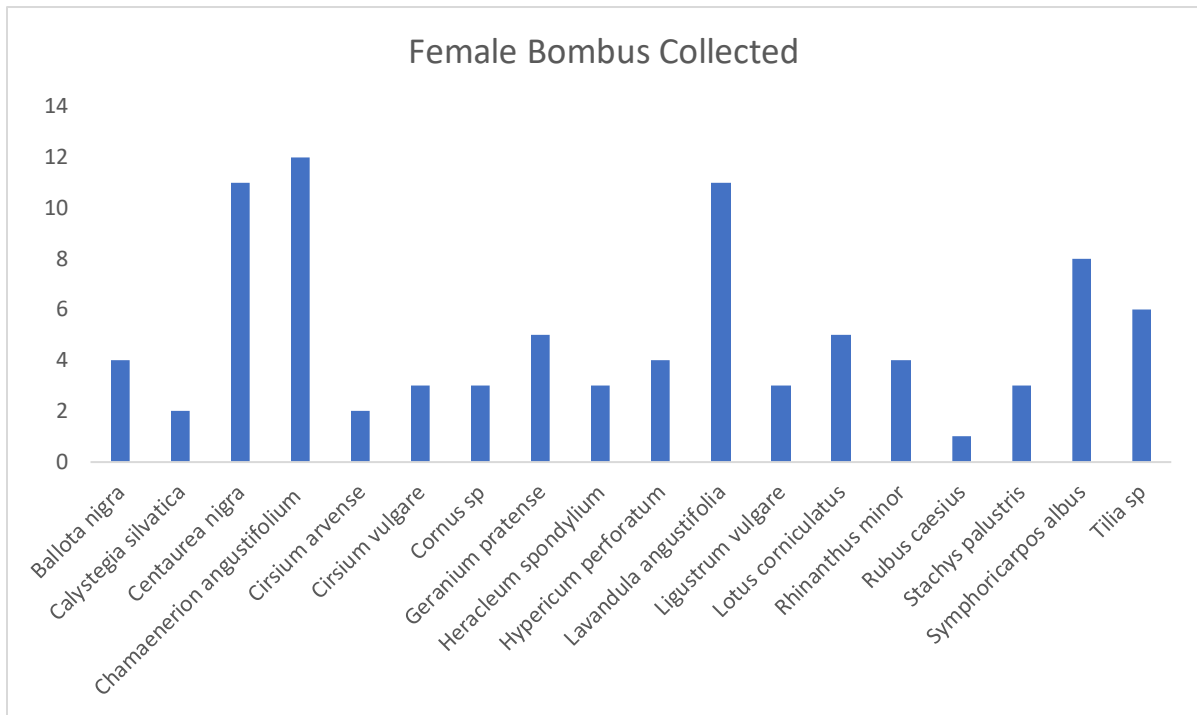


Figure 7 and 9 show the plant choices of the bees that are specific to their sex. There was a clear difference in the number and range of collected female bees. Eighteen different plant

species were sampled, with female species visits and 12 different plant species with male visits.

There were notable differences in the plant species that were visited by the different sexes of bees and a range of reasons that could lead to this choice of behaviour.

Female bees preferred plants that were in early or peak bloom, whereas male bees were generally found on plants that were towards the end of their blooming cycle in several plant species. Examples of this were noted in *Lavandula angustifolia* (English Lavender) at two sites in close proximity to each other with similar environmental factors such as temperature. One of these sites contained *L. angustifolia*, which was early in its bloom, while the other was more mature. At the mature site, 9 out of 11 bees sampled were males, whereas in the newly blooming *L. angustifolia*, all six of the samples taken were females. An additional example is *Chamaenerion angustifolium*, where all 12 of the samples taken in June 2023 were female, while samples that had been collected last year in August were mainly male.

Different patterns depending on species

There was also some variation found in the behaviour of different species, and which flowers showed a preference for. A small selection of flowers, such as *Jacobea vulgaris* and *Cirsium vulgare*, was only visited by *B. lapidarius*, while some plant species, such as *Centaurea nigra*, were visited by a range of species, with this example having five different species.

Because different bee species have different foraging times, there might have been a different distribution of species if a longer sampling time was used. For example, *Bombus pratorum* (early bumble bee) is active from March to June, while *Bombus lapidarius* has a much later foraging season from April to November. This difference in foraging season could help reduce competition between species and allow plants with different blooming seasons to be pollinated, but more research is needed to assess how significantly different factors such as

pollen and nectar quantity or quality influence the different behaviours of the different species.

Because not all species were genotyped, we could not draw any concrete comparisons regarding the differences between the findings from the collected data.

Behavioural Experiments

Behavioural experiments showed that bees did not react to single compounds in vials that were placed in the arena. However, it cannot be concluded that the bees were in an unnatural environment and were placed under stressed conditions. To reduce this, the bees were cooled and then released, but this did not prevent them from becoming disoriented.

In artificial flowers placed among natural ones, a negligible difference was found between the bees' behaviour towards the different concentrations, and only a few bees approached the flowers, but none landed on them. There were no data on the concentrations of naturally produced compounds; therefore, a range of 0.71M - 0.0071 μ M (alpha-pinene) and 0.72M - 0.0072 μ M (d-limonene) was used for experiments with both high and low concentrations of the compounds. The use of the blank vial with no scent also had no visiting bees, which demonstrated that there was no difference in the preference of the bees from having a single compound to no compound in the artificial flowers.

After experimenting with different concentrations, a sucrose reward was added to the artificial flowers using an Eppendorf tube in the centre, and was 1 g dissolved in 20 ml of deionised water. The addition of a sugar reward had no impact on the attraction of the bees to the plants, and there is a possibility that this concentration was too high; therefore, further research is needed to identify the optimum concentration.

These results also did not change based on the colour or material of the artificial flower used, including white, yellow, blue, and purple paper, and white and blue plastic. A range of flower

shapes were also used to replicate a range of natural flowers, but this had no effect on attracting bees.

In all cases, the flowers were visited by a range of other flies, including the patches containing scent and the sugar reward, and there was little difference in flower visitation based on colour or scent. This shows that the artificial flowers had some attractive properties to other insects, and were unlikely to be attracted to the colour of the paper or a specific scent.

Discussion

The preference for natural flowers is clear from the results because of the lack of visitation of the artificial flowers in comparison to the natural ones, including the different forms of colour and scent, which implies that there are many factors that contribute to a bee's preference for flowers and that their sensory systems are highly refined to be able to note the difference from a distance away..

The difference observed in the preferences between female and male bees aligns with previous studies on bees, including those conducted in a semi-natural meadow, which aligned the behaviours to the possibility of their different nutritional needs. Females have additional roles in male bees, which include collecting pollen for their young, whereas the primary role of male bees is to find a mate. This creates a higher calorific need for females and leads them to forage at a higher rate, and a reason for them to be highly specific when choosing a flower to gain a reward. (Roswell et al, 2019).

This would also explain the higher abundance of female bees in comparison to males, and there is scope for investigation into the preference of female bees for flowers in earlier blooms. This could be due to the quantity of compounds released by the early flowers and females with higher levels of sensitivity, or alternatively, there could be a higher quantity or

quality of pollen within early flowers. This would also help explain the high numbers of pollen baskets on *Bombus* which were found on these freshly bloomed flowers. The lack of pollen in the artificial flowers could have been a contributing factor preventing female bees from visiting them, as there was a lower level of reward in comparison to the natural ones. Male bees could have the potential to prefer nectar to sucrose solution when faced with this option, which could have contributed to their lack of visitation. As the paper and plastic flowers did not have floral or nectar evaporation, there is a chance that this contributed to the bees associating the flowers with a lack of rewards. Alternatively, the bees were not given time or training to associate these plants with reward; therefore, unlike the natural flowers, there was a higher risk involved in choosing these plants. One limitation of this study is that pollen production per flower has a positive correlation with floral display size, which is a factor that was not investigated in these experiments and could have an impact on the choice of flowers due to pollen content or alternative factors (Nicolay et al., 2023).

The flowers also lacked some properties that allowed bees to choose their plants, including the texture of the paper and plastic trays, and the electric field that is observed around natural plants. There is evidence that bees are able to detect these properties (Harrap, Hempel de Ibarra et al., 2021) which contributes to their motivation to visit flowers with these features. These features are harder to replicate in artificial flowers, so techniques should be further developed to allow them to be investigative factors.

These experiments were only carried out on a limited number of species that were not genotyped, over a period of six weeks, and within one region. These results imply that they cannot be applied to all *Bombus spp.*, and that if further experiments were carried out to look at species-specific results, it might be that some of the factors discussed have variations based on species. As different *Bombus spp.* have a range of different foraging seasons (Goodwin, 1995), there is the potential for experimentation to be carried out from early spring to autumn

to observe a wider range of peak seasons and to collect a wider variety of volatile samples to observe if certain compounds are more prevalent at different times of the year. There is also the possibility that if the artificial flowers were introduced throughout the entire foraging season, then the bees could be trained to associate them with a reward and be less likely to use previous experiences to choose which plants to pollinate.

Conclusion

Overall, it was found that bees did not have a strong attraction to single-scented compounds or a particular flower colour and that they relied on having a strong multi-modal experience to allow them to choose flowers. This is more specific to bees than to other insects, such as several fly species, which are attracted to artificial flowers in a range of forms.

However, there was a noticeable difference in the preference of flowers between the different sexes of bees, which indicates that the bees have varying and specific preferences, and with further development of the artificial flowers, there is the potential to identify more closely how each species prefers colour or olfactory scent by creating a more realistic copy of the flowers to reduce the strong preference for natural flowers.

The implications of this study can aid in the improvement of biodiversity and horticulture by providing evidence of the difference in preference of plant species, and therefore the benefit of having diverse access to different species of plants by bumblebees.

Future Work

The scope for future work within the study includes conducting a more in-depth analysis of the specific molecular volatiles within each plant species and creating a network to indicate the preferences of certain olfactory scents for specific species or sex of bees. There could also

be experimentation on the wavelength of reflected light of plants that bees show a preference to , to allow more close representation of the artificial plants.

To further this investigation, the bees could be trained to respond to a range of scents and then observe which one showed the most preference for. There could also be development into the behaviour of bees when floral blends are used in comparison to single compounds to further explore how having a mixture of available scents contributes to the multimodal experience of the bees.

Additionally, artificial flowers could be further developed by creating a humid environment within them and by experimenting with different materials to replicate the texture of natural flowers more closely. This would allow us to state which aspect of flowers is most influential in allowing a bee to distinguish between natural and artificial flowers, as well as the properties that have the most significance in visitation choice. There could also be the addition of other properties, such as pollen; however, additional experiments are needed to determine the exact pollen counts for flowers

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