Floral Categorization in Bumblebees

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Abstract

In nature, pollinators must navigate fields of resources presenting a variety of features, differing in shape, size, colour, etc. Foraging on a flower by flower basis is slow and maladaptive. Instead foragers must be able to differentiate between rewarding and unrewarding floral species while also generalizing learned information between flowers of the same species. The ability to categorize stimuli occurs on several levels of abstraction, laid out by Herrnstein (1990). In order to categorize objects, animals must first be able differentiate between them without memorizing each stimulus separately. Consequently, objects can be grouped by physical characteristics through perceptual categorization, or, on a more abstract scale, by the function they serve to the animal. This thesis explores the bees' ability to categorize flowers, following the levels of categorization to answer two questions: Can bees form categories? And how abstract can their categorization become? There has been limited investigation previously in categorization in bees, and no research done on invertebrates addresses categorization beyond physical features. The bees' ability to form categories was evaluated with four experiments of preference: 1) similar-but-different judgements; 2) perceptual categorization; 3) simple and mediated generalization; and 4) functional relevance. Results show firstly that bees can generalize characteristics within flower species, but also differentiate individual flowers. Secondly, bees can form perceptual categories, and while they rely on physical floral features for categorization, bees also demonstrated preliminary abilities for functional generalization as well. These results provide an explanation to natural foraging techniques adopted by the bees. The adaptive nature of categorization allows foragers to find resources more efficiently and better prepare in changing environments.

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Overview

A typical meadow can be a confusing array of scents and colours. A forager’s ability to distinguish and sort all the incoming information is shaped by both ecological circumstances and the course of evolution. By combining both these factors, the field of cognitive ecology examines how cognitive processes such as decision making and processing are shaped by environmental contexts to increase individual fitness (Dukas, 1998; Chittka & Thomson, 2001). For instance, bees can use colour cues (Zhang, Bartsch, & Srinivasan, 1996) and motor sequences (Mirwan & Kevan, 2015) to navigate different spatial arrangements, and use visual stimuli to learn certain movements (Collett, Fry, & Wehner, 1993). Bees can also detect changes in pollen qualities and nectar concentration in the environment and change their behaviour accordingly (Townsend-Mehler, Dyer, & Maida, 2011; Leonhardt & Blüthgen, 2012). There have also been preliminary investigations into metacognition in honeybees, where bees chose to opt out of performing difficult discriminations when presented with a choice (Perry & Baron, 2013).

Coevolution has allowed pollinators and plants to interact using a variety of signals, leading to an increase in plant diversities in both nature and in human agriculture. Many plant species rely on pollinators rather than wind to transfer their pollen between individuals, while pollinators benefit by obtaining resources such as nectar from the flowers (Memmott, Craze, Waser, & Price, 2007). While odour cues allow bees to approach flowers that are not in sight (Riffell, 2011), visual cues also serve as important markers to the values of rewards and their location. As a result, natural flowers vary in features such as colour, shape, size, and odours. In comparison, wind-pollinated plants produce flowers that carry no scents, patterns, or colours (Culley, Weller, & Sakai, 2002).

Despite the mutual relationship between pollinators and plants, the fitness costs to the two are not the same. While nectar serves to attract pollinators, the production of nectar may use up to 37% of the plant's available energy (Southwick, 1984) without necessarily increasing reproductive benefits (Pyke, 1991). On the other hand, the survival of bee colonies depends on the amount of stored nectar and pollen. As a result, foragers must find the most rewarding flower to offset energy costs from the search (Pyke, 1978). Foraging bees must face the decision of which flower out of many options to visit in the shortest possible time. Eusocial insects, such as bumblebees, provide a unique evolutionary outlook in that fitness is determined by the colony and not the individual (Wilson, 1975). Because the survival and reproductive success of the whole colony depends on the resources from the workers, foragers are still motivated to gather resources despite being satiated themselves. In a complex and changing environment with multiple potential resources, bees must be able to accurately and efficiently identify profitable sources of nectar or pollen.

Individual flowers change their reward values over short periods of time (Heinrich, 1979). Resources can be depleted over the course of a day by conspecifics, and flowers that were previously rewarding may become unrewarding as they age. Remembering reward values on a flower by flower basis appears maladaptive. Instead, correctly identifying floral species could indicate more stable resource levels. If one individual flower is depleted, others within the species are still rewarding. Identifying rewarding resources requires more than memorizing individual floral characteristics.

Early bee research had assumed bees to be incapable of "thinking" and to be governed only by instinct as a result of having small brains (von Frisch, 1962). However, despite a small brain size, bees have a rich behavioural repertoire necessary for a social lifestyle (Benard, Stach, &

Giurfa, 2006). Social bees cannot survive for long outside the colony, and must return to the hive after foraging. This often means navigating back over several kilometers in changing landscapes using landmarks and celestial cues (Wehner & Rossel, 1985), and visiting hundreds of flowers in quick succession (Ribbands, 1949). Identifying important cues and ignoring noise would appear adaptively crucial for the foragers.

Sorting incoming information about hundreds of individual flowers allows for faster identification of relevant rewarding resources. Evidence for flower classification can be found in natural foraging behaviour, specifically flower selection techniques such as floral constancy. These behaviours give indications of mechanisms such as attention and memory demands, and the ability to discriminate and generalize behaviour. This review will (1) outline natural bee behaviours that indicate the use of categorization during foraging through flower selection and preferences, (2) illustrate some of the key cognitive mechanisms necessary for identifying and categorizing flowers, such as memory and attention, and (3) describe the different forms of categorization. To avoid repetition, some concepts are elaborated further in the introductions for each experiment.

1 Flower selection

A forager can encounter up to five plant species per second in meadows with species such as trefoil, meadow vetchling, cow vetch, thistles, and purple loosestrife (Chittka, Gumbert, & Kunze, 1997). As a result, strategies for managing information for rewarding stimuli are necessary to reduce the amount of detail to which it must attend.

1.1 Foraging preferences

1.1.1 Colour

Certain floral features act as cues to foragers as predictors of nectar content. Foragers with no previous experience on flowers appeared to have preferences for certain floral colours (Briscoe & Chittka, 2001; Chittka & Wells, 2004). Colour is a reliable indicator, as it is not affected by angle of approach, while shape or symmetry are. As a result, colour information is ranked higher than spatial information, and foragers learn mainly the colour of the target resource (Ronacher, 1992; Giurfa, Backhaus, & Menzel, 1995; Lehrer and Campan, 2005). Overall, bees showed an innate preference for blue (420 nm) (Menzel., 1967; Giurfa et al., 1995; Dyer et al., 2007). While experience can override the innate preferences, bees also reverted back to their unlearned colour preferences when rewards are similar across species (Banschbach, 1994; Gumbert, 2000). Testing demonstrated that bees preferred flowers from the violet-blue range (Raine, Ings, Dornhaus, Saleh, & Chittka, 2006), which has been found to have higher nectar content in various environments, such as the Mediterranean and Atlantic Islands (Chittka, Ings, & Raine, 2004). Similarly, floral colour signals evolved to fit the visual capabilities of their pollinators, with trichromatic flowers for bees, and tetrachromatic flowers for avians (Shrestha, Dyer, & Burd, 2013).

1.1.2 Multi-sensory cues

While producing one cue would be simpler and more cost-effective to produce, flowers tend to use multi-sensory displays to influence pollinator behaviour (Leonard, Dornhaus, & Papaj, 2011). For instance, odor cues can facilitate learning of floral colours in bumblebees, and bees were more likely to exhibit constancy to flowers producing odor signals. Multiple cues reduce uncertainty and increase learning speeds (Leonard et al., 2011). These multiple signals prevent any missed opportunities for foragers that may bypass or mistake one cue as noise (Cnaani, Thompson, & Papaj, 2006).

1.1.3 Social cues

Attention to conspecific behaviour allows for reduction of costs through trial-and-error sampling in the foragers' environments. However, the presence of conspecifics might also indicate a drained resource. Social learning increases intraspecific competition and limits alternative and independent sampling (Avarguès-Weber, Lachlan, & Chittka, 2018).

For bumblebees, naïve foragers prefer inflorescences that are occupied by conspecifics, and decide upon observing other bees whether or not a flower is profitable (Worden & Papaj, 2005). Bees rely on social cues when there is a lack of information about the flower (Leadbeater & Florent, 2014) or when investigation is costly (Saleh, Ohashi, Thomson, & Chittka, 2006). However, conspecific cues are also flexible and can be ignored in areas of high competition (Plowright et al., 2013). Social cues are harder to ignore than other perceptual floral cues, however this may be a result of familiarization in the nest or Pavlovian conditioning, as other bees are live and more salient (Avarguès-Weber et al., 2018).

1.2 Flower Constancy

Flower constancy is defined as a foraging behaviour that favors one or a few species of flowers, and can be found in several species of bees (Chittka, Thomson, & Waser, 1999; Raine & Chittka, 2007; Rossi, Santos, Salvarrey, Arbulo, & Invernizzi, 2015). Because flowers of a species share similar rewards and physical features, identifying similar flowers would be an effective method for efficient foraging. Although other flowers in the area may be potentially rewarding, there is a higher chance of finding rewards by sticking to known rewarding species.

1.2.1 How constancy forms

Bumblebees cannot communicate sources of food directly to other members of the colony (Free & Butler, 1959). As a result, the scent of predominant pollen in a nest induces bees to seek the same scent when they are foraging (Free, 1970; Dornhaus & Chittka, 1999). Flower odors are species specific, so the effects of their constancy are influenced by the colony as well as the flowers available. Even inconsistent bees habitually visit the same two or more species for several trips, with the types of pollen returning from the mixed loads remaining similar with each trip (Free, 1970). Bees also use the taste of pollen within the nest to determine which flowers to visit (Muth, Francis, & Leonard, 2016).

Bees have limited foraging repertoires but must navigate a field of diverse flowers. As a result, bees exhibiting no specialization have lower foraging success rates and difficulties extracting resources compared to bees displaying constancy to only a few floral species (Heinrich, 1976). Flowers producing the most food had the most bees foraging on it, and switching is rarely observed. Specializing in specific flowers allows bees to increase their efficiency on collecting resources from specific morphs (Darwin, 1895).

The response does not appear to be an innate preference for specific flowers, as individuals from the same species display flower constancy on different plant species. Additionally, foragers ignored other flower species in the area that were equally rewarding (Waser, 1986). Because it is rare for only one rewarding floral species to dominate an area, skipping over potential resources would not maximize energy intake rate (Chittka et al., 1999).

Naïve bees spontaneously choose one colour and become constant to it (Hill, Wells, & Wells, 1997). Individuals not trained to visit other flower colours failed to sample or even land on the alternatives. However, flowers with multiple cues encourage more constancy than just colour alone (Gegear & Laverty, 2005). Honeybees showed constancy, even when rewards differed in quality, quantity, and frequency (Wells & Wells, 1983). Individual constancy persists regardless of hivemate behaviour (Wells & Wells, 1983). As a result, single resources would not be overwhelmed by all the hive's foragers competing for the same nectar (Heinrich, 1975; Wells & Rathore, 1994).

Bees respond more to changes in nectar concentration rather than nectar volume (Cnaani, Thomson, & Papaj, 2006; Bandschbach, 1994) to maximize energy. Sugar concentration can be immediately assessed upon landing, but volume cannot, and is also less likely to be affected by exploitation from other foragers.

1.2.2 How constancy breaks apart

Despite the bees' natural tendency to specialize on one or a few specific flowers, flower constancy is not always advantageous and on occasions, breaking constancy allows for better foraging. During a single trip, bumblebees in general were less constant than honeybees to one species of inflorescence (Free, 1970). Maximizing energy intake during foraging depends not only on the reward obtained, as mentioned above, but also the costs of obtaining the reward (Laverty, 1994). If alternatives are at different distances, most bees tended to maximize energy by visiting the closest flower (Marden & Waddington, 1981).

As mentioned before, the more complex the flower, the more constancy the bees exhibited in comparison to simpler flowers (Laverty, 1994). Simpler flowers elicit less constancy in bumblebees. A background of similarly coloured flowers decreased foraging accuracy in bumblebees more than one of dissimilar flowers (Goulson, 2000), suggesting preferences may be based on similarity judgements. Bees were also able to learn to switch colours based on specific times of day. Bees switched between blue and yellow flowers depending on which choice was rewarding during which time of day (Gould, 1987), and flower choice is necessarily dependent on the features of the flower.

Flower constancy is only an adaptive foraging method if switching between distinct species is costly. Although bees have exhibited innate preferences for specific flower qualities, such as colour, odor, and social cues, the effects of learning on innate foraging preferences can be seen through the formation and dissolution of flower constant behaviour. Having considered bee behaviour in a natural environment, the next step is to consider how the behaviour may occur.

2 Cognitive mechanisms for category formation

2.1 Attention

A forager’s environment does not consist solely of rewarding or unrewarding flowers. Filtering out noise, or irrelevant perceptual stimuli leads to more accurate identification of floral cues and greater foraging success. A balance must be achieved between expanding search opportunities while narrowing down attention to only specific cues. This is particularly important for smaller brains as efficient information processing would rely upon accurate filter processes (Mackintosh, 1965).

Two different search strategies have been identified: serial search and parallel search. Serial search requires attention directed to one item at a time in an environment, while parallel search attends to all items simultaneously. Serial search strategies were favored by honeybees (Spaethe, Tautz, & Chittka, 2006), where the forager searches every flower in the area until it finds the target. This search method lends to faster and more accurate foraging when more target resources occupy an area. However, serial searches can also lead to more mistakes when more distractors are present. On the other hand, bumblebees appear to rely on parallel search strategies (Morawetz & Spaethe, 2012). Although it is a slower search method when more targets are available, it unaffected by the number of distractors available, thus exchanging speed in some instances for higher foraging accuracy.

Visual search in animals is sometimes assumed to be restricted to one target at a time, and this limitation has previously been used to explain flower constancy (Dukas & Real, 1993). However, bumblebees have been found to identify two different colours simultaneously in a field of distractors (Nityananda & Pattrick, 2013). Bees also have been shown to detect and discriminate simple visual stimuli as quickly and accurately as humans and primates (Nityananda, Skorupski, & Chittka, 2014), but for complex patterns and similarly coloured objects, active scanning is required to correctly identify the stimuli, where bees make side to side scanning movements while approaching a target (Ings, Wang, & Chittka, 2012).

Bottom-up attention processes are considered standard for insects where salience of cues influences attention. For instance, bees' preferences for colours on the blue range of the spectrum can interfere with learning tasks (Morawetz, Svoboda, Spaethe, & Dyer, 2013). However, learning can modify selective attention in bees, suggesting also a top-down approach (Nityananda, 2016). Bees trained to associate colours with an aversive quinine solution became better at identifying and avoiding those colours (Chittka, Dyer, Bock, & Dornhaus, 2003), and while bees naturally prefer global information (i.e. the whole picture rather than individual features), they can be trained to attend to local information over global information (Avarguès-

Weber, Dyer, Ferrah, & Giurfa, 2015). As a result, previous learning can influence a forager's search behaviour.

2.2 Memory

To recognize familiar flowers, memorized components of the flower must be recalled and matched to the flower in question. A more detailed recall indicates more chances of mismatch between the current flower and that from previous experience. To allow for efficient foraging, memory must also be flexible enough to allow for distortions, such as those from wind, occlusion, or approach direction (Benard et al., 2006). Flower constancy is often explained through cognitive shortfalls, such as limited memory storage and retrieval (Gegear, 2005). These limitations steer foragers to favour identifying and generalizing a small number of floral features rather than memorizing large numbers of inflorescences (Chittka, Thomson, & Waser, 1999). Instead of relying on memory to identify individual flowers, memory storage can be maximized with generalization between certain floral components.

In early research, limitations in short term memory provided an explanation towards flower constancy. Flowers display multiple signals towards pollinators, and only a selected number of specific cues can be kept in working memory, resulting in a limited number of flowers visited (Leonard et al., 2011). Delays have been found in the retrieval of sensory cues of flowers that have not been visited recently (Bar-Shai et al., 2004). This is especially true when switching between floral species of different morphologies as handling time for each motor pattern increases between switches (Chittka & Thomson, 1997).

Similarity judgments may also lower cognitive load during foraging. Bumblebees were more selective when there was more variation among flowers of multiple traits rather than in just a single trait (Gegear & Laverty, 2005). A limited ability to process visual information from many flowers simultaneously may have selected for preferences of particular visual features of known rewarding flowers instead.

However, constraints in memory come with advantages. In addition to the different reward levels provided by floral species, the foraging environment may also change quickly over the course of the day. While it may appear adaptive for bees to learn associations between floral traits and rewards quickly, learning speed was a trade off for efficient memory retrieval. For instance, when bees were trained to learn new contradictory information, their memory performances were poorer than bees that were given only a delayed recall task (Chittka, 1998). Additionally, bees that learned one task (associating one colour with a location) had better memory retrieval than those that learned two (two colours with two locations) (Chittka & Thomson, 1997).

Large individual differences exist between learning performances of bumblebees, indicating that selection pressure may not always favor faster learning. While artificial selection can produce measurably faster learners within a few generations (Scheiner, Page, & Erber, 2001), naturally occurring populations are stabilized at the intermediary level (Raine et al., 2006). Because the environment is changing rapidly, new associations are constantly necessary to find rewards. Once information is stored in the long-term memory, it is hard to eliminate (Chittka, 1998). Having a larger long-term memory storage may interfere with learning changes in the environment. Instead, limiting the information input to only the most constant and salient elements may speed up retrieval and learning new cues in the long run.

2.3 Differential vs Absolute Conditioning

Training methodology has been found to influence the degree in which bees can discriminate stimuli. Two different forms of conditioning are often discussed: absolute and differential. Absolute conditioning involves the learning of a stimulus with no alternative present, while differential conditioning includes both rewarded and nonrewarded stimuli. With absolute conditioning, bees were found to assign more weight to learning the lower parts of a pattern, while differentially conditioned bees learned both (Giurfa et al., 1999). Bees trained on differential conditioning also learned both the reinforced stimulus in an excitatory way, and the nonreinforced stimulus in an inhibitory way.

Training through differential conditioning allows for discrimination of finer details than absolute conditioning. For instance, bees were able to discriminate finer colour features when trained with differential conditioning (Dyer & Chittka, 2004). Discrimination between perceptually similar stimuli was possible after differential but not absolute training (Giurfa, 2004). Furthermore, through differential conditioning, subjects are able to learn relational attributes between the stimuli rather than just absolute features.

3 Categorization

3.1 Definition

The abovementioned basic cognitive mechanisms and adaptive foraging preferences build into the more complex process of categorization, making use of the foragers' attention, memory, and learning methods to group objects in nature into known categories. Objects in the environment are often ambiguous and variable, and behaving appropriately towards unknown stimuli requires accurate predictions of behavioural consequences. While a small set of stimuli can be classified together through rote memorization, it would likely be a challenge to remember the consequences of every new stimulus in the forager's environment, particularly when it comes at a high cost to the individual, such as encountering predators. Simplifying information from a complex and changing environment will reduce cognitive demand and allow animals to adapt behaviours faster and more appropriately (Zayan & Vauclair, 1998).

Comparative research has given definitions of categorization in the past. Categorization has been defined as the ability to group distinguishable objects or events based on a family resemblance, and treat them as equivalent (e.g. Troje, Huber, Loidolt, Aust, & Fieder, 1999). It is considered a process of determining what things belong together (Zentall, Gallizio, & Chritchfield, 2002). Animals demonstrate categorization by responding appropriately to novel stimuli because they resembled others for which the appropriate response is already known (Vauclair, 2002). While most definitions of categorization are broad, categorization can be broken down into different forms.

Herrnstein (1990) exemplified a framework for five levels of stimulus control, increasing in abstractness. The first two levels included a lack of discrimination and categorization by rote. At the basic level, the animal appears to be transferring learning to the novel stimuli, but only because it cannot differentiate between the novel and the familiar. At the second level, the animal is unable to find common elements between the stimuli, and each individual stimulus is memorized.

Following categorization by rote at the third level is the open-ended categories, where objects are grouped by their perceptual features. The animal is capable of extracting common physical features of each object, and also transferring their learning onto novel objects that exhibit those features.

The fourth level is functional or conceptual categorization, where animals can group together objects that serve a common purpose. In this case, the objects do not necessarily need a physical resemblance to one another to be considered similar.

Finally, the highest level is abstract or relational categorization, where behaviour is transferring within relationships between objects. Examples of relationships could be spatial, where subjects could be trained to prefer objects that are above or below one another, or a comparison, where subjects have to identify objects based on sameness or difference.

Although Herrnstein frames the levels of stimulus control as hierarchical, rote memorization and the inability to discriminate are often not considered to be categorization (e.g. Zentall, Wasserman, Lazareva, Thompson, & Ratterman, 2008). For our purposes, categorization begins at the third level of open-ended categories, where tests of discrimination and generalization must be satisfied to ensure categorization does not depend on rote memory or confusion. In order to form categories, the animal must first be able to discriminate between two stimuli or groups of stimuli. Additionally, it must also be able to perceive some similarity between stimuli belonging to a group.

However, because abstractness of the stimuli groups are not always measurable, open-ended categories in this case would include both perceptual and abstract forms of categorization. Indeed, animals have been shown to perform both perceptual and abstract categorization successfully, but advanced forms of functional categorization has been largely unsuccessful (Shettleworth, 2010). Because of this, functional categorization will be considered the highest level of categorization in keeping with other authors (e.g. Zentall et al., 2008). More concrete examples for each level of categorization can be found in the following sections or in Experiments 2-4.

3.2 Discrimination and Generalization

In tests of categorization, animals are challenged with new stimuli, outside of the range of stimuli with which they were trained to categorize. As new resources can be encountered in rapid succession, being able to respond adaptively to a novel stimulus based on experience demonstrates flexible behaviours. The transfer of learning onto novel stimuli is also known as stimulus transfer and combines several of the basic cognitive processes mentioned previously (Giurfa & Menzel, 2013). To find targets in the environment, foragers must be able to identify the features, or any physical characteristic, of a flower and search the surrounding stimuli for those features. Extracting information and applying it onto novel situations is a necessary adaptation within any changing environment.

The transfer of learning from one known stimulus onto novel stimuli requires several processes. Firstly, the individual must be able to discriminate between types of stimuli. Discrimination training often involves reinforcement for different responses for each stimulus or group of stimuli. This could involve identifying differences between two or more flower species where only one is rewarding. In this case, not only are the features of a specific flower important, but also the features that differentiate the rewarding species from all others. Even training with single stimuli must also involve discrimination, as the animal must be able to differentiate it from other environmental cues (Shettleworth, 2010). For instance, a forager can be trained with only one flower, but must be able to identify the flower from its surroundings.

Once the forager has identified the features specific to the rewarding flower, generalization must occur in the presence of distortions of the original flower, or noise and interference from the environment, such as changes in position. Discrimination often involves repeated showings of the same images (Shettleworth, 2010). However, no repetition is necessary for generalization to occur. This requires learning, memorization, and evaluation of perceptual input (Giurfa & Menzel, 2013). Generalization typically takes place between perceptually similar inputs, where stimuli that are close together on a perceptual scale are treated as equivalent, and allows for flexible responding for novel objects. The absence of generalization between category members indicates only rote memorization is used. Previously categorization by rote has been mistaken for higher levels of categorization, where subjects appeared to be identifying each stimulus correctly by category membership, but in reality, were simply memorizing each stimulus and the response appropriate to it. For instance, pigeons were trained with images of cats, flowers, cars, and chairs, and could peck the correct key lights at each demonstration during testing (Herrnstein, Loveland, & Cable, 1976). Similar results have been found for people present/absent discrimination tasks (Herrnstein & Loveland, 1964) and images of bird species (Roberts & Mazmanian, 1988). However, pigeons have been shown to learn the contingencies of up to 320 unrelated images and remember it after 2 years (Vaughan & Greene, 1984). As images are repeated over training sessions, memorization of each picture and its outcome could have simply been a result of discrimination performance with the assigned categories being of no importance (Roberts & Mazmanian, 1998).

However, generalization is also possible as a result of a lack of discrimination, where the novel stimuli are too close perceptually for the forager to discriminate between them. For categorization to exist, foragers must see the novel flowers as also different from the original, but also extract the similar features from known flowers to apply onto novel flowers. Rewarding resources can also share features with unrewarding ones and being able to discriminate between groups of shared reward consequences would eliminate foraging time lost during confusion.

3.2.1 Picture-Object Correspondence

Correspondences between 2D and 3D stimuli can be used to examine discrimination and generalization abilities. For animals, pictures would not always be representative of the objects they portray. Although lacking in stereo vision, bees have been found to demonstrate an ability to perceive relative distances. For instance, bees can detect edges and extract depth cues from motion parallax (Werner, Stürzl, & Zanker, 2016) and can measure distance using optic flow (Esch, Zhang, Srinivasan, & Tautz, 2001). At smaller visual angles, bees can differentiate objects based upon dimensionality (Kapustjansky, Chittka, & Spaethe, 2009). While foragers do not come across pictures in nature, picture-object relationships are similar-but-different, akin to challenges found in environments where foragers must distinguish between groups of similar stimuli that also differ individually in reward.

To recognize and find association between photographs and 3D objects, the subject must not confuse the two (i.e. the subject sees the difference between the object and photo and treats them as the different) nor see them as independent (i.e. the subject sees the resemblance between the object and photo). This draws parallels to the first two levels of stimulus control identified by Herrnstein (1990), where categorization can be mistaken for either a lack of discrimination or rote memorization. In order to transfer behaviour in categorization tasks, foragers must be able to identify every individual stimulus as a new entity, while also generalizing similar aspects between them. As a result, there must be some similarities found between the object and the corresponding photograph while also recognizing they are not the same (Fagot, Martin-Malivel, & Dépy, 2000). Previous research demonstrated that bees are able to pass tests to reject both confusion and independence (Thompson & Plowright, 2014). With training, bees were able to discriminate between corresponding objects and photographs, while also recognizing similarities between the two. The similar-but-different features in pictures and objects give insight into the discrimination and generalization abilities in foragers that are necessary for categorization. Additionally, pictures and objects are similar but differ in more than a specific set amount of features, which is more representative of flowers found in nature.

3.3 Open-ended categories

3.3.1 Perceptual

Once subjects can identify the different-but-similar aspects of the stimuli, it follows that they can generalize behaviour onto different variations of that group of stimuli. Because generalizing behaviour speeds up foraging, it would be advantageous to use perceptual similarities to circumscribe classes of unlimited members using the contingencies of just a few. Considered to be at the basic level of categorization, perceptual learning requires subjects to be able to discriminate between different groups of stimuli, while also generalizing the similarities within them (Keller & Shöenfeld, 1950; Benard et al., 2006). This ability involves the sorting of stimuli into classes that share one or more properties and respond appropriately to each class. Typical perceptual categorization studies consist of initial training with stimuli containing categorical attributes, and then testing the subjects for transfer of learning on novel stimuli. For instance, bees trained to discriminate between two colours can be given patterns containing one or the other, with one colour being rewarded, while the other is not. To verify if bees can extract the common colour within each category, they are given novel patterns containing the common colours, and tested for preference. Positive transfer of learning indicates category formation based on colour. The development of perceptual learning is controlled largely by the animals' capabilities of discrimination or primary stimuli generalization. As a result, rejecting rote memorization requires testing for generalization between category members. Perceptual categories are open-ended and can have limitless instances of related stimuli, including those not available in the animals' natural environment.

In many instances, an open-ended categorical test usually requires the subject to learn one or a few attributes, such as symmetry. For instance, bees were able to categorize objects based on the absence or presence of bilateral symmetry (Giurfa, Eichmann, & Menzel, 1996). Bees were trained to prefer either symmetrical or asymmetrical patterns and were able to transfer learning onto novel symmetrical or asymmetrical patterns. After experience with training stimuli, bees were able to extrapolate the feature of symmetry and apply it onto novel patterns.

Similar results have been found with other patterns as well, including orientation preferences (van Hateran, Srinivasan, & Wait, 1990). Bees were trained to discriminate two gradients of different orientations, with one orientation being rewarding and the other non-rewarding. When tested with novel patterns, bees chose the appropriate orientation that corresponded with the rewarding orientation during training.

While many studies reveal positive learning transfer to novel stimuli in bees, a natural foraging environment requires more complex strategies than simply identifying and extracting one common feature in all rewarding resources. Although categorization of features could occur across different sensory inputs, visual learning presents an ecologically relevant context for social insects, where natural environments require learning cues for the hive and food source locations (Avarguès-Weber, Deisig, & Giurfa, 2011).

Category formation occurs by similarity of features, but it is difficult to specify the exact features used to classify stimuli (Zentall et al., 2008). Categories can exist for polymorphous stimuli, where the objects are not linked by any specific characteristic, but generalization can still occur to novel stimuli (Lea & Harrison, 1978). Often, a combination of several features is required to discriminate between rewarding and non-rewarding flower species, and additionally, not all members within a species could share all the features. Recognition of stimuli appears to be general rather than specific. For instance, a forager capable of perceptual categorization would recognize a generic prototypical flower rather than specific individual flowers (Herrnstein, 1990). This holds true even when individual recognition is necessary, such as when identifying a specific landmark leads to generalization towards other perceptually similar landmarks, suggesting that natural contingencies tend towards open ended categories.

Success on categorization tasks has been found for instances involving these more complex features. Honeybees were able to sort photographs belonging to distinct classes of landscapes, plant stems, and flower shapes (Zhang et al., 2004). These photographs differ in individual, low-level features, making it impossible for bees to pick out one or two features to generalize. According to the prototype theory, in such instances, the different features learned from experience could be assembled to build a generic pattern representation (Avarguès-Weber et al., 2011). This representation would then be used to respond to novel stimuli. However, experimental evidence is limited for the formation of prototypes in animal research. For instance, no difference in discrimination occurred between prototypical and non-prototypical stimuli in birds (Watanabe, 1988; Jitsumori, 1993). In research with baboons, prototypes were classified more accurately than other exemplars, but category membership did not involve prototypical representations (Dépy et al., 1997). Although some attempts have been reported, category performance was expressed under prototypical stimuli representing typical features of the categories rather than less typical exemplars.

3.3.2 Relational

Relational categories include stimuli grouped together through a common relationship, such as “same or different”, and “above or below”, rather than physical features of the stimuli involved. Because of this, an individual stimulus alone cannot form an abstract category as it requires a comparison, such as more than, bigger than, etc. This also leads to the formation of open-ended classes with unlimited membership.

Abstract categories were once thought to characterize the mental divide between humans and all other species (Penn, Holyoak, & Povinelli, 2008). More recently however, evidence of same-different learning has been found in bees as well. Typical same-different experiments showed bees trained with reward on a sample stimulus, and subsequently given a choice between an identical stimulus and an alternative. One group of bees received a reward for choosing the identical while the other was rewarded for choosing the alternative. Results showed that bees trained along one element also transferred learning onto other elements. For instance, honeybees trained to make same or different judgements for colour also transferred behaviour onto odour stimuli (Giurfa et al., 2001). Along the same lines, a more recent study with bumblebees trained bees to perform same-different discrimination tasks using either colour or line grating stimuli (Brown & Sayde, 2013). Bees were given pairs of two different stimuli sets (colour or line grating) in either the same or different configurations. Bees could transfer both from colour to line grating and vice versa.

Flower constancy has been found to interfere with relational judgments previously (Wiegmann, Wiegmann, MacNeal, & Gafford, 2000). Individual bees that are more constant were more likely to generalize absolute features, (i.e. a flower of a particular size) rather than relational features (i.e. choosing the smaller flower in a group). Environmental factors that promoted constancy may inhibit relational categorization, and bees may be using simple associative learning instead.

3.4 Categorization by shared consequences

While open-ended categories are based upon perceptual similarity, psychological representations of the contingencies of reinforcement are not necessarily tied to physical elements. Members of a functional or conceptual category have more than perceptual attributes in common (Shettleworth, 2010). While only simple generalization is required for open-ended categories, this level of categorization relies on mediated or secondary generalization (Delius, Jitsumori, & Siemann, 2000), where behaviour is transferred between stimuli because of shared consequences, or functional equivalence, such as food items, predators, or landmarks, rather than physical attributes. As a result, the connection between the stimuli in a category is based on learning (Herrnstein, 1990).

In order for category members to be linked by concepts, there must be a generalization of behaviour over all members, and this must apply beyond perceptual similarity (Zayan & Vauclair, 1998). It is possible that members with a perceptual category described previously could also be related based upon functional equivalence as well as physical properties, and concepts may also be required for open-ended categorization. It is important to note that the term “concept” may be vague in literature and has no generally accepted definition (Zentall et al, 2008), however, categories are often defined in terms of human language concepts (Watanabe, et al., 1993). For categorization, Herrnstein (1990) defined concept as a differential response to a set of stimuli. The grouping may be open-ended or limited. Responding generalizes to all stimuli within the set regardless of perceptual similarity. For that reason, concept learning has been attributed to other forms of categorization as well (i.e. Giurfa & Menzel, 2013). The main difference is that classes here are arbitrary and associative. The stimuli themselves may have little to no common features but are associated to common responding by previous experience towards a common contingency, such as toys (Feuerbacher & Rosales-Riuz, 2017).

Because responding is similar for all members of the category, there is no gradual decrease of responding along a perceptual scale as found in only generalization (Pastore, 1987). However, transfer tests for open-ended categories involve novel stimuli that exist on a perceptual or relational scale. As a result, it becomes difficult to separate the responding as a result of simple generalization over conceptual learning. Often, novel stimuli are ideal exemplars of each grouping. For instance, transfer tests to novel stimuli for symmetry all share the same symmetrical elements, rather than existing on a perceptual scale of stronger or weaker symmetry. Responses towards novel stimuli in these cases are often similar to responses to the familiar stimuli. On the other hand, categorization by shared consequences does not involve transfer onto novel stimuli, and responding is only due to previous experience. For instance, a functional category of food would only consist of things previously experienced to be food, and cannot transfer onto novel objects where the association is not yet made. This allows for more accurate testing on conceptual learning rather than generalization.

3.4.1 Functional equivalence

Functional equivalence is not to be mistaken for formal stimulus equivalence, or Sidman’s equivalence. Based on the experiment by Sidman (1971), formal equivalence requires the subject to demonstrate reflexivity (after training to select a photograph of flower A in the presence of flower A, one would select the photo of flower B in the presence of flower B), symmetry (after training to select the photo of flower B in the presence of flower A, one would select the photo of flower A in the presence of flower B), and transitivity (if trained to select the photo of flower B in the presence of flower A and a photo of flower C in the presence of flower B, one would select a photo of flower C in the presence of flower A). While evidence for formal equivalence has been demonstrated in sea lions (Shusterman & Kastak, 1993) and chimpanzees (Yamamoto & Asano,1995), very little investigation has been conducted in other non-human species. Although formal equivalence has yet to be tested in invertebrates, indirect evidence is available for functional equivalence in bees using serial reversal training.

3.4.2 Serial Reversals

It is difficult to test whether an animal is categorizing based on concepts with no physical similarities, since in nature, most objects sharing a function also tend to share perceptual similarities. For instance, in other species, such as pigeons, performance in categorization tasks was faster and more accurate when there was some shared similarity between the groupings (Watanabe, Lea, & Dittrich, 1993). Additionally, as previously noted, instances of rote memorization had been mistaken for categories. Because this level of categorization relies on previously experienced contingencies rather than physical features, the category can no longer be open-ended and transfer tests to novel stimuli would not be possible, making it also difficult to distinguish rote memorization from categorization. However, generalization within categories has been investigated using serial reversal training to eliminate the possibility of rote memorization.

Previous research has found evidence of conceptual categories in other species. Studies of functional categorization ensure that members only share a specific associated response, such as a reward or reinforcer. For instance, baboons and chimpanzees successfully formed functional classes by sorting stimuli into groups of food and non-food, despite the stimuli within a group having little to no similar features with one another (Savage-Rumbaugh, Rumbaugh, Smith, &

Lawson, 1980; Bovet & Vauclair, 1998). Similar findings for dolphins were identified (von Fersen & Delius, 2000). Dolphins were trained with reward contingencies on two sets of tones, where one set was rewarded while the other was not. Following serial reversal training, the dolphins could identify the contingency for each set despite the tones having more similarity between sets rather than within sets.

Reversal learning can also demonstrate the influence of reward contingencies on perceptual categories as well, as it allows groups of stimuli to be considered perceptually relevant or irrelevant without specific discrimination training. For instance, in a previous study, pigeons were trained to categorize two groups of slides. Then they were given the mirror reversals of each slide, with reversed reward values. Initially, pigeons generalized behaviour to the mirror images, but after a couple reversals, they responded appropriately depending on orientation (Vaughan & Greene, 1984). Although the slides generalized to their mirror images based on similarity, the subjects used orientation as a category based upon reward contingencies.

A typical serial reversal task involves repeated reversals of the reward contingencies between two sets of stimuli, after an initial discrimination training. The animal must adopt a win stay-lose shift strategy, where once they try the last rewarded response with no reward, they shift to the other response. Optimal performance on the task involves one error between each reversal set (Shettleworth, 2010).

Early work on reversal learning in bees had mixed results (e.g. Menzel, 1969; Couvillon & Bitterman, 1986). More recently, bees have been tested on serial reversals, revealing a reduction in errors and improved performance across successive reversals (Strang & Sherry, 2014). Individuals who learned the initial association faster were also faster at reversing the association and retaining the information for longer periods (Raine & Chittka, 2012). Bees were capable of relational categorization, which is traditionally considered to be more sophisticated than functional categorization (Herrnstein, 1990). It would follow that a lower level of categorization should also be possible with bees.

4 Plan of Study

While serial reversals give us insight on the learning flexibility in bees, rapid reversals in quick succession are rarely found in natural floral species. Once natural flowers are past their blooming period, no further reward reversals can occur within the foragers' lifetimes. Very little investigation has been done for mediated generalization in bees in a more natural context. Rather than serial reversals, the use of one reversal has been proposed to examine category membership (Lea, 1984). After the subjects are trained to discriminate between two clusters of stimuli, the reward values of one stimulus from each cluster are switched – what was rewarding becomes unrewarding and vice versa. Subsequent testing on the remaining stimuli reveals whether the subject will respond to them as if they also changed their reward values. When the reward values of the original stimulus have changed, behaviour should transfer to all remaining stimuli within that cluster. For instance, in an example from Herrnstein (1990), acorns vary, but for a creature that relies on acorns, they would likely resemble each other. If acorns were to suddenly become bitter, after tasting one or a few bitter acorns, is bitterness associated with acorns in general? A similar problem can be found in bumblebees. If a rewarding species becomes unrewarding, are all members of the species treated as unrewarding?

The following studies will examine if bumblebees are able to categorize based upon simple generalization, and if so, whether they do so in conjunction with mediated generalization in categorization tasks. By using what is known about generalization in picture-object correspondence tasks, categorization will be examined with 2D and 3D stimuli. While photographs may not exist in natural settings, mechanisms of matching and generalizing between several features allows for a more natural behaviour than categorization tasks focussing only on a single feature. In nature, several flowers of the same species may look identical with subtle changes, as would objects as they become degraded into photographs.

Following the logical steps in stimulus control outlined by Herrnstein (1990), categorization can only occur if bees can discriminate between groups of flowers while generalizing within them. Experiment 1 examines if bees can spontaneously generalize between a photograph and an object, while also being able to differentiate between the two without training. Transfer of learning will be tested both from a photograph to an object and vice versa.

Once both discrimination and generalization are established, the following study uses a single reversal test to examine perceptual categorization capabilities. Unlike tests that require generalization onto novel stimuli, single reversal tests can be used even when novel stimuli are not included and successful completion would also mean rejection of rote memorization during the task. First bees were tested on perceptual categorization where corresponding photographs and objects are grouped under the same reward conditions, where one photo-object pair is rewarding, and the other is not (experiment 2). If one stimulus switches reward, will behaviour generalize towards the corresponding object or photograph?

Following this, bees were tested for mediated generalization in one of two ways. The first is using perceptually different stimuli, where the object and the corresponding photograph of another object share a common reward (experiment 3), and adopts the same methodology as experiment 2. Either the photographs or objects are removed and the remaining stimuli switch reward consequences. The bees were tested for transfer on the removed stimuli. This reveals whether or not bees can transfer learning with perceptually different stimuli, whether they rely on memory, or whether they generalize between similar stimuli despite different predicted outcomes.

The final study used three perceptually different flowers to examine if sharing reward consequences creates a similarity between stimuli (experiment 4). Following experiment 3, it is still uncertain whether or not stimuli with shared reward consequences are considered to have a relationship to the bees. By removing the element of perceptual similarity, results from experiment 4 will determine if bees have some degree of learning transfer between flowers of shared consequences and give an indication of the mechanisms typically employed during functional categorization. A brief outline of all four experiments are shown below in Table 1.

Similar methodology between the studies allowed for better comparison between the results. A radial arm maze was used for all studies, where 3D and 2D stimuli used for training were placed on the ends of the corridors with feeders containing reward, but bees were tested on unrewarding stimuli individually. The first ten choices of each bee were recorded during testing, where a choice was recorded when contact was observed between the bee and the stimulus, back wall of the corridor, or the feeder.

Table 1: Outline of all experimental designs

		Experiment 1	Experiment 2	Experiment 3	Experiment 4	
Discrimination Training	Phase 1	OBJECT X+/ OBJECT Y-	OB.X PH.X+/OB.Y PH.Y-	OB.Y PH.X+/OB.X PH.Y-	Object X Object Y + / Photo X Photo Y- Vs Object X Photo X+/ Object Y Photo Y-	
	Phase 2		OB.Y+/OB.X-	OB.X+/OB.Y-		
Test	Test 1	OBJECT X vs PHOTO X	PHOTO X vs PHOTO Y	PHOTO X vs PHOTO Y	Test 1	OB. Z vs PH. Z
	Test 2	PHOTO X vs PHOTO Y			Test 2	Ph. X2 vs PH. Y2
					Test 3	OB. Z vs PH. X2

OB. X is Object X, OB. Y is Object Y, OB. Z is Object Z

PH. X is Photo X, PH. Y is Photo Y

The four experiments will demonstrate the bees' abilities to categorize with increasing levels of abstraction, starting with basic generalization and discrimination abilities to investigations in functional categorization. Because categorization is rarely examined in insects, this will clarify the extent of categorization processes in bumblebees based upon models that have already been established in other animal psychology literature.

Experiment 1

Different-but-Similar Judgments by Bumblebees

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Abstract

This study examines picture perception in an invertebrate. Two questions regarding possible picture- object correspondence are addressed for bumblebees (*Bombus impatiens*): (1) Do bees perceive the difference between an object and its corresponding picture even when they have not been trained to do so? (2) Do they also perceive the similarity? Twenty bees from each of four colonies underwent discrimination training of stimuli placed in a radial maze. Bees were trained to discriminate between two objects (artificial flowers) in one group and between photos of those objects in another. Subsequent testing on unrewarding stimuli revealed, for both groups, a significant discrimination between the object and its photo: discrimination training was not necessary for bees to detect a difference between corresponding objects and pictures. We obtained not only object-to-picture transfer, as in previous research, but also the reverse: picture-to-object transfer. In the absence of the rewarding object, its photo, though never seen before by the bees, was accepted as a substitute. The reverse was also true. Bumblebees treated pictures as “different-but-similar” without having been trained to do so, which is in turn useful in floral categorization.

Introduction

Because there is natural variability in the visual appearance of individual flowers within a species, managing distortions of an original rewarding stimulus is a requirement for successful foraging. However, recognition of key floral characteristics is necessary to differentiate a rewarding species from other environmental stimuli. Bees are flower constant (Chittka, Thomson, & Waser, 1999; Raine & Chittka, 2005, 2007; Rossi, Santos, Salvarrey, Arbulo, & Invernizzi, 2015), having natural tendencies to exclusively visit certain flower species and prefer matching stimuli. As a result, foraging strategies must include different-but-similar judgements, or the extracting of shared attributes from a rewarding stimuli group, and distinguishing those features from those of unrewarding stimuli groups. These processes may be important in picture-object recognition mechanisms. Although bees do not encounter photographs in nature, counterpart photos and objects are used here to assess different-but-similar judgments. Pictorial stimuli, or floral patterns, are often used in lieu of real world objects in bee research [e.g., photos of flowers (Plowright et al., 2013), artistic representations of flowers (Chittka & Walker, 2006), colour drawings of flowers (Zhang, Srinivasan, Zhu, & Wong, 2004)] though little is known about picture processing, especially among invertebrates. As noted by Gould (2002), to simply place cues in the vicinity of food and call them “flower learning cues” can lead to mischaracterizations of flower learning processes. To understand pictures as representations of an object, an association from one to the other must be made (Salsa & de Mendoza, 2007). Fagot, Martin-Malivel, and Dépy (2000) described certain obstacles, such as confusion and independence, to an individual’s picture perception. Confusion indicates the subject’s inability to distinguish between an object and its 2D representation. For instance, pigeons (*Columba livia*) displayed attacking behaviours to a photograph of a conspecific as they would to a live model (Looney & Cohen, 1974). Identical behavioural responses were shown to both pictures and objects.

On the other hand, independence, where the subject is unable to find similarities between the object and corresponding picture, is demonstrated by the lack of behavioural responses expected to be elicited from objects to pictures or vice versa. For instance, pigeons that were capable of identifying individual humans were unable to transfer the recognition to photographs of the individuals (Dittrich, Adam, Ünver, & Güntürkün, 2010). Picture-object correspondence can be defined as a transfer or generalization between 2D and 3D stimuli, where too much correspondence results in confusing objects and photos, while too little means the subject fails to see the relation between the two (Weisman & Spetch, 2010).

Picture recognition is not always reliable, and even among vertebrates, object-to-picture correspondences may fail (e.g., Dittrich et al., 2010). Behavioural responses towards a 3D stimulus do not indicate successful identification of the stimulus’s corresponding photograph. A number of cues, such as depth, colour, luminosity, and size, are misrepresented in pictorial stimuli for animal studies due to technical constraints (e.g., Delius, Emmerton, Hörster, Jäger, & Ostheim, 2000). With the increase in research on bee cognition in recent years, results often rely on behavioural responses to pictorial floral patterns in place of real flowers. For instance, simple 2D designs are used to replicate complex elements, such as symmetry (Plowright, Evans, Leung, & Collin, 2011; Rodríguez, Gumbert, Hempel de Ibarra, Kunze, & Giurfa, 2004), petal arrangements (Lehrer, Horridge, Zhang, & Gadagkar, 1995; Séguin & Plowright, 2008), and spatial frequency (Horridge, 1997). However, behavioural responses to images do not necessarily indicate subjects equate the stimuli with natural floral cues, and accurate processing of images is often a learned ability (Delius et al., 2000).

While there is little investigation in picture-object correspondences in insects, previous research has found insects to be capable of transferring learning of the same features in different stimuli groups. Honeybees (*Apis mellifera*) were capable of making different-but-similar judgments with mirror images (Gould, 1988). Bees were first trained to discriminate between two patterns, consisting of the same features in different arrangements, and then tested for behavioural responses towards their mirror images.

In one test, bees chose the previously rewarding stimulus over its mirror image, ruling out confusion. At the same time, the bees also preferred the mirror image over a novel stimulus. Despite the perceptual differences between the two, mirror images were accepted as a substitute in the absence of a rewarding stimulus. Gould's method of assessing similarity and difference judgements has been used in subsequent research with similar findings using mirror images (Korneluk & Plowright, 1995), and left-right transformations (Plowright, 1997; Stach & Giurfa, 2001), and we adopt the method in the present study.

Honeybees also demonstrated positive learning transfers from a stimulus set to novel stimuli that share common features with original stimuli (Benard, Stach, & Giurfa, 2006). For instance, grouping of similar colours is possible (Benard & Giurfa, 2008; Dukas & Waser, 1994) and stimuli within the same colour gradients were treated as similar. Additionally, honeybees could discriminate between non-floral Picasso and Monet painting styles (Wu, Moreno, & Tengen, 2013) with some ability to transfer learning onto novel stimuli. Discriminations between natural scenes, such as trees in forests (Dyer, Rosa, & Reser, 2008), and flower shapes (Zhang et al., 2004), have also been found.

Previous research on picture-object processing showed bumblebees (*Bombus impatiens*) to be capable of learning to differentiate between an object and photo with training (Thompson &

Plowright, 2014). Furthermore, learning from object discrimination was also transferred to the corresponding photographs. After an initial discrimination training with two objects, bees preferred the corresponding image of the previously rewarding object over that of the previously unrewarding object.

This study expands further on the previous research by Thompson and Plowright (2014). We examined whether bees could discriminate between counterpart photos and objects even in the absence of prior discrimination training, or whether they confused them. It is possible that picture-object discriminations are like some other pattern discriminations in being so difficult for bumblebees that they require some prior training (e.g., Perreault & Plowright, 2009). If the discrimination training between pictures and objects could be dispensed with, one research obstacle in floral categorization would be removed. Such a result would suggest that in nature, some of the floral discriminations necessary for categorization could be made without associative learning.

Methods

Subjects

Four colonies of bumblebees (*Bombus impatiens*) were used for this study, supplied by Koppert Biological Systems Canada. The bees had no experience outside the colony prior to the experiment. The colonies were fed pollen ad libitum and trained with 1:2 sugar to water solution by volume in a radial arm maze. Individual bees were labelled with coloured numbered tags glued to the thorax. A total of 20 bees from each colony were tested.

Materials

The colonies were contained in a plastic cubic box, with a 40-cm tube connecting the container to a rectangular wooden walkway, topped with glass plates. A tunnel of wire mesh connected the walkway to an opening in the center of the maze floor. The radial arm maze consisted of 12 corridors (14 cm long and 15 cm high), which opened into a central area (17 cm diameter) from an entrance (6 cm wide). Though the angle of approach from the entrance of the corridor to the flower at the end of the corridor could not be controlled, the corridors did restrict the angle of approach. Only 8 of the 12 corridors were in use for the experiment, with the remaining 4 entrances blocked by opaque Plexiglass® dividers. The maze had a clear, Plexiglass® cover (53 cm diameter). The opaque grey walls of the maze were also of the same material. Feeder troughs were located at the end of the corridors, and filled with sugar solution or left empty (Figure 1).

Artificial flowers or photographs were also attached to the end of the corridors, with one stimulus per corridor, and bees were able to fly to and touch the stimuli (Figure 2). The stimuli were randomly placed before each training session to ensure no location cues were used. The experiment used two types of synthetic fabric flowers (a single yellow flower (X) and a pink cluster of flowers (Y)). The corresponding colour photographs of each flower type were also used (Figure 3). Four copies of both photos or both objects were used during training and testing. The same objects and photos were used from the Thompson and Plowright (2014) study. The photographs were taken using a Panasonic DMC-FZ20 camera and printed with a Canon MP560 ink jet printer with a grey background (8.5 cm x 10.5 cm). Flowers are photographed so that photos and objects would be at the same angle when approaching each corridor. High frequency

(> 40kHz) light ballasts (Sylvania Quicktronic T8 QHE4x32T8/112 with fluorescent bulbs Sylvania model FO32/841/XP/SS/EC03) were positioned directly above the maze.

Procedure

The bees were trained in groups and foraged freely in the maze for 3 – 5 hrs every day for three weeks. The bees were labelled with coloured number tags on the thorax as they left the maze and monitored individually. A choice was made when a bee extended its proboscis into the feeders. A new choice was recorded once the bee exited the current corridor and entered another or the same corridor to feed. Satiated bees would leave the maze and go back to the colony, and then return to the maze again for another foraging trip. As with the Thompson and Plowright (2014) study, an individual was selected for testing if it had foraged for two consecutive days and made eight consecutive choices on rewarding stimuli in a given day, three of which had to be different corridors. Should a bee extend the proboscis in an unrewarded feeder, it had to make eight more consecutive choices on the rewarding feeders to qualify for testing. The observer noted each time a bee lands on a feeder. After each day of training, the walls and floor of the maze were rinsed and left to dry overnight to eliminate sugar residues.

Upon reaching the training criteria, bees were tested individually. The testing stimuli were placed with empty unrewarding feeders. Choices were recorded when the bee touched either the feeders, stimuli (flower or photograph) or the back wall of the corridor. The bee had to have exited the corridor before another choice was recorded. The first ten choices of each bee were recorded.

Design

Two conditions were tested in the experiment, with two colonies trained on the object discrimination (artificial flowers) and two on the photograph discrimination. “Object” and “Photo” are heretofore abbreviated as “Ob.” and “Ph.”, respectively. In the first condition, the single yellow flower was the rewarding (+) stimulus (Ob. X+), while the cluster of pink flowers was the unrewarding (–) stimulus (Ob. Y–) (Table 2). Four copies of each stimulus were placed in the maze. The first colony of bees was trained in that scenario and twenty bees were selected for testing, in two groups of ten. Bees were randomly selected for each of the two groups. The first group was tested using the flower that was rewarding during training, and its corresponding photograph (Ob. X vs. Ph. X). The second group was tested against photographs of the corresponding flowers (Ph. X vs. Ph. Y) to examine if bees accepted the photo in the absence of the previously rewarding stimulus, and learning was transferred from the object to the photo. The stimuli were then counterbalanced (Ob. X–, Ob. Y+) for a second colony (see Figure 4 for a sample of the design). Again, twenty bees were tested: half with the previously rewarded flower and its corresponding photograph (Ob. Y vs. Ph. Y), and the other half with both sets of photographs (Ph. X vs. Ph. Y).

In the second condition, the photograph of X was rewarding (Ph. X+) while the photograph Y was unrewarding (Ph. Y–) (Table 2). Bees were tested either with the previously rewarded photo and its corresponding object (Ph. X vs. Ob. X), or with the two objects (Ob. X. vs. Ob. Y) (see Figure 4 for a sample of the design). The procedure was later counterbalanced for a fourth colony (Ph. X–, Ph. Y+) (Table 2) with the bees tested on the photograph and object (Ph. Y vs. Ob. Y) or objects corresponding to the photographs (Ob. Y vs. Ob. X). Ten bees were used for each set of tests, and the first ten choices of each bee were recorded.

Statistical Analyses

Because the data were binomial with replication within subjects (each having 10 choices), a replicated Goodness of Fit test (Sokal & Rohlf, 2012) was used to compare choice proportions to a theoretical chance value of 0.5. The G_P value determined if the group proportions differed from the theoretical chance value (50:50), while the G_H value tested for individual differences. G values were compared to the χ^2 value to test for significance.

Results

Discrimination Training Between Two Objects

Figure 5 outlines the results of both tests when the rewarded stimulus (S+) was an object. There was no confusion between the object that had been rewarded during training and its corresponding photograph. The choice proportions for the object were significantly greater than chance, both for Object X ($G_P = 61.35$, $p < 0.001$, df = 1) and Object Y ($G_P = 21.98$, $p < 0.001$, df = 1). The subjects' first choice favored the previously rewarding object, with 10 of 10 on Object X and 7 of 10 bees on Object Y. No individual differences were detected ($G_H = 10.33$, p = 0.32, df = 9; GH = 5.77, p = 0.76, df = 9, for Object X and Y respectively).

Furthermore, bees were capable of transferring learning from the S+ to the corresponding photograph, which was accepted as a substitute in the absence of the S+. In the absence of Object X that had been rewarded during training, Photo X was chosen over Photo Y ($G_P = 20.04$, $p < 0.001$, df = 1). Similarly, in the absence of Object Y that had been rewarded during training, Photo Y was chosen over Photo X ($G_P = 11.79$, $p < 0.001$, df = 1). All 10 bees chose Photo X as their first choice when Object X had been rewarding, and 9 of 10 bees chose Photo Y as their first choice when Object Y had been rewarding. No significant individual differences were found ($G_H = 10.39$, p = 0.32, df = 9; $G_H = 9.57$, p = 0.39, df = 9 respectively).

Discrimination Training Between Two Photos

When the bees were trained with photographs, as shown in Figure 6, there was no confusion between the photo that had been rewarded during training and its corresponding object. The choice proportions for the photograph were significantly greater than chance, both for the Ph. X+/Ph. Y– group ($G_P = 5.82$, $p = 0.016$, $df = 1$), with 6 of 10 bees choosing Photo X as their first choice, and the Ph. Y+/Ph. X– group ($G_P = 21.98$, $p < 0.001$, $df = 1$), with 8 of 10 bees choosing the Photo Y as their first choice. No significant individual differences were found. ($G_H = 4.18$, $p = 0.90$, $df = 9$; $G_H = 6.98$, $p = 0.64$, $df = 9$ respectively).

Furthermore, bees were capable of transferring learning from the S+ to the corresponding object, which was accepted as a substitute in the absence of the S+. In the absence of Photo X that had been rewarded during training, Object X was chosen over Object Y ($G_P = 21.98$, $p < 0.001$, $df = 1$), with 9 of 10 first choices on Object X. Similarly, in the absence of Photo Y that had been rewarded during training, Object Y was chosen over Object X ($G_P = 13.26$, $p < 0.001$, $df = 1$), with 7 of 10 bees choosing Object Y as the first choice. Again, no significant individual differences were found ($G_H = 12.69$, $p = 0.18$, $df = 9$; $G_H = 11.95$, $p = 0.23$, $df = 9$ respectively).

Discussion

In this study on picture-object correspondence in an invertebrate, we developed a way of assessing how bumblebees spontaneously treated a photograph of an artificial flower, having never seen the photograph, and the artificial flower corresponding to the photograph, having never seen the flower. We devised an operational way of testing for their ability to differentiate the two and yet detect a similarity. Following the discrimination training on objects or on photos, half of the bees received one test (for the difference judgement) and half received the other (for the similarity judgement). Given that both subgroups succeeded, and there was no cue as to which of the two tests would be administered, we conclude that all bees could have made either judgement.

The two counterbalanced conditions first tested for unlearned discriminations between a corresponding object and photo. After discrimination training between two objects or photos, bees were tested for preferences between a previously rewarding stimulus and its 2D or 3D counterpart. All bees had a strong preference for the previously rewarding stimuli over the novel counterpart object or photograph, thus demonstrating a capability of discriminating objects and photographs without training or prior exposure to both stimuli. As a result, discrimination training is not necessary for the bees to perceive the difference between photos and objects. This aspect of discrimination will be useful in future studies of categorization. Because many differences exist between objects and photos, being able to discriminate between the two is not surprising. However, as the perceptual system of bumblebees differs greatly from that of humans, testing for difference judgments was necessary to confirm the expectations.

When examining independence (i.e., the inability to see similarities between objects and photographs), the bees were tested with only corresponding photos or objects of the training stimuli and the original S+ and S– were absent. The bees transferred learning from the rewarding object to its corresponding photo as with previous research (Thompson & Plowright, 2014) and also from the photo to its object. In the absence of the rewarding object, the corresponding photo was taken as a replacement. The same holds true for rewarding photos, where the corresponding object was preferred as a substitute in the photo’s absence. Bees were able to find a similarity both when information is added from a photograph to an object, and when an object is degraded to a photograph.

While the previous study (Thompson & Plowright, 2014) reported success with a picture-object discrimination on which bumblebees had been trained, here we found no prior discrimination learning was necessary. Moreover, prior experience with photographs was not necessary for the discrimination. Also, there were no significant individual differences found, indicating a possible homogeneous different- but-similar picture-object perception in bumblebees, likely due to the strict training criteria required for testing. The implications from this study are neither to license the use of 2D stimuli, nor to argue for its discontinuation. Rather, it is to underscore the caution, made by others (e.g., Spetch & Friedman, 2006; Weisman & Spetch, 2010) against untested assumptions regarding how pictures of real objects are treated in animal cognition.

A number of differences exist between objects and their photographs, facilitating discriminations between the two. Elements, such as colour, can alter from an object to a photo, and picture capture devices are often designed for human vision only. Photos were also printed on a grey background. Additionally, cues from tactile manipulation of both objects and photos were available, which might be used in discriminating between objects and photos (Chittka, 1998; Chittka & Thompson, 1997). Despite the differences present between the photographs and the objects, the bees were still able to perceive similarities between the two. This is possible for all counterbalanced conditions, indicating that the qualities of the objects and photographs do not differ too greatly for similar judgements to be impossible.

While picture object recognition mechanisms may appear to have little ecological relevance to bees in nature, individual flowers within a resource-rich floral species differ from one another, and it would be disadvantageous for a foraging bee to remember details of a single flower rather than common features of the entire species. Here, the photo or object counterparts of the rewarding stimuli might be considered another inflorescence within the same species. Thus, the results do not necessarily entail that bees view images as representations of objects.

In previous research, no transfer in bees was found when images were degraded to drawn pictures (Thompson & Plowright, 2014), possibly due to a lack of shared features between the images and objects. The differences between a line drawing and an object may be too large for similar features to be perceived. Even among humans, pictorial stimuli are not necessarily treated as representations of their object counterparts (Deregowski, 2000), and behavioural responses are not uniformly defined within groups. While it is unknown whether or not pictures are recognized as representations of objects to bees, experience with the pictures or objects are important in picture recognition (Aust & Huber, 2010; Watanabe, 2000). In this case, previous training experience on pictures and objects leads to behavioural transfers to the counterpart stimuli. In addition, our results underscore the importance of current context: how bees treated stimuli at the time of testing depended not only on their reinforcement history but also on the alternative that was presented.

Although it is possible that only the most salient cue, such as shape or colour, is attended to in discrimination tasks, bumblebees did generalize learning from at least one artificial flower to a black and white silhouette (Thompson & Plowright, 2014). Moreover, bees have been found to learn multiple elements simultaneously (Ronacher, 1998), suggesting a possible set of features, such as a particular configuration of cues, to be necessary for picture-object identification and categorization (Avarguès-Weber, Deisig, & Giurfa, 2011; Avarguès-Weber, Portelli, Benard, Dyer, & Giurfa, 2010).

Because of the perceptual similarities between an object and its corresponding photograph, discriminations and learning transfers between the two may be a result of categorization processes used to identify floral species in nature. Open-ended categories may be possible, where stimuli are grouped based on similar features (Herrnstein, 1990). Previous research has identified the use of open ended categories in honeybees (e.g., Avarguès-Weber et al., 2010; Zhang et al., 2004). Moreover, categorization of floral colour was found in bumblebees (Dukas & Waser, 1994). Subjects not only discriminated within and between groups of stimuli, but also generalized to novel stimuli. However, further research is needed to confirm the role of open-ended categorization in picture-object recognition.

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Experiment 2

A transferrable change in preferences of floral patterns by bumblebees through reward reversal

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Abstract

This study examines the use behavioural transfer across perceptually similar stimuli in bumblebees (*Bombus impatiens*) and addresses whether foraging judgments about a floral stimulus can change in a way that contradicts direct previous experience with that stimulus. Twenty bees from each of four colonies underwent discrimination training of stimuli placed in a radial maze. Bees were trained to discriminate between two corresponding object and photograph pairs of artificial flowers, where one object and its corresponding photo were rewarding, while another object and its corresponding photo were unrewarding. Following discrimination training, one stimulus from each pair (either the object or the photo) was removed. The predictive reward values of the remaining stimuli were either switched for one group or stayed the same for another. Subsequent testing on the removed stimuli revealed foraging preferences to shift based on experience with the other stimulus in the group. For instance, bees treated a previously unrewarding object as rewarding after learning that the corresponding photograph had become rewarding. Foraging decisions depend not only on previous experience with stimuli, but also category membership.

Introduction

In a natural environment, foragers can encounter several different floral species within a single trip. Because even a slowly flying bumblebee can be exposed to several inflorescences a second (Chittka, Thomson, & Waser, 1999), strategies for managing information for rewarding stimuli seem necessary to reduce amount of detail to which it must attend. By generalizing perceptually similar stimuli within a floral species, the forager can obtain rewards more quickly than if it had to learn the characteristics of each individual flower one by one.

Bees are flower constant (Chittka et al., 1999; Raine & Chittka, 2007; Rossi, Santos, Salvarrey, Arbulo, & Invernizzi, 2015), in that they restrict foraging to a few available floral species. Moreover, bumblebees are also more selective when there is variation among flower types in multiple traits rather than in just a single trait (Gegear & Laverty, 2005). This suggests a natural bias to forage on perceptually similar stimuli. In nature, members within a floral species are not identical and the forager must be able to compare and generalize stimuli based on shared attributes. Effective foraging must allow foragers to adjust to distortions while still correctly identifying features that characterize rewarding floral species. Thus, behavioural transfer between similar floral stimuli within a species despite differences between individual flowers is necessary for flower constancy to exist.

Previous research has found evidence of generalization between perceptually similar stimuli. For instance, honeybees were found to be capable of grouping visual images of different flower shapes, plant stems and landscapes (Zhang, Srinivasan, Zhu & Wong, 2004), as well as properties of floral features such as bilateral symmetry (Giurfa, Eichmann, & Menzel, 1996), orientation and layout (Avarguès-Weber, Deisig, & Giurfa, 2011; Horridge & Zhang, 1995), and configuration (Avarguès-Weber, Portelli, Benard, Dyer, & Giurfa,, 2010; Stach, Benard, &

Giurfa, 2004). Honeybees can even discriminate between representations of Monet and Picasso paintings, and generalize their learning to new paintings of the same style (Wu, Moreno, Tangen & Reinhard, 2013). While most of the research centers on honeybees, bumblebees were also found to prefer stimuli of similar colours (Gumbert, 2000). Bumblebees were able to generalize foraging behaviours to other stimuli sharing a similar colour, while ignoring irrelevant features (Dukas & Waser, 1994).

Previously, different-but- similar judgements have been examined in bumblebees using corresponding objects and photographs (Thompson & Plowright, 2014; Xu & Plowright, 2016). Bees were able to generalize between 3D stimuli and their 2D representations based on perceptual similarities, despite also recognizing the two as being different. In the absence of a rewarding object or photograph, its counterpart was accepted as a substitute. This eliminates the possibility of preferences due to a lack of discrimination. The current study takes advantage of the different-but-similar judgments to further investigate the role of behavioural generalization based on perceptual similarities.

Generalization is usually studied by testing subjects on novel stimuli following discrimination training (see Benard, Stach & Giurfa, 2006). In the present study, we took a different approach and instead examined behaviour towards learned members of a perceptually similar group through reward reversals. Serial reward reversals had been used previously to examine behavioural flexibility in bees (Strang & Sherry, 2014). By reversing the reward contingencies between stimuli, changes in performance can be measured across reversals. However, rapid reward reversals in quick succession are rarely if ever found within natural floral species. For instance, after natural flowers (e.g. thistles, lilacs, sunflowers) are past their blooming period, they are finished for the season. Within the lifetime of bumblebee foragers, there will be no further reward value reversals.

The use of one reversal has been proposed to test inter-stimulus behavioural transfer (Lea, 1984): after animals are trained to discriminate between two clusters of stimuli, the reward values of one stimulus from each cluster are reversed—what was rewarding becomes unrewarding, and vice-versa. Subsequent testing with the remaining stimuli reveals whether the animals respond to them as if they too had changed their reward value. When the reward value of the original stimulus has changed, behaviour should also change for the remaining stimuli. The current study adopts this method. By way of an analogy, we describe an example outlined by Herrnstein (1990). Acorns vary, but for a creature that relies on acorns, they probably resemble each other. Leaving aside for the moment the question of how it is that an animal comes to treat acorns in the same way, suppose that acorns were to become bitter. After tasting one or a few bitter acorns, is bitterness associated with acorns in general? Here we ask a similar question regarding the behaviour of bumblebees: if an unrewarding flower such as sunflower becomes rewarding, then is another sunflower treated as also having become rewarding? We modelled this situation in the laboratory in its simplest form: two rewarding flowers of one type, two unrewarding flowers of another type, and one reversal of the reward values.

Methods

Subjects

Seventy six bumblebees (*Bombus impatiens*) from eight colonies, supplied by Koppert Biological Systems Canada, were used for this study. The colonies were fed pollen ad libitum and trained with 2:1 sugar to water solution by volume in a radial arm maze. Individual bees were labelled with numbered tags on the thorax. The bees had no pre-experimental experience outside the colony.

Materials

All materials used are the same as experiment 1, with the exception of the flowers and photographs.

Artificial flowers or pictures (Figure 7) were attached to the feeders on the walls, with one stimulus per corridor. Feeder troughs were located at the end of the corridor and either filled with sugar solution or left empty. The 2D and 3D portions refer to all parts of the floral stimuli with the exception of the source of nectar, which for our artificial flowers was a three dimensional feeder trough. During training and testing, bees were able to touch the stimuli while feeding. All stimuli were placed randomly before each training session to eliminate the use of location cues. Four stimuli were used: a single yellow flower (Object X, heretofore referred to as Ob. X) and a cluster of blue flowers (Object Y, heretofore referred to as Ob. Y) and their corresponding photographs (Photo X and Photo Y abbreviated to Ph. X and Ph. Y). Yellow and blue to the human eye are well differentiated by bumblebees (e.g. Ings, Raine, & Chittka, 2009). While bees have been found to have an innate preference for blue, learned colour preferences often override innate preferences, unless generalizations are no longer possible (Gumbert, 2000). The artificial flowers were made of synthetic fabric. The photographs (8.5 cm x 10.5 cm) were taken using a Panasonic DMC-FZ20 camera and printed on a grey background with a Canon MP560 ink jet printer.

Spectral reflectance curves of the objects and their photos are shown in Figure 9. To obtain these curves, the total diffuse spectral reflectance factors, $\rho(\lambda)$, were measured on a Perkin-Elmer

Lambda-19 UV/VIS/NIR spectrophotometer equipped with a diffuse reflectance integrating sphere accessory RSA-PE-19 in accordance with quality system procedure PAR-119 version 1.02. The measurements were performed at ambient temperature (23 ± 1)°C and for a relative humidity of (15 ± 1)%. The data were recorded from 300 nm to 700 nm with a fixed spectral bandpass of 5 nm. The measurement geometry was 8° incidence, hemispherical collection (8°:t), with the specular component included. For the diffuse reflectance factor measurements, representative test samples of the objects and photos were prepared or identified. For the two flower picture samples, the test area included all colours and the samples were backed with a black backing. For the fabric flower specimens, eight layers of fabric were sandwiched together alternating dark and light coloured areas to give an opaque specimen. The irradiated area of each test sample was approximately 8 mm x 18 mm. Independent measurements (a minimum of three) were taken on each test sample, and averaged.

Design

Bees underwent an initial discrimination training (Phase I) with all four stimuli for two weeks. One picture-object pair was rewarded (+) while the other was not (–). After the initial training was complete, one stimulus from each pair (either the photograph or the object) was removed. Trained bees then underwent either an experimental or control condition for an additional five days (Phase II). Phase II began once at least 10 bees had completed Phase I successfully. For the experimental group, the remaining stimulus would switch reward values – the stimulus that was previously rewarding would become unrewarding and vice versa. In a separate control colony, the reward values of those stimuli would remain the same. For both colonies, the bees were then tested on the removed stimulus (see Figure 8 for a sample of the design). The procedure was repeated four times in counterbalanced conditions with a total of eight colonies (see Table 3).

Procedure

Upon leaving their colony, bees were trained in groups for three to five hours a day and choices were recorded for each bee individually for 15 days. A choice was made when a bee extended its proboscis into the feeders, and a new choice was recorded only after bees exited the current corridor. For consistency with previous research, an individual was selected for testing if it had foraged for two consecutive days and made at least eight consecutive choices on rewarding stimuli in both Phases I and II. Bees that chose unrewarding feeders must restart the training criterion. The bee also had to have foraged on both the rewarding photo and object at least twice. Previous research suggested that bees are able to learn discrimination tasks following two trials (Riveros & Gronenberg, 2012), and learning speed was higher in species with less prominent innate colour preferences (Ings, Raine, & Chittka, 2009). Upon reaching training criterion for two training phases, bees were tested individually with empty feeders. Choices were recorded when the bee touches the stimulus, feeder, or back wall of the maze. The bee must have exited the corridor before another choice could be made. The first ten choices of each bee were recorded.

Statistical Analyses

A first set of analyses compared the choice frequencies, out of 10, of each colony to a theoretical proportion. Because the data were binomial with replication within subjects (each having 10 choices), a replicated Goodness of Fit test (Sokal & Rohlf, 2012) was used to compare choice proportions to a theoretical chance value of 0.5. The G_P value determined if the group proportions differed from the theoretical chance value (50:50), while the G_H value tested for individual differences. G values were compared to the χ^2 value to test for significance.

A second set of analyses was used to compare the switched and non-switched groups. Because the data were binomial, a logistic model was fitted to the choice frequencies using SPSS 22. We compared groups receiving the same Phase I training but different Phase II training, as well as groups receiving the same Phase II training but different Phase I training.

Results

Switching the Reward Values of Objects

Object & Photo X+ / Object & Photo Y–

Figure 10a outlines the results of reinforcement reversals using the objects for bees trained with an initially rewarding Ob. X and Ph. X. When reward values of Ob. X and Ob. Y were switched, bees preferred Ph. Y over Ph. X ($G_P = 18.20$, df = 1, $p < .001$) with no significant individual differences ($G_H = 7.44$, df = 9, $p = .59$). Even though Ph. X was last experienced as rewarding, that photo was now avoided. On the other hand, when the objects' reward values remained the same, bees preferred Ob. X over Ob. Y ($G_P = 69.32$, $p < .001$) with no significant individual differences ($G_H = 9.74$, df = 9, $p = .37$).

Object & Photo Y+ / Object & Photo X–

Figure 10b illustrates the results of reinforcement reversals using the objects for bees initially trained with Ob. Y and Ph. Y as rewarding. When the reward values of Ob. Y and Ob. X were switched, bees preferred Ph. X over Ph. Y ($G_P = 7.62$, df = 1, $p = .006$) with no significant individual differences ($G_H = 8.64$, df = 8, $p = .37$). The opposite was true when the reward values of the objects remain the same, where bees preferred Ph. Y over Ph. X ($G_P = 13.85$, df =1, $p < .001$), with no significant individual differences ($G_H = 10.47$, df = 7, $p = .016$).

Between Group Comparisons

There was a significant difference in choice proportions between whether or not the objects had their reward values switched ($\chi^2 = 104.5$, df = 1, p < .001), with the switched groups preferring the photograph of the rewarding object in Phase II over the photograph that was previously rewarding in Phase I. Additionally, there was a significant interaction, showing that the effects of switching were more pronounced in the bees trained with a rewarding X ($\chi^2 = 7.38$, df = 1, p < .006).

Another comparison examining the effects of Phase II training in relation to Phase I training showed that there was a significant difference between results for the non-switched and switched groups for colonies receiving the same Ob. X+/Ob. Y– training in Phase II ($\chi^2 = 15.47$, df = 1, p < .001). Bees trained initially with Ob. X+ and Ph. X+ demonstrated a stronger preference for Ph. X when Ob. X was rewarding in Phase II than bees trained initially with Ob. Y+ and Ph. Y+. No significant differences were found between the remaining two colonies receiving Ob. Y+/Ob. X– during Phase II ($\chi^2 = 0.21$, df = 1, p = .65).

Switching the Reward Values of Photos

Object & Photo X+ / Object & Photo Y–

The effects of reinforcement reversals on photographs are shown in Figure 11a for bees trained on an initially rewarding Ob. X and Ph. X. When the reward values of both photos were switched, the choice proportions of Ob. Y and Ob. X did not differ from chance ($G_P = 1.44$, df = 1, p = .23), though the non-significant bias was in the expected direction of favouring Ob. Y. No significant individual differences were found ($G_H = 3.46$, df = 9, p = .94). A different pattern emerges, however, when the reward values of the photos remain the same, where bees had a strong preference for Ob. X over Ob. Y ($G_P = 46.97$, df = 1, p <; .001), again with no significant individual differences ($G_H = 6.02$, df = 8, p = .64).

Object & Photo Y+ / Object & Photo X–

Figure 11b shows the results for reinforcement reversals using photographs for bees trained initially with a rewarding Ob. Y and Ph. Y. A stronger difference was noted, where the switching of the photos' reward values showed bees to prefer Ob. X over Ob. Y ($G_P = 21.98$, df = 1, p < .001) with significant individual differences ($G_H = 24.96$, df = 9, p = .003). All bees had choice proportions at or above chance level on Ob. X with the exception of one bee, which avoided the object, choosing it three times out of ten. In comparison, when the reward values of the photos remained unchanged, bees preferred Ob. Y over Ob. X ($G_P = 41.38$, df = 1, p < .001), with no significant individual differences ($G_H = 5.51$, df = 9, p = .79).

Between Group Comparisons

As before, there was a significant difference between the switched groups and non-switched groups for object preference ($\chi^2 = 84.08$, df = 1, p < .001). The switched groups preferred the object corresponding to the photo that had been rewarding in Phase II, and not the object that was rewarding in Phase I. No significant effect of the Phase I training was obtained ($\chi^2 = 2.95$, df = 1, p = .08), and there was no significant interaction with switching/non-switching ($\chi^2 = 0.95$, df = 1, p = .33). A significant difference was found between preferences for colonies receiving the same Ph. Y+/Ph. X– training in Phase II ($\chi^2 = 29.62$, df = 1, $p < .001$). Bees initially trained with Ob. Y+ and Ph. Y+ had a stronger preference for Ob. Y when Ph. Y was rewarding in Phase II than bees trained initially with Ob. X+ and Ph. X+. There were no significant differences between two colonies receiving Ph. X+/Ph. Y– in Phase II training ($\chi^2 = 2.04$, df = 1, $p = .15$).

Discussion

Identifying and grouping flowers that vary in appearance over space and time holds ecological relevance to bees. Although photographs do not appear in their natural environments, the photographs may be viewed as another floral resource within a floral species. When the reward values remained the same in both training sessions, bees consistently chose the stimulus that was previously associated with reward. However, changes in the significance of one stimulus within our groups (X flowers and Y flowers) also changed behaviours toward the other member of that group.

When an object in an unrewarding picture-object pair became rewarding, the bees preferred the photo of that rewarding object, despite never having encountered the photo as rewarding. Moreover, bees rejected the photo that they learned as previously rewarding in favour of the photo corresponding to the last rewarding object. The same holds true for photographs: an object previously learned to be unrewarding became more attractive if the photograph of the object became rewarding. By adjusting their behaviour immediately to changes in one member of the category, bees can more easily adapt to changing environments without having to relearn anew the value of every flower.

Our methods required labour intensive and time consuming training, training again, and then testing of the same individually labelled bees. Although the amount of exposure varied among individuals in group training depending on when they reached criterion level, no significant individual differences were found in any of the colonies, with the exception of one colony for which the behaviour of one bee stood out from that of the rest. Because both experimental and control groups received the same initial training and testing regimen, differences in behaviour could be attributed to the switching of reward values.

In cases of differential learning, trained preferences towards rewarding stimuli were accompanied by learned avoidance of non-rewarding stimuli (Giurfa et al., 1999). Following initial training, bees not only learned to prefer stimuli in the rewarding cluster, but also avoided stimuli that were from the unrewarding cluster. The learning transfers observed here from switching the reward values demonstrated that not only were previous preferences to rewarding stimuli disregarded, but also previous learning of avoidance to specific stimuli can be offset by experiencing reward from different stimuli in the same category.

Our results stand in contrast to those obtained in previous research on vertebrates (Delius, Jitsumori, & Siemann, 2000). Despite successful training in pigeons to classify different stimuli into groups, they showed little to no transfer after the reversal training, even with stimuli that were perceptually similar (e.g. Astley & Wasserman, 1998; Fersen & Lea, 1990; Jitsumori, 1993), though with multiple successive reversals, transfer has been obtained (Vaughan, 1988) The current study, however, revealed strong behavioural changes towards stimuli following just one reversal. In spite of the mixed results in vertebrates, bees were capable of successfully transferring learning. Given the difficulties in species comparisons, we can only speculate as to the many possible causes of the difference in performance. One possibility is that the types of objects that are classified differ across studies. With pigeons, categories used have been both unnatural (e.g. cars, chairs) and natural (e.g. trees). In our case, however, not only were the categories natural (i.e. flowers), but they were categories that are crucially important for survival.

Having focused on how bumblebees treat members of our floral groups—what they do—we now interpret these results in terms of a well-known framework for understanding categorization—how they do it. Inherent in this classification scheme (Herrnstein, 1990) is the explicitly comparative approach. Virtually all species, and not just ours, face categorization problems—they confront objects to which they must react appropriately; objects such as stones and nests and worms and grains for which there is natural variability in appearance. The ability to categorize stimuli has turned up most everywhere it has been sought (Herrnstein, 1990, p.138). How do animals do it? The underlying processes leading to categorization may differ among species (Chittka & Jensen, 2011). Five levels of categorization, at increasing levels of abstractness, have been proposed: (1) The first shows the organism behaving in the same way towards members of a cluster because it fails to discriminate among the stimuli. For example, chimps that initiate social behaviour upon viewing their reflections in a mirror indicates a lack of discrimination between their reflection and another animal. (2) The second, categorization by rote, consists of memorizing exemplars as a list. Herrnstein (1990) gives the example of learning the names of the stops on the Boston subway system’s “Redline”. (3) Beyond rote learning, at the third level, is open-ended categorization. Organisms discriminate between different groups of stimuli while generalizing within them (Keller & Shönfeld, 1950; Benard et al., 2006). By virtue of the perceptual distances between stimuli, organisms have a means of categorizing new objects. For instance, pigeons can be trained to distinguish between photos containing trees or not, and can classify new exemplars (Herrnstein, 1979). (4) The fourth level, that of concepts, involves sorting by function—the use of perceptual similarity is not needed. While the lack of perceptual generalization may appear to be rote memorization, category members are connected by their associated consequences: generalization is “mediated” through a common consequence. Though the term “concept” has been used inconsistently in the literature, “associative concept learning”, as defined by Zentall, Wasserman, Lazareva, Thompson and Rattermann (2008), involves grouping arbitrary stimuli based upon a common outcome. For example, in this issue, Feuerbacher and Rosales-Ruiz (2017) examine the formation of the concept “toy” by a dog through a common response of playing tug-of- war with a variety of different objects. (5) Finally, at the fifth level, the subjects would be capable of categorizing based upon abstract relations (e.g. same/different, inside/outside) among concepts. The conceptual abilities of Alex the Parrot are a case in point (Pepperberg, 1987): Alex learned to identify the number of objects in sets of two to six objects and generalized to new sets.

The results here showed bees to change their behaviour despite previous experience based on the similarities between the photos and objects, eliminating the possibility of rote memorization as a foraging strategy. Additionally, because bees could differentiate between the 2D and 3D stimuli (Xu & Plowright, 2016), the behavioural changes with a reward reversal are evidence of the use of categories in foraging. This opens the possibility of the use of open-ended categories based on Herrnstein’s descriptions. If so, it remains to be determined what perceptual cues were used. Several aspects of the objects such as texture, size and shape of the original object would differ when degraded to a photo. Colour photographs are adapted to human vision and indeed the spectral characteristics of the objects and their photos, while similar, were also different. Although much of previous categorization research varied the stimuli along one trait, such as colour (Dukas & Waser, 1994), bees had also grouped stimuli that varied along multiple traits (e.g. Zhang et al., 2004). It seems plausible that bees learned multiple features simultaneously (Ronacher, 1998), and that a set of features may be required for categorization (Avarguès-Weber, Deisig, et al., 2011).

So far, the current results give evidence of simple generalization, where bees generalized their behaviour between stimuli having physical similarities. However, we do not discount the possibility of mediated generalization (Shettleworth, 2010): the generalization based on the common associations with an outcome that is at the heart of concept formation (Herrnstein's level 4). Behavioural transfer between the photo and object could be a result of a common consequence between the two, in which they both give the same reward and would be considered equivalent. This equivalence implies only functional equivalence: by virtue of training in which cues become associated with the same consequence, they come to function as substitutes for each other (Hall, 1996) and is not to be confused with formal equivalence, or "equivalence classes", where subjects are tested for the emergence of the mathematical relations of symmetry, reflexivity, and transitivity (see for example in this issue, Plazas & Cortés, 2017).

Grouping of floral stimuli might still occur even when no perceptual similarities are perceived. After all, non-rewarding flowers that do not physically resemble other rewarding flowers are still all flowers insofar as they are candidate food sources, as opposed to being nestmates or predators. Future research, along the lines of several studies described in this issue (Sturdy et al., 2017; Vonk & Leete, 2017) might extend our work to categorization by function of floral stimuli that bear little perceptual resemblance to each other. Evidence of abstract categorization (Herrnstein's level 5) has been found in previous research, where bees grouped stimuli based upon abstract relations such as sameness/difference (Brown & Sayde, 2013; Giurfa, Zhang, Jenett, Menzel, & Srinivasan., 2001) and above/below (Avarguès-Weber, Dyer, &

Giurfa., 2011). Time will tell whether the abilities of bees in categorization tasks remain more striking than any of their limitations.

Experiment 3

Learning transfers in Bumblebees within Perceptually Different Flower Groups

Abstract

This study examines the use of behavioural transfer across perceptually different stimuli in bumblebees (*Bombus impatiens*) and addresses whether category formation of floral stimuli is based upon perceptual floral features or rewards available from floral groupings. Previous experiments have found that bees transfer learning based on category membership, but little is known as to how membership is formed. Eight to ten bees from each of four colonies underwent discrimination training between two groups of stimuli in a radial arm maze. Bees were trained to discriminate between two object and photograph pairs of artificial flowers, where the object and its corresponding photo were placed in different reward groups, so that one object is rewarding while its corresponding photo is unrewarding, while another object is unrewarding and its corresponding photo is rewarding. Following discrimination training, one stimulus from each pair (either the object or the photo) was removed. The predictive reward values of the remaining stimuli were either switched for one group or stayed the same for another. Subsequent testing on the removed stimuli revealed foraging preferences to shift based on its similarity to the last rewarding stimulus. Despite training to discriminate between corresponding photo and object pairs, bees still generalized between them. Bees rely on perceptual similarity judgments to form categories even when the similarity is not beneficial.

Introduction

The ability to generalize is required for any categorization task, which takes two different forms. The first is simple generalization, where behaviour is transferred to other stimuli sharing similar characteristics. Often these characteristics reflect perceptual features of the objects. Because most categorization tasks involve a transfer test onto novel stimuli, much of previous research provides evidence for simple generalization in bees. Honeybees could group visual stimuli on the basis of specific features such as symmetry (Giurfa, Eichmann, & Menzel, 1996), configuration (Avarguès-Weber, Portelli, Benard, Dyer, & Giurfa, 2010), and orientation (Avarguès-Weber, Deisig, & Giurfa, 2011). Behavioural transfers can also be found for tasks involving several features. Bees were able generalize and group flower shapes, plant stems, landscapes and trees (Zhang, Srinivasan, Zhu & Wong, 2004). They also demonstrated the ability to differentiate between Monet and Picasso paintings and to generalize the learning to novel paintings (Wu, Moreno, Tangen & Reinhard, 2013). Similarly, bumblebees have also demonstrated successful simple generalization both with single feature tasks, such as colour (Gumbert, 2000; Dukas & Waser, 1994) and with more complex stimuli, such as behavioural transfers of preferences from 3D artificial flowers to their photos and vice versa (Thompson & Plowright, 2014; Xu & Plowright, 2016).

Under other circumstances, using mediated generalization involves generalizing flowers on the basis of a common association, such as reward association between two flowers. Stimuli in this case are grouped together because they share a common function to the animal. This should be possible without perceptual similarities, but it is often more difficult to distinguish between perceptual and mediated generalization within a natural setting, as objects that share functions also tend to share similar features. Mediated generalization has been found with sorting food and non-food items by pigeons (Watanabe, 1993), Japanese monkeys (Tsutsui, Hosokawa, Yamada, Iijima, 2016), and baboons (Bovet & Vauclair, 1998). Animals were trained to sort the items correctly, then tested for transfer with appropriate items that were not part of the training.

Similarly, objects in a bee's environment would serve distinct functions to the foragers. Apart from food resources, bumblebees can recognize landmarks (Cartwright & Collett, 1983), and nestmates and other conspecifics (Foster, 1992). Mediated generalization has yet to be studied in bumblebees. However, generalization based on shared consequences rather than perceptual features would be advantageous in natural environments, not only for sorting objects based on function, but also when dealing with floral mimics. Because mimics have developed signals that hinder discrimination, visual discrimination is slower when not accompanied by odour cues (Kunze & Gumbert, 2001; Leonard, Dornhaus, & Papaj, 2011). Additionally, odour cues are learned only when paired with a reward, and not in non-rewarding flowers (Palottini, Estravis Barcala, & Farina, 2018), making odour recognition a potentially unreliable source of information in mimic recognition. When odour cues are not available, the use of mediated generalization between perceptually different flowers could aid in discriminating rewarding flowers from mimics.

Bumblebees have been shown to learn floral associations even when perceptual similarity becomes unreliable. For instance, bees demonstrated behavioural flexibility using serial reversals (Strang & Sherry, 2014). Additionally, bees have been found to learn fine discriminations between colours and patterns given differential rather than absolute training (Giurfa, 2004; Avarguès-Weber, Sanchez, & Giurfa, 2010), where bees trained with rewarding and unrewarding stimuli performed better than those given exposure to just a rewarding stimulus. As a result, bees are capable of learning two similar flowers as different, despite their similarities. The next step, therefore, would be the determine whether or not that learning can be transferred between members of a floral category.

It is still uncertain whether or not bumblebees are capable of mediated generalization, or if they rely on perceptual features solely during foraging trips. Previously, bees were found to generalize behaviour to perceptually similar objects (Xu & Plowright, 2017). Bees were trained to group photos and objects based on picture object pairs for yellow flowers and blue flowers, where one pair was rewarding and the other unrewarding. When one member of the group changes reward values, bees transferred their learning about one member to all members of the group. For instance, when 3D yellow flowers and photographs were rewarding, and the photo suddenly became unrewarding, bees also avoided the 3D flower and treated it as having become unrewarding as well despite only experiencing it was rewarding. This behavioural transfer occurs even when bees have experienced the objects as unrewarding previously, suggesting that perceptual similarity provides a stronger cue than learned consequences.

Here, we will use the same methodology, with one difference: the stimuli will be grouped with minimal perceptual similarity during training. The situation models encounters with floral mimics where only one of two flowers that are perceptually similar is rewarding. Bees are trained with two perceptually dissimilar stimuli that are rewarding, while the more perceptually similar stimuli are separated with different reward values. One flower from each group is removed and the remaining flowers have their rewards values switched. Bees are then tested to learning transfers on the removed flowers. Through this, we will examine how bees will treat perceptually similar stimuli that have different reward consequences, and how learning can transfer when those reward consequences change. If bees are able to categorize flowers without any perceptual similarities, then learning transfers will occur based upon the initial reward values of the flowers, as found in Experiment 2, where if one of two flowers change reward values, the other would also be treated as having changed value as well. However, if bees require some perceptual similarities between flowers to categorize them, then generalization will occur based on the level of perceptual similarity between the stimuli.

Methods

Subjects

Thirty six bumblebees (*Bombus impatiens*) from four colonies, supplied by Koppert Biological Systems Canada, were used for this study. The colonies were fed pollen *ad libitum* and trained with 2:1 sugar to water solution by volume in a radial arm maze. Individual bees were labelled with numbered tags on the thorax. The bees had no pre-experimental experience outside the colony.

Materials

Materials used are the same as experiment 2.

Design

This study uses a similar design as the one described in Table 3. The difference here is that bees were trained with perceptually different flowers in Phase I (Table 4), whereas in Table 3, bees were trained with counterpart objects and photos of one flower type. Bees underwent an initial discrimination training (Phase I) with all four stimuli for two weeks. Photo X and Object Y were rewarded (+) while their corresponding object and photograph counterparts (Photo Y and Object X) were not (–). After the initial training was complete, one stimulus from each pair (either the photograph or the object) was removed. Trained bees then underwent either an experimental or control condition for an additional five days (Phase II). Phase II began once at least 10 bees had completed Phase I successfully. For the experimental group, the remaining stimulus would switch reward values – the stimulus that was previously rewarding would become unrewarding and vice versa. In a separate control colony, the reward values of those stimuli would remain the same. For both colonies, the bees were then tested on the removed stimulus (see Figure 12 for a sample of the design). The procedure was repeated four times in counterbalanced conditions, where two colonies received during Phase II training on objects but not photos, and the other two were trained on the photos but not objects, to make a total of four conditions.

Procedure

Upon leaving their colony, bees were trained in groups for three to five hours a day and choices were recorded for each bee individually for 10 days. A choice was made when a bee extended its proboscis into the feeders, and a new choice was recorded only after bees exited the current corridor. For consistency with previous research, an individual was selected for testing if it had foraged for two consecutive days and made at least eight consecutive choices on rewarding stimuli in both Phases I and II. Bees that chose unrewarding feeders were required restart the training criterion until they met criterion. The bee also had to have foraged on both the rewarding photo and object at least twice.

Upon reaching training criterion for two training phases, bees were tested individually with empty feeders. Choices were recorded when the bee touched the stimulus, feeder, or back wall of the maze. The bee must have exited the corridor before another choice could be made. The first ten choices of each bee were recorded.

Statistical Analyses

A first set of analyses compared the choice frequencies, out of 10, of each colony to a theoretical proportion. Because the data were binomial with replication within subjects (each having 10 choices), a replicated Goodness of Fit test (Sokal & Rohlf, 2012) was used to compare choice proportions to a theoretical chance value of 0.5. The G_P value determined if the group proportions differed from the theoretical chance value (50:50), while the G_H value tested for individual differences. G values were compared to the χ^2 value to test for significance.

A comparison between Experiments 2 and 3 test results was run using Crosstabs in SPSS. The non-switched conditions in both experiments that share the same Phase II training were compared to examine if there is an effect of Phase I training in the case where bees from both experiments had preferences based upon perceptual similarity.

Results

Switching the Reward Values of Objects

Figure 13a outlines the results of reinforcement reversals using the objects for bees. When reward values of Ob. X and Ob. Y were switched so that the previously unrewarded Ob. X in Phase I was now rewarding and Ob. Y becomes unrewarding, bees preferred Ph. X over Ph. Y ($G_P = 18.2$, df = 1, $p < .001$) with significant individual differences ($G_H = 20.53$, df = 9, $p = .01$). Bees varied between favouring Ph. X and having no significant preferences. No bees showed a preference for Ph. Y over Ph. X that significantly deviated from the group. Bees did not transfer learning from Ob. X to Ph. Y or Ob. Y to Ph. X. On the other hand, when the objects' reward values remained the same, bees showed no preference in their preference for the photos (G_P = 2.18, df =1, p = 1.39) with no significant individual differences (G_H = 9.18, df = 9, p = .42).

Switching the Reward Value of Photos

Figure 13b outlines the results of reinforcement reversals using the photos for bees. When reward values of Ph. X and Ph. Y were switched so that the previously unrewarded Ph. Y in Phase I is now rewarded and Ph. X becomes unrewarding, bees preferred Ob. Y over Ob. X (G_P = 29.88, df = 1, $p < .001$) with no significant individual differences (G_H = 6.5, df = 10, p = .77). Despite having experienced both Ob. Y and Ph. X as rewarding in Phase I, bees failed to act as if the change in reward in Ph. X had also occurred in Ob. Y. On the other hand, when the photos' reward values remained the same, bees preferred Ob. X over Ob. Y (G_P = 12.001, df = 1, $p < .001$) with significant individual differences (G_H = 37.14, df = 8, $p < .01$). Half the bees showed a strong Ob. X preference, while another half had no significant preferences for either.

Comparisons to Experiment 2

Comparisons to the previous experiment would reveal changes to the bees' similarity judgment as a result of reward groups. Bees trained in the non-switched object conditions had a significant difference in preferences between Experiment 2 and 3 when tested with photos (χ^2 = 23.98, df = 1, p<.001). When given Ob. Y rewarding and Ob. X unrewarding in Phase II without a switch occurring, bees from Experiment 2 had a significantly higher preference for Ph. Y than bees from Experiment 3. There were marginally significant differences for groups tested on objects (χ^2=3.67, df=1, p=.05), where bees from Experiment 2 had a higher preference for Ob. X than bees from Experiment 3, when trained with a rewarding Ph. X in Phase II without a switch occurring. While bees preferred perceptually similar stimuli in both experiments, the preference for similarity was stronger when Phase I training specifically required it.

Discussion

Objects in a bee's environment can serve different functions to the bee, such as floral resources or landmarks. Additionally, food resources serve different functions, depending on the amount of pollen or nectar it gives, where pollen serves as a source of protein for feeding larvae while nectar serves as a source of carbohydrates for energy. While bees may not specialize for either pollen or nectar foraging (Russell, Morrison, Moschonas, & Papaj, 2017), they are still able to differentially forage for pollen or nectar depending on the needs of the colony (Fewell & Winston, 1992; Plowright, Thomson, Lefkovitch, & Plowright, 1993; Plowright, Cohen-Salmon, Landry, & Simonds, 1999). As such, it stands to reason that the bees' foraging techniques reflect an ability to generalize between the functions of the objects in their environment. In the present study, bees preferred the photograph or object most perceptually similar to the last rewarding stimulus experienced, regardless of shared consequences. Bees chose the counterpart object or picture of the rewarding flower in Phase II regardless of the associations learned in Phase I. As with previous research (Xu & Plowright, 2016), learning from one flower can be transferred to another without the forager having the same experience on the second flower. Unlike the previous study, bees were trained specifically to discriminate between two similar flowers, but bees still grouped flowers based on perceptual similarities. As a result, bees do not transfer learning between stimuli groups if the perceptual similarities between members of two different groups of flowers are too large. Instead, bees rely on generalization between perceptually similar stimuli.

Because of differential training in Phase I, learned preferences towards rewarding stimuli would also be accompanied by learned avoidance of unrewarding stimuli (Giurfa et al., 1999). As a result, bees learning the attributes of a rewarding object would also learn to avoid attributes towards its unrewarding photograph counterpart, and vice versa. While the group in the switched conditions have experienced the preferred stimulus as rewarding previously in Phase I, this was not the case for the non-switched conditions. Despite learning specifically to differentiate between the photographs and objects of the same flower, the bees still made choices based on physical similarities during testing.

The methodology is similar to that in previous research (Xu & Plowright, 2017), and involves labour intensive and time consuming training. Finer discriminations often involve a speed tradeoff and take longer to learn (Dyer & Chittka, 2004b). While it could be argued that with more training, the bees would eventually be able to group the different objects and photos as belonging to the same category, such a method would be impractical and unlikely to occur in natural settings.

In line with previous research, where bees were able to discriminate between fine details in stimuli, bees here were able to differentiate similar flowers when trained to do so (Dyer & Chittka, 2004). However, as shown by the transfer tests, bees still categorized based on perceptual similarity. While bees are able to make fine discriminations, it appears to be context specific and training would therefore, not be applicable in other situations. Categorization relies upon innate preferences for similarity and is not solely based upon learning and experience.

Context specific foraging has been demonstrated using serial reversals. Through experience in multiple reversals, bees have been able to transfer learning between perceptually different stimuli (Strang & Sherry, 2014). However, bees have never been shown to reject similar stimuli in favor of different ones based on reward consequences, although this ability has been found in other animal studies, such as dolphins (von Fersen & Delius, 2000). While bees are capable of transferring learning between perceptually different stimuli, the addition of similar features between groups may limit this ability.

Selection in mimicry has favoured being confusing to the foragers from the original source. In this study, the photographs and objects are distinguishable to the bees even without prior experience in both (Xu & Plowright, 2015). Instead, the learning transfers demonstrated in this study might indicate an inability to transfer to perceptually different stimuli in the presence of similar stimuli rather than a lack of discrimination between the photographs and objects. Objects in nature that appear similar usually share consequences. Even in cases when the opposite is true, where flowers that appear different give the same rewards, perceptual similarities still play a part in flower selections. For instance, tomato and blueberry flowers may be both rewarding to a bee, but each flower still shares similarities to others of its species.

Groupings of floral stimuli can occur despite contradictory experience. Bees that have learned to differentiate between two flowers in one context can treat them similarly in another context. However, the results do not suggest that bees are incapable of categorization by function, but rather, bees require some degree of perceptual similarity to make judgments regarding categories. In comparison to results from Experiment 2, bees have a weaker preference for the perceptually similar flower during the test when no switch occurs. For instance, bees trained with a rewarding Ob. Y in Phase II do not have as strong a preference for Ph. Y as they did in Experiment 2, indicating that perceptually different flower groupings had some effect on their similarity judgments.

Moreover, bees in this experiment not only had to transfer learning in the absence of perceptual similarity but were also tested to do so in spite of perceptual similarity, making the task more difficult than those in previous research (e.g. Dyer & Chittka, 2004). Some research suggests that bees can be trained to attend to and generalize specific portions of patterns (Fauria, Colborn, & Collett, 2000; Dukas & Waser, 1994). Further research can determine whether or not bees can categorize by flower function using perceptual similarity, and the degree in which innate similarity judgments influence categorization. By removing the added difficulty of perceptual similarities between groups, bees may have an easier time grouping flowers with little perceptual similarity. The next step to examining mediated generalization involves looking into the bees' abilities to generalize specific floral characteristics based upon reward values rather than simply through similarity, and whether the reward values of the flowers influence how bees perceive similarities between group members.

Experiment 4

The Role of Function in Perceptual Similarity Judgments in Bumblebees

Abstract

This study examines the effects of learning on behavioural transfers, specifically whether floral similarity judgments change based on the way flowers are grouped through rewards. Fifteen bees from each of eight colonies underwent discrimination training between two groups of stimuli in a radial arm maze. All bees were exposed to the same two picture-object pairs, and were trained to discriminate either pictures from objects, or one pair from the other. Bees were then chosen for one of three transfer tests: a) for choice preferences between photos and objects; b) for choice preferences between a picture-object pair; and c) for learning transfers with perceptual similarity as a confounding variable, where bees are expected to choose the previously rewarded floral dimensionality, but in the presence of another perceptually similar flower, and vice versa. Results revealed that bees transferred learning to different flowers depending on the flower groupings given during training, but preferences for perceptual similarity remained even when the similarity was contrary to the training. Bees are able to group flowers based on the relevant features learned through experience rather than just through perceptual similarity, but also do not spontaneously discount similar flowers during foraging.

Introduction

Generalizations between floral species are advantageous to foragers to minimize foraging time and energy. Bees are flower constant, where they restrict foraging to one or a few species of flowers (Chittka et al., 1999; Rossi et al., 2005). However, it is unclear how bees determine different floral species. Given the results from experiment 3, bees preferred perceptually similar stimuli despite being trained to choose otherwise. From this, two possible foraging methods may lead to the formation of a category: perceptual generalization and mediated generalization.

Using perceptual generalization, bees may select flowers during a foraging trip that most resemble the rewarding flowers already experienced and ignore other resources, where perceptually similar flowers that are unrewarding are still considered part of the rewarding category. Under these circumstances, bees are unable to form functional categories. In typical foraging environments, the flowers that most resemble a rewarding species are most likely other members of the species, and foraging based on simple generalization would increase efficiency where there is a low chance of failure. Previous research in bees has found evidence of perceptual generalization of simple, one-feature, generalizations, such as symmetry (Plowright, Evans, Leung, Collin, 2011), and of more complex stimuli, such as landscapes, flower shapes (Zhang, Srinivasan, Zhu, Wong, 2004), or painting styles (Wu, Moreno, Tangen, Reinhard, 2013). Bees have also been shown to categorize colours. Bees that were trained on two rewarding colours generalized their behaviour to all novel colours within the spectral range while bees that were trained on only one colour did not (Benard & Giurfa, 2008).

However, colour generalizations have been shown also apply to specific flower parts. Bees trained on two bicoloured patterns were able to generalize based on one of the two colours while ignoring the other (Dukas & Waser, 1994). If bees are capable of extrapolating specific floral features and generalizing them based upon experience, this would indicate that the reward values of the flowers visited impacts the categories that are formed.

This leads to the possibility of mediated generalization, where with enough experience, bees can extrapolate the common elements in the rewarding flowers and generalize based on specific features. Objects in the forager's environment are known to have different roles for the forager. Through mediated generalization, objects sharing similar roles are categorized together. For instance, bees were able to use landmarks to determine locations of foraging grounds (Cheng, Collett, Wehner, 1986; Kheradmand, Cassano, Gray, & Nieh, 2018) or the colony (Brünnert, Kelber, Zeil, 1994) and must be able to differentiate landmarks from rewarding flowers. Also, flowers can differ in resources such as pollen and nectar and must also be differentiated based on the colony's needs, where more pollen is collected with more brood present (Free, 1967) and low pollen storage levels (Fewell & Winston, 1992). This would indicate that mediated generalization does play a part in perceptual category formation by determining which features are needed to form the category and would allow for more accurate foraging to meet colony demands.

Although objects in a natural environment can serve a different functional relevance to a forager, only members of a floral species would normally be observed to change reward values simultaneously. As a result, while two perceptually different flowers may both be originally rewarding, the loss of reward from one flower may not always indicate no reward from another floral species. Nonetheless, this does not indicate that sharing a reward consequence has no effect on the bees' ability to categorize. Mediated generalization may still be possible; however, category formation in the absence of any perceptual similarity may be a difficult task for the bees.

In the following study, we aim to investigate whether or not bees rely entirely on perceptual generalization during foraging, or if they can be trained to ignore the more salient perceptual similarities for features that are more functionally relevant. The experiment uses the same four stimuli found in Experiments 2 and 3, but instead will be grouped by the experimenter in two different ways. Along with grouping flowers by X’s and Y’s as was done previously, we will also be grouping objects and photos of those objects separately (e.g. objects as rewarding and photos as unrewarding). Object X and Photo X, for instance, either belong to the same group or to different groups. Bees will be tested on the counterparts of a rewarding object and a novel object.

The goal is to examine whether bees will be able to determine group relationship based on different features of a flower (i.e. the relation between an object and photograph pair vs the relation between 3D and 2D stimuli), and whether having more similarities between groups interfere with how bees categorize flowers that would otherwise belong together. The addition of novel stimuli allows us to examine learning transfers while keeping the learned consequences of the testing stimuli as both unrewarding. The use of novel stimuli allow for investigations of learning transfers onto new objects, where memory alone is insufficient. Previous research on categorization showed that in the absence of learning transfers onto novel objects, subjects may be using memory to sort various items. For instance, pigeons were found to successfully sort hundreds of photographs of squiggles and scenery into random arbitrary categories and remember the stimuli when tested a year later (Vaughan & Greene, 1984). Other birds, such as nutcrackers, are also able to remember hundreds of locations for food caching purposes (Kamil & Balda, 1985). However, a category concept of certain objects implies that the animal should be able to classify new instances of that object that it has not seen before. Learning transfer tests allow for further investigation as to whether subjects are sorting based on rote memories or higher levels of categorization.

Methods

Subjects

One hundred and twenty bumblebees (*Bombus impatiens*) from eight colonies, supplied by Koppert Biological Systems Canada, were used for this study. The colonies were fed pollen *ad libitum* and trained with 2:1 sugar to water solution by volume in a radial arm maze. Individual bees were labelled with numbered tags on the thorax. The bees had no pre-experimental experience outside the colony.

Materials

Materials used are the same as in experiments 2 and 3 with new stimuli added for testing. Flowers used for testing include a white flower (Ob. Z), a yellow flower different from the Ob. X (Ob. X2), and their corresponding photographs (Ph. Z, and Ph. X2) and an additional photograph of a cluster of blue flowers (Ph.Y2) (Figure 14). Novel Ob. X2 and Ph. X2 are the same as Ob. X and Ph. X used in Experiment 1.

Design

Bees underwent training to discriminate between the two photos (Ph. X and Ph. Y) from the two objects (Ob.X and Ob. Y), with one pair being rewarding (+) and the other unrewarding (-). The trained bees were assigned to one of 3 discrimination tests on unrewarding stimuli between: 1) a novel photo and object (Ob. Z and Ph. Z), 2) two novel photos resembling the training stimuli (Ph. X2 and Ph. Y2), and 3) a perceptually novel stimulus, and another stimulus perceptually resembling the training stimuli (Ph. Z and Ob. X2, or Ob. Z and Ph. Y2, depending on the rewarded stimulus during training).

In a separate control colony, photo and object X were rewarding while photo and object Y were unrewarding, as a comparison to the previous procedure (see Figure 15 for an example of the design). The bees underwent the same tests as above. A total of 10 bees were used for each test, with 5 bees from two different colonies. The procedure was repeated two more times in counterbalanced conditions with two colonies per condition for a total of eight colonies (see Table 5).

The first two tests determine whether or not bees are able to extrapolate specific features of categories and generalize them. In test 1, bees trained to discriminate objects and photos would be able to treat Ob. Z and Ph. Z differently while bees trained to discriminate between X and Y would not. The same holds true for test 2, where bees trained on X and Y would show a preference for either X2 or Y2, but those trained on objects and photos would not.

The third test examines the influence of perceptual similarity on categorization and how similarity judgment can change with experience. To successfully pass the test, bees trained with discriminations between 2D and 3D stimuli must be able to disregard more salient perceptually similar flowers in favour of other 2D or 3D stimuli. Bees trained to discriminate by flower type (X vs Y flowers) will act as a control group. As experiment 3 demonstrates, the similarities of flowers between groups may influence the bees’ abilities to categorize within a group. Bees trained with objects as rewarding will be compared to bees trained with Y as rewarding, and both groups are tested on choices between a novel object (Object Z) or a novel Y photo (Photo Y2). Similarly, bees trained with photos as rewarding will be compared to bees trained with X as rewarding, and both groups are tested on choices between a novel photo (Photo Z) or a novel X object (Object X2). Bees that were able to ignore the similarity between the stimuli within each

X and Y group would choose the novel flower of the correct dimension while those who cannot would fail the test. Bees trained to discriminate between X and Y will only need to generalize based on perceptual similarities, as have been shown in Experiments 1-3.

Procedure

Upon leaving their colony, bees were trained in groups for three to five hours a day and choices were recorded for each bee individually for 15 days. A choice was made when a bee extended its proboscis into the feeders, and a new choice was recorded only after bees exited the current corridor. For consistency with previous research, an individual was selected for testing if it had foraged for two days and made at least eight consecutive choices on rewarding stimuli in both Phases I and II. Bees that chose unrewarding feeders restarted the training criterion. The bee also had to have foraged on both the rewarding photo and object at least twice during the training period.

Upon reaching training criterion for two training phases, bees were tested individually with empty feeders. Choices were recorded when the bee touched the stimulus, feeder, or back wall of the maze. The bee must have exited the corridor before another choice could be made. The first ten choices of each bee were recorded.

Statistical Analyses

A first set of analyses compared the choice frequencies, out of 10, of each colony to a theoretical proportion. Because the data were binomial with replication within subjects (each having 10 choices), a replicated Goodness of Fit test (Sokal & Rohlf, 2012) was used to compare choice proportions to a theoretical chance value of 0.5. The G_P value determined if the group proportions differed from the theoretical chance value (50:50), while the G_H value tested for individual differences. G values were compared to the χ^2 value to test for significance.

A second set of analyses look into whether a) bees who alternate visits between rewarding stimuli during training perform better during tests than those that are more constant towards one stimulus; b) bees that take more days to learn the training perform differently than those that take fewer days. An independent t-test was used with the data fitted to a cluster model.

All other analyses done between colonies and between conditions used Crosstabs in SPSS.

Results

Results of Tests 1-3

Test 1 results for dimensionality

As shown in Figure 16, bees trained with objects as rewarding had a significant preference for Object Z over Photo Z ($G_P = 7.95$, df = 1, p = .004) with no significant individual differences ($G_H = 11.07$, df=9, p =.27). Bees trained with rewarding photos had a significant preference for Photo Z over Object Z ($G_P = 21.98$, df =1 , p < .001) with no significant individual differences (G_H=15.32, df=9, p=.08). However, bees trained with either X rewarding or Y rewarding both showed no significant preferences for either Object Z or Photo Z ($G_P = 0.36$, df=1, p = .55; for both groups) with no significant individual differences for either a rewarding X ($G_H = 6.59$, df = 9, p=.68) or Y (G_H=2.49, df=9, p = .98). No preference was expected and none was obtained.

Test 2 results for flower type

Bees trained with a rewarding Object and Photo X had a significant preference for Photo X2 over Photo Y2 (G_P =82.88, df =1, p < .001) with no significant individual differences (G_H=14.01, df=9, p=.12). Similarly, bees trained with a rewarding Object and Photo Y had a significant preference for Photo Y2 over Photo X2 (G_P=57.64, df=1, p < .001) with no significant individual differences (G_H= 9.25, df=9, p=.41). Bees had no significant preferences for either Photo X2 or Photo Y2 when they were trained to prefer either objects (G_P = 0, df =1, p =1) or photos (G_P = .64, df =1, p = .42) with no significant individual differences for the group trained with rewarding objects (G_H=4.9, df=9, p=.84). However, there was a significant heterogeneity in the group trained on rewarded photos (G_H = 18.54, df = 9, p = .03), where half the bees were at chance level while the other half preferred either X or Y. Upon closer inspection, significant individual differences were found at the colony level in that condition for one of the colonies (G_H = 12.95, df =4, p=.01), but not the other (G_H = 3, df =4, p=.55), indicating possible innate X and Y preferences in individual bees in the absence of experience. However, this is not the case for photos or objects as they appear to be equally appealing to bees trained on either X or Y (Figure 17).

Test 3 results for similarity judgments

The test for similarity judgments gives the bees trained to discriminate between dimensionality a novel flower with more salient perceptually similarity to the training flowers as a distractor. The bees must choose the novel stimulus of the correct dimensionality while ignoring more salient perceptually similar flower.

As shown in Figure 18, bees trained with either Photos + or Xs + were tested on Object X2 and Photo Z in Test 3. Both testing stimuli are new and have never been used in training. Bees trained to prefer X over Y had a significant preference for Object X2 over Photo Z (G_P=61.35, df =1, p < .001) with no significant individual differences (G_H=12.04, df= 9, p=.21). Bees trained to prefer photos over objects had no significant preference for either Object X2 or Photo Z (G_P = 1.97, df = 1, p =.16) with no significant individual differences (G_H=15.8, df=9, p=.07).

Bees trained with either Objects + or Ys + were tested on Photo Y2 and Object Z in Test 3. As before, testing stimuli are new and have never been encountered by the bees during training. Bees trained to prefer Y over X had a significant preference for Photo Y2 over Object Z as expected (G_P=33.25, df= 1, p < .001), with no significant individual differences (G_H=6.28, df=9, p=.71). Bees trained to prefer objects over photos had no significant preference for either Photo Y2 or Object Z (G_P = 1.97, df= 1, p = .16), with no significant individual differences (G_H=1.97, df=9, p=.99).

Between-Group Analyses

General preferences

Bees trained to prefer objects over photos were significantly different from bees trained to prefer Y over X in choice proportions for all tests. Bees trained with rewarding objects chose objects significantly more than bees trained to prefer Y in Test 1 (χ^2=4.57, df=1, p = .032) Bees trained to prefer Y chose Y2 significantly more than those trained to prefer objects in Test 2 (χ^2=29.78, df=1, p<.001). Bees trained to prefer Y and those trained to prefer objects also differed significantly on preferences in Test 3 (χ^2=10.051, df=1, p=.002).

Bees trained to prefer photos over objects had significantly different choice proportions from bees trained to prefer X over Y in all tests. Bees trained to prefer photos had significantly higher preferences for photos than those trained with a rewarding X in Test 1 (χ^2=14.08, df=1, p<.001). Bees trained to prefer X had higher choice proportions for X2 than those trained to prefer photos in Test 2 (χ^2=36.63, df=1, p<.001). Bees trained to X and those trained with photos also differed significantly in Test 3 (χ^2=42.54, df=1, p<.001).

Photos rewarding vs Objects rewarding

No significant differences were found between choices of bees trained with photos rewarding and objects rewarding for Tests 1 and 2. Bees in both groups were equally proficient at selecting the correct novel photo or object accordingly in Test 1. Test 2 showed no significant preferences for both groups (see tests 1 and 2 above). However, a slight significant difference was found between the groups in Test 3 (χ^2=3.92, df=1, p=.048). Bees trained with rewarding photos selected the correct dimension in Test 3 more often than bees trained with rewarding objects did.

Flower type vs Dimension training

Bees trained to group using flower types (X vs Y) were compared to bees trained to group using dimensions (3D vs 2D) in how they well they performed in the respective tests. Bees trained on X and Y chose the corresponding X2 or Y2 flower in Test 2 more often than bees trained on 2D and 3D flowers chose the corresponding object or photo Z in Test 1 (χ^2=25.113, df=1, p<.001).

Colony differences

No significant differences were found between colonies given the same training and testing, including the condition where significant individual differences were found. Individual differences cannot be attributed to a colony effect (see Table 6 for details).

Individual factors

Effects of foraging proportions

Bees chosen for testing must have visited each of the two rewarding flowers at least twice. However, bees differ in the proportion of visits to each flower. Despite this, no significant results were found for all tests and conditions between their test results and the proportion of visits to each flower during training (see Table 6 for details). Bees that foraged more consistently on one of the flowers did not perform better or worse than bees that often switched between rewarding flowers.

Effects of training days

How quickly the bees learned the training did not appear to affect their results during testing for all but one test, where bees showed a slight significance (see Table 6 for details). Bees that learned the task faster did not perform better than bees that took more training days to learn the task, but also most bees finished the training in 4 days or less. The effect may be more apparently had bees had a larger difference in their training days.

Preference changes

No differences were found when comparing between the bees' first and last five choices (see Table 6 for details). Bees did not appear to change their preferences over the course of the testing period for any condition or test. Errors made during the testing phase remained constant throughout the 10 choices.

Discussion

When bees are trained to discriminate between flower types, they always choose the flowers that most resemble the training flower types, ignoring differences in dimensionality (2D and 3D). Bees trained to discriminate based on dimensionality were able to generalize to other flowers of the correct dimensionality, while also showing no overall preference for flower types.

Although they were able to generalize to the corresponding object or photo Z during tests with novel stimuli, bees trained on dimensions (2D vs 3D) were not able to do the same when one of the choices also had a higher resemblance to the training stimuli (Test 3). At the same time, while bees did not spontaneously choose the option of the correct dimension during Test 3, they also did not spontaneously choose the option with the highest perceptual similarity. Bees instead viewed both options as equally appealing. Bees trained on flower type, however, did not, and had strong preferences for the correct flower type presented. Previous experience on the flowers appears to dictate the level of similarity perceived by the foragers. Novel flowers that would not have appeared rewarding now do because of experience on different rewarding flowers.

Our methods include strict training criterion on individually labelled bees. Arguably the training methods selected for the best bees to perform for the tests. However, bees that were able to finish training earlier did not differ significantly from bees that were slower at learning the task. Similarly, bees that switched more between rewarding training stimuli also did not perform significantly better than bees that were more constant on one flower. As a result, individual differences in training experience did not lend an advantage to specific bees, and all bees performed similarly as a result of the training. Differences in preferences from each condition are a result of the experience on stimuli offered during training, and not as a result of innate preferences.

How well the bees perform during the tests appear to depend on the level of perceptual similarity of the training flowers, where flower groups with higher perceptual similarity present less challenge for the bees to categorize. Bees trained to select objects or photos were compared to bees trained to select X or Y flowers. Although all groups were able to pick the correct dimension or flower type in Tests 1 and 2, bees trained on X and Y flowers had a significantly higher rate of selecting the right stimulus than those trained on dimensions. Similarly, bees trained to prefer photos chose the correct dimension in Test 3 significantly more often than bees trained on objects did. Bees trained on flower type had high preferences for the correct flower type whenever it was presented. While bees can generalize specific floral features, the ability becomes limited when the flowers have fewer perceptual similarities.

Test performance for bees trained on dimensions differed based on the test given. When presented with novel 3D and 2D flowers with no additional degree of similarity in Test 1, bees generally chose the stimulus of the correct dimension than those given Test 3, where perceptual similarity was added as a confounding variable. Choices made by the bees can also be a result of environmental factors as well as experience. For instance, bees were found to increase selectivity of flowers when there were more choices present (Austin, Horack, & Dunlap, 2018). Bees also generalized colours based on levels of similarity, where similar novel colours were preferred over less similar alternatives (Gumbert, 2000). Here, a similar situation is observed, where bees were able to choose flowers based on the trained dimensions but were unable to do so when other perceptually similar flowers were present. Flower choice depends not only on the bees' experience on rewarding and unrewarding flowers, but also the alternative options available in the environment, and their degree of similarity to known flowers.

While differential conditioning has been useful to condition minute discriminations in bees previously (e.g. Avarguès-Weber, de Brito Sanchez, & Giurfa, 2010; Giurfa, 2004), bees do not necessarily learn rewarding and unrewarding flowers equally. Here, the bees had more difficulties transferring learning to novel objects when objects were rewarded than transferring learning to novel photos when trained with rewarding photos, suggesting that either the photos or the objects had a stronger within-group perceptual similarity than the other. Bees trained with rewarding photos performed better in Test 3 than bees trained with rewarding objects, despite both groups receiving the same stimuli during training. Most avoidance learning research in bees has focussed on odour cues rather than visual cues (e.g. Tedjakumala & Giurfa, 2013; Wright et al., 2010), however, while bees were able to learn avoidance of specific unrewarding flowers, this discrimination is limited when flower selection is limited (Goulson, Chapman, & Hughes, 2001). As a result, learning a preference does not yield the same results and learning an avoidance. In this case, learning Ph+ is not the same as learning Ph-.

Bees trained to discriminate objects and photos did not significantly prefer Object or Photo Z in the presence of X2 or Y2, however, they did significantly choose the correct novel object or photo when presented with another Z. Despite the fact that they cannot completely ignore the salient floral features, they also do not immediately choose the most salient similarity. Categories, therefore, are flexible, and new flowers are grouped not only on their features, but also on a forager's previous experience and the alternatives available.

These results give an indication to the possibility of functional category formation in bumblebees. Bumblebees can extrapolate and generalize specific floral details to novel flowers, but the ability becomes unreliable if there are more salient perceptual similarities present as a distractor. However, to rely on only perceptual categorization would indicate the bees take into account on the perceptual characteristics of the flower rather than the reward values. Instead, bees do not spontaneously prefer flowers based only on perceptual similarity either, and in cases where both group membership and perceptual similarities interfere, bees will visit both possibilities with no overall preference for one over the other. Bees appear to categorize based on both the reward values of the category members and the similarities of the flowers available in the area. Overall, bees rely on perceptual similarity to form categories, but the similarity judgments change based on previous experience and the function of the flower.

Conclusions

This thesis aims to explore categorization in bumblebees as an explanation for certain natural foraging behaviours, such as flower constancy. The four experiments contribute to the existing research and expand on the details of the levels of categorization found on categorization in bumblebees. Bees demonstrated the ability to both discriminate between two groups of flowers while also generalizing between them. Previous literature on picture-object correspondences in animals outline similar requirements in similarity and difference judgments for recognition of pictures and objects. The use of photos and objects for bees allows for further investigation into the generalization and discrimination processes that compose the building blocks of categorization.

Experiment 1 found bees to be able to discriminate between photos and objects of two flower types without prior training, while at the same time, being able to generalize picture to object and vice versa. Bees accepted the counterpart photo or object of the rewarding flower in its absence. Bees treat counterpart objects and photos as the same, but also are able to discriminate between them, indicating that the generalization between the objects and photos are not a result of confusion or inability to discriminate. At the same time, they are not treated as separate or independent, indicating that bees do not rely on rote categorization while foraging.

Experiment 2 showed how bees respond in changing environments. When one of the flowers in a grouping changes reward values, bees treated the other as also having changed reward values despite never having experienced so. Through this, bees were confirmed to possess perceptual categorization abilities.

Experiment 3 examined possible functional categorization in bumblebees, and followed a similar design to Experiment 2 with perceptually different flowers grouped with the same reward values, while perceptually similar flowers are separated into different reward groups. Bees were unable to generalize their learning between flowers sharing little perceptual similarity, and categorized the flowers based upon perceptual similarity. At the same time, the perceptual similarities between the reward groups might cause interference with the ability to categorize in cases of little perceptual similarity.

Following up, Experiment 4 showed that bees were able to extrapolate specific details of flowers and categorize using those features. However, perceptual similarities between groups interfered with the bees' generalizations. Bees chose both the flowers with matching features to the rewarding groups and the flowers that resembled the learned rewarding flowers in other similar characteristics. For instance, bees were able to find the 3D flower when given novel 3D and 2D stimuli, but not when given novel 3D and novel Y stimuli, in which case, bees see both the 3D and the Y flowers as equally desirable. The floral categories formed depend on the floral features as well as the resource selections available.

The floral stimuli used have multiple cues that could be generalized. The bees could be using cues of colour, shape, symmetry, or even smaller details like the shape of the petals, to generalize between groupings. Additionally, different bees may use different cues for categorization. While salient floral cues may allow for simple generalization of flower features, a more complex environment may require attentional shifts to less salient but more relevant features to find resources. This could further explain how natural foraging strategies such as flower constancy both take form but also break down.

Discussion on methodology

Social cues

While all materials remained the same between testing and training periods, bees would have a different experience during the testing phase. Training was done in groups while testing was individual, indicating a lack of social cues in the testing environment. As demonstrated in previous research, bees use social cues as an indicator for the flowers visited. Bees rely on conspecific information in environments lacking in other information (Leadbeater & Florent, 2014). As bees experience high rewards during foraging, they will ignore social cues to forage based upon experience (Jones, Ryan, & Chittka, 2015). As a result, new bees would generally rely on conspecifics to gain information on rewarding flowers, however trained bees would likely have limited use of social cues.

Naturalistic limitations

The experiments described try to imitate naturalistic settings as much as possible, but laboratory environments are restrictive in recreating natural elements. For instance, there is a lack of UV light in the lab. However, even in natural settings, flowers reflecting pure ultraviolet light are rare and lower in intensity than other reflections on the spectrum (Chittka et al., 1994). While ultraviolet light falls inside the bees’ colour vision, it does not appear to have more importance as other wavebands, and bees show no innate preferences for ultraviolet reflections (Kevan, Chittka, & Dyer, 2001). As a result, UV light does not appear to have any more impact over the bees’ learning abilities than other colours. Additionally, the floral colours used (blue, yellow, and white to humans) were found to have no foraging preference effects following learning in bees (Arnold, Savolainen, & Chittka, 2009).

Flowers in the experiments also gave unlimited rewards, allowing bees to fill their stomachs after one visit. In nature, bees often drink from several flowers before returning to the hive. Bees were found to have higher responses to higher sugar concentrations rather than volume, and even in cases where flowers differed in volume, some bees still visited the less rewarding flower (Cnaani, Thompson, & Papaj, 2006). In such cases, volume and concentration of nectar is based on the relative amounts available in the environment. Here, the large volume of sugar water does not appear to influence the bees foraging. Additionally, shorter foraging trips have been linked to greater colony growth (Westphal, Steffan-Dewenter, & Tscharntke, 2006), but no evidence suggests it influences flower choice.

Flowers in natural environments are also prone to exploitation, and thus would not always contain a fixed volume of nectar. As such, bees visiting flowers may find some flowers to have large volumes of nectar while others within the same species contain none. Previously, consistent rewards were found to be good for rapid acquisition of behaviours, while inconsistent partial rewards promoted a resistance to behavioural extinction (Jenkins & Stanley Jr., 1950). Bees were found to be sensitive to variation in reward distributions, where the presence of reward variation inhibits their rate of learning, as estimation of nectar qualities can influence foraging decisions, however, the level of variation has no effect on learning (Dukas & Real, 1993(b)). Additionally, foragers shortened visits to flower species when the nectar volume was variable (Biernaskie, Carter, Hurly, 2002).

Similarly, the flowers used here resemble flowers found in nature. However, bees have been found to generalize between objects that are not typically found in their environments as well, such as human faces (Avarguès-Weber et al., 2010). As shown in the current studies, bees don’t immediately treat objects and photographs as the same, despite being able to generalize between them. Further investigation could determine if bees are more biologically prepared to categorize stimuli found in their natural environments than those that are not.

Significant contributions

Results in relation to foraging behaviour

Categorization can further the explanation for flower constancy in nature, where bees with experience on one or a few species of rewarding flowers would generalize to other perceptually similar flowers in the area. The presented results show how similarity judgments can influence floral category membership. Bees are able to extrapolate features and generalize them with experience, but also rely on perceptual similarity to make foraging choices. As a result, categorization may be responsible for flower constant behaviour in bees, where bees visit the same few species of flowers after discriminating between and generalizing within floral categories. Because foragers of the same species were constant on different flowers (Darwin, 1895), constancy to specific flowers does not appear to be an innate preference, and could be a result of categories learned with individual experience.

Flower choice does not depend upon the experience of the forager but also the resource alternatives available. Floral mimics were more effective when the deceptive flower was interspersed with rewarding flowers in the environment (Katz & Essenberg, 2018). As demonstrated in Experiments 3 and 4, strong similarities between groups interfere with category formation. As a result, mimicry is more successful in environments where discrimination between groups is harder for foragers to make.

Experience also dictates the preference of the flowers. As shown in Experiment 4, different groupings during the training resulted in different preferences for flowers, despite all bees being exposed to the same flower during training. Experience of reward groups in the field may also result in changes in preferences. During foraging, the grouping of rewarding flowers, rather than simply the flowers themselves, can influence foraging decisions. Whereas previously categorization in bees focussed on discrimination training and generalizations to novel stimuli (e.g. Giurfa et al., 1996; Stach & Giurfa, 2005) here, bees changed their preferences when flowers are given different reward groupings, despite being exposed to the same flowers. Bees do not just rely on simple generalization, and the way flowers are experienced must also be considered.

Results in relation to cognition

Because of limitations in memory and recall, categorization can be used as a method to lower the cognitive load required during foraging. Preferences for a few relevant features to foraging allows the forager to access a wider array of resources without having to remember the details of every resource. Experiments 2 and 3 show that bees were able to generalize based on similarity even when they have experienced similar flowers as unrewarding. As a result, bees rely more upon perceptual generalization than memory during foraging trips.

While bees are able to discriminate fine features of different stimuli (e.g. Dyer & Chittka, 2004), this discrimination is not made spontaneously without training. Bees also appear incapable of finer discriminations when given other similar flowers as a confounding variable. Fine discriminations found in laboratory environments are unlikely to be replicated in more complex natural environments.

Results in relation to categorization

Perceptual categories have been found in previous bee research. As mentioned previously, honeybees were able to discriminate and generalize between various sets of perceptual stimuli (e.g. Giurfa et al., 1996; Zhang et al., 2004; Wu et al., 2013; Avarguès-Weber et al., 2010).

While bumblebee research is more limited, evidence for perceptual and relational categories in bumblebees also exist (e.g. Dukas & Waser, 1994; Brown & Sayde, 2013), and studies also point to similarity and difference judgements in bees (e.g. Plowright & Korneluk, 1995; Perreault & Plowright, 2009). However, higher levels of categorization have yet to be investigated in bumblebees.

The current studies revisits the levels of categorization as outlined in previous works (Herrnstein, 1990; Zentall et al., 2002), offering a more complete picture of categorization in comparative research. While categorization has been more extensively studied in honeybees (e.g. Benard, Stach, & Giurfa, 2006), research in categorization is more limited in bumblebees. Additionally, the studies give insight on possible higher levels of categorization in bumblebees, beyond generalizations of perceptual similarities.

Practical applications of categorization research

Honeybees alone were found to be insufficient pollinators in the agriculture settings, leading other foragers such as bumblebees to play a crucial role in pollination (Free, 1993; Watanabe, 1994). Bees however, face a challenge in finding flowers and navigating the foraging landscape both in agricultural regions and in the wild. Recent declines in insect-pollinated plants have been found, leading to possible declines in the bee populations (Carvell et al., 2006). The use of categorization behaviours can be used in addition to current conservation research on crop preferences (e.g. Pywell et al., 2006). Certain forms of crop rotations in agricultural settings have been linked to negative effects on local bumblebee populations (Osgathorpe, Park, Goulson, Acs, & Hanley, 2006). The current research lends to understanding of how bees use categorization to navigate changing environments, and could be useful in ameliorating current agricultural practices, like crop rotations, that may be detrimental to native bee populations.

Future directions

Future research given the current results presented could take one of two main directions: a) a further examination into the categorical processes of the bees and how stimuli are categorized, or b) investigation in whether bees are capable of performing higher levels of categorization tasks.

Prototypes and exemplars

As shown through the results, bees are capable of extrapolating specific details of flowers and generalizing them. Generalizations to novel stimuli can occur even when no specific features are available to be generalized (Lea & Harrison, 1978). The prototype theory suggests that with experience different features from various rewarding flowers can be assembled as a representative pattern (Avarguès-Weber et al., 2011). On the other hand, an exemplar-based model suggests that foragers categorize based on similarities to known rewarding and unrewarding flowers.

Learning at the exemplar level or learning at the feature level are not mutually exclusive to one another (Makino & Jitsumori, 2007). The use of family resemblances allows for maximization of the information from learning of the two levels. The use of artificial categories can determine if bees are able to form categories structured by family resemblance. Artificial categories in previous research use polymorphous stimuli with features that were independent of one another. Flowers in natural foraging environments have features that do not correlate to one another perfectly. Properties of family resemblance relationships involve highly variable flowers that may have no features in common structured by a similarity network with features correlating to one another in each category. Prototype effects were first examined in human studies using polymorphous stimuli. Categorical performance was examined between prototypical stimuli representing the central tendency of the categories than other less typical exemplars.

Polymorphous categories were found in previous comparative studies where pigeons were able to successfully learn a category while human participants could not (Makino & Jitsumori, 2007; Lea & Harrison, 1978). Similarly, baboons were found to classify stimuli better when given prototypes than exemplars, whereas humans did not (Dépy, Fagot, & Vauclair, 1997). The bees' capabilities in generalizing relevant similarities suggest the use of a prototype during categorization, but also suggest the use of exemplars as a point of generalization.

Superordinate categories

While functional categories can be examined through behavioural transfer from one member of a category to all members of a functional-equivalence class (i.e. a group of stimuli that became interrelated through common behavioural functions), another approach involves investigating the formation of superordinate categories. Flowers of a rewarding species may all perceptually resemble one another in nature, but not all rewarding flowers are of the same species, nor resemble other rewarding flowers in any way. The superordinate category of rewarding flowers would encompass subgroups of all species of flowers that were found to be rewarding. While subgroups may share perceptual similarity between members, superordinate categories are unlikely to be based on perceptual resemblance (Astley & Wasserman, 1999). As a result, evidence of superordinate categories also confirms the use of functional categorization.

Superordinate categorization has been found in other animal studies. For instance, pigeons were able to classify a series of photographs as depicting natural or artificial objects (Lazareva, Freiburger, & Wasserman, 2004). In natural environments, flower constant bees do not necessarily visit only one species of flower (Free, 1970) and have been found to switch specialities as well (Waser, 1986). Current results indicate that the way bees receive reward influences their similarity judgments. From this, concepts of rewarding or unrewarding resources may exist without any perceptual similarities to link them.

Recent research on invertebrates, particularly bees, revealed abilities in problem solving (Mirwan & Kevan, 2014), social learning (Leadbeater & Chittka, 2009) and even emotional processing (Perry, Baciadonna, & Chittka, 2016). These abilities, once thought to be found only in other creatures with big brains, are also possible in animals with smaller brains. Complex cognitive abilities evolved through selection pressures to allow animals to better navigate their environments. The ability to categorize flowers functions much the same way for bees, as an easier method to understanding the resources available in a natural setting.

References

Arnold, S. E., Savolainen, V., & Chittka, L. (2009). Flower colours along an alpine altitude gradient, seen through the eyes of fly and bee pollinators. *Arthropod-Plant Interactions, 3*(1), 27-43.

Astley, S. L. & Wasserman, E. A. (1998). Novelty and functional equivalence in superordinate categorization by pigeons. *Animal Learning & Behavior, 26*, 125-138.

Aust, U., & Huber, L. (2010). Representational insight in pigeons: Comparing subjects with and without real-life experience. *Animal Cognition*, *13*, 207–218.

Austin, M. W., Horack, P., & Dunlap, A. S. (2018). Choice in a floral marketplace: the role of complexity in bumble bee decision-making. *Behavioral Ecology*, *30*(2), 500-508.

Avarguès-Weber, A., de Brito Sanchez, M. G., Giurfa, M., & Dyer, A. G. (2010). Aversive reinforcement improves visual discrimination learning in free-flying honeybees. *PLoS One*, *5*(10), e15370.

Avarguès-Weber, A., Deisig, N., & Giurfa, M. (2011). Visual cognition in social insects. *Annual Review of Entomology, 56*, 423-443.

Avarguès-Weber, A., Dyer, A. G., Ferrah, N., & Giurfa, M. (2015). The forest or the trees: preference for global over local image processing is reversed by prior experience in honeybees. *Proceedings of the Royal Society of London B: Biological Sciences, 282*(1799), 20142384.

Avarguès-Weber, A., Dyer, A. G., & Giurfa, M. (2011). Conceptualization of above and below relationships by an insect. *Proceedings of the Royal Society of London B: Biological Sciences, 278,* 898-905.

Avarguès-Weber, A., Lachlan, R., & Chittka, L. (2018). Bumblebee social learning can lead to suboptimal foraging choices. *Animal behaviour, 135*, 209-214.

Avarguès-Weber, A., Portelli, G., Benard, J., Dyer, A., & Giurfa, M. (2010). Configural processing enables discrimination and categorization of face-like stimuli in honeybees. *Journal of Experimental Biology, 213,* 593–601.

Bandschbach, V. S. (1994). Colour association influences honey bee choice between sucrose concentrations. *Journal of Comparative Physiology A, 175*(1), 107-114.

Bar-Shai, N., Samuels, R., Keasar, T., Motro, U., & Shmida, A. (2004). Flight durations in bumblebees under manipulation of feeding choices. *Journal of Insect Behavior, 17*(2), 155-168.

Benard, J., & Giurfa, M. (2008). The cognitive implications of asymmetric color generalization in honeybees. *Animal Cognition, 11*, 283–293.

Benard, J., Stach, S., & Giurfa, M. (2006). Categorization of visual stimuli in the honeybee *Apis mellifera. Animal Cognition, 9*(4), 257-270

Biernaskie, J. M., Cartar, R. V., & Hurly, T. A. (2002). Risk-averse inflorescence departure in hummingbirds and bumble bees: could plants benefit from variable nectar volumes?. *Oikos, 98*(1), 98-104.

Bovet, D., & Vauclair, J. (1998). Functional categorization of objects and of their pictures in baboons (Papio *anubis*). *Learning and Motivation, 29*(3), 309-322.

Briscoe, A. D., & Chittka, L. (2001). The evolution of color vision in insects. *Annual Review of Entomology, 46*(1), 471-510.

Brown, M. F., & Sayde, J. M. (2013). Same/different discrimination by bumblebee colonies. *Animal cognition, 16*(1), 117-125.

Brünnert, U., Kelber, A., & Zeil, J. (1994). Ground-nesting bees determine the location of their nest relative to a landmark by other than angular size cues. *Journal of Comparative Physiology A*, *175*(3), 363-369.

Carvell, C., Roy, D. B., Smart, S. M., Pywell, R. F., Preston, C. D., & Goulson, D. (2006). Declines in forage availability for bumblebees at a national scale. *Biological conservation, 132*(4), 481-489.

Cheng, K., Collett, T. S., & Wehner, R. (1986). Honeybees learn the colours of landmarks. *Journal of Comparative Physiology A*, *159*(1), 69-73.

Chittka, L. (1998). Sensorimotor learning in bumblebees: long-term retention and reversal training. *Journal of Experimental Biology, 201*(4), 515-524.

Chittka, L., Dyer, A. G., Bock, F., & Dornhaus, A. (2003). Psychophysics: bees trade off foraging speed for accuracy. *Nature, 424*(6947), 388-388.

Chittka, L., Gumbert, A., & Kunze, J. (1997). Foraging dynamics of bumble bees: correlates of movements within and between plant species. *Behavioral Ecology, 8*(3), 239-249.

Chittka, L., Ings, T. C., & Raine, N. E. (2004). Chance and adaptation in the evolution of island bumblebee behaviour. *Population Ecology, 46*(3), 243-251.

Chittka, L. & Jensen, K. (2011). Animal cognition: concepts from apes to bees. *Current Biology, 21*, R116-119.

Chittka, L., Shmida, A., Troje, N., & Menzel, R. (1994). Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. *Vision Research, 34*(11), 1489-1508.

Chittka, L., & Thomson, J. D. (1997). Sensori-motor learning and its relevance for task specialization in bumble bees. *Behavioral Ecology and Sociobiology, 41*(6), 385-398.

Chittka, L., Thomson, J. D., & Waser, N. M. (1999). Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften, 86*, 361–377.

Chittka, L., & Thomson, J. D. (Eds.). (2001). *Cognitive Ecology of Pollination: Animal Behaviour and Floral Evolution*. Cambridge University Press.

Chittka L., & Walker J. (2006) Do bees like Van Gogh's Sunflowers? Optics & Laser *Technology, 38*, 323–328

Chittka, L., & Wells, H. (2004). Color vision in bees: mechanisms, ecology and evolution. *Complex Worlds from Simpler Nervous Systems*, 165-191.

Collett, T. S., Fry, S. N. & Wehner, R. (1993). Sequence learning by honeybees. *Journal of Comparative Physiology A, 172*, 693-706.

Cnaani, J., Thomson, J. D., & Papaj, D. R. (2006). Flower choice and learning in foraging bumblebees: effects of variation in nectar volume and concentration. *Ethology, 112*(3), 278-285.

Couvillon, P. A., & Bitterman, M. E. (1986). Performance of honeybees in reversal and ambiguous-cue problems: Tests of a choice model. *Learning & behavior, 14*(3), 225-231.

Culley, T. M., Weller, S. G., & Sakai, A. K. (2002). The evolution of wind pollination in angiosperms. *Trends in Ecology & Evolution, 17*(8), 361-369.

Darwin, C. (1895). *The effects of cross and self fertilisation in the vegetable kingdom*. New York, NY: D. Appleton and Co.

Delius, J. D., Emmerton, J., Hörster, W., Jäger, R., & Ostheim, J. (2000). Picture perception in pigeons. In J. Fagot (Ed.), Picture perception in animals (pp. 1-27). Philadelphia, PA: Psychology Press Ltd.

Delius, J.D., Jitsumori, M., & Siemann, M. (2000). Stimulus equivalences through discrimination reversals. In C. Heyes & L. Huber (Eds.), The evolution of cognition (pp 103–122). Cambridge, MA: MIT Press.

Dépy, D., Fagot, J., & Vauclair, J. (1997). Categorisation of three-dimensional stimuli by humans and baboons: search for prototype effects. *Behavioural Processes, 39*(3), 299-306.

Deregowski, J. B. (2000). Pictorial perception: Individual and group differences within human species. In J. Fagot (Ed.), Picture perception in animals (397 – 430). Philadelphia, PA: Psychology Press Ltd.

Dittrich, L., Adam, R., Ünver, E., & Güntürkün, O. (2010). Pigeons identify individual humans but show no sign of recognizing them in photographs. *Behavioural Processes*, *83*, 82–89.

Dornhaus, A., & Chittka, L. (1999). Insect behaviour: Evolutionary origins of bee dances. *Nature*, *401*(6748), 38.

Dukas, R. (Ed.). (1998). Cognitive ecology: the evolutionary ecology of information processing and decision making. University of Chicago Press.

Dukas, R., & Real, L. A. (1993). Learning constraints and floral choice behaviour in bumble bees. *Animal Behaviour, 46*(4), 637-644.

Dukas, R., & Real, L. A. (1993) (b). Effects of nectar variance on learning by bumble bees. *Animal Behaviour*, *45*(1), 37-41.

Dukas, R., & Waser, N. M. (1994). Categorization of food types enhances foraging performance of bumblebees. *Animal Behaviour, 48*, 1001–1006.

Dyer, A. G., & Chittka, L. (2004). Bumblebees (Bombus terrestris) sacrifice foraging speed to solve difficult colour discrimination tasks. *Journal of Comparative Physiology A, 190*(9), 759-763.

Dyer, A. G., Rosa, M. G. P., & Reser, D. H. (2008). Honeybees can recognise images of complex natural scenes for use as potential landmarks. *Journal of Experimental Biology*, *211*, 1180–1186.

Dyer, A. G., Whitney, H. M., Arnold, S. E., Glover, B. J., & Chittka, L. (2007). Mutations perturbing petal cell shape and anthocyanin synthesis influence bumblebee perception of Antirrhinum majus flower colour. *Arthropod-Plant Interactions*, *1*(1), 45-55.

Esch, H. E., Zhang, S., Srinivasan, M. V., & Tautz, J. (2001). Honeybee dances communicate distances measured by optic flow. *Nature*, *411*(6837), 581.

Fagot, J., Martin-Malivel, J., & Dépy, D. (2000). What is the evidence for an equivalence between objects and pictures in birds and nonhuman primates? In J. Fagot (Ed.), Picture perception in animals (pp. 295 – 320). Philadelphia, PA: Psychology Press Ltd.

Fauria, K., Colborn, M., & Collett, T. S. (2000). The binding of visual patterns in bumblebees. *Current Biology*, *10*(15), 935-938.

Fersen, L. V. & Lea, S. E. (1990). Category discrimination by pigeons using five polymorphous features. *Journal of the Experimental Analysis of Behavior*, *54*, 69-84.

Feuerbacher, E. N., & Rosales-Ruiz, J. (2017). Can dogs learn concepts the same way we do? concept formation in a German Shepherd. *International Journal of Comparative Psychology, 30*, 1-25.

Fewell, J. H., & Winston, M. L. (1992). Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera L*. *Behavioral Ecology and Sociobiology*, *30*(6), 387-393.

Foster, R. L. (1992). Nestmate recognition as an inbreeding avoidance mechanism in bumble bees (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, 238-243.

Free, J. B. (1967). Factors determining the collection of pollen by honeybee foragers. *Animal Behaviour*.

Free, J. (1970). The Flower Constancy of Bumblebees. *Journal of Animal Ecology, 39*(2), 395-402.

Free, J. B. (1993). *Insect pollination of crops* (No. Ed. 2). Academic press.

Free, J. B., & Butler, C. G. (1959). Bumblebees. London: Collins.

Gegear, R. J. (2005). Multicomponent floral signals elicit selective foraging in bumblebees. *Naturwissenschaften, 92*(6), 269-271.

Gegear, R. J., & Laverty, T. M. (2005). Flower constancy in bumblebees: a test of the trait variability hypothesis. *Animal Behaviour, 69*, 939–949.

Giurfa, M. (2004). Conditioning procedure and color discrimination in the honeybee *Apis mellifera*. *Naturwissenschaften, 91*(5), 228-231.

Giurfa, M., Backhaus, W., & Menzel, R. (1995). Color and angular orientation in the discrimination of bilateral symmetric patterns in the honeybee. *Naturwissenschaften, 82*(4), 198-201.

Giurfa M, Eichmann B, & Menzel R. (1996). Symmetry perception in an insect. *Nature, 382*, 458-461.

Giurfa, M., Hammer, M., Stach, S., Stollhoff, N., Müller-Deisig, N., & Mizyrycki, C. (1999). Pattern learning by honeybees: conditioning procedure and recognition strategy. *Animal Behaviour, 57,* 315-324.

Giurfa, M., & Menzel, R. (2013). Cognitive components of insect behavior. In Handbook of behavioral neuroscience (pp. 14-25). Amsterdam: Elsevier.

Giurfa, M., Zhang, S., Jenett, A., Menzel, R., & Srinivasan, M. V. (2001). The concepts of ‘sameness’ and ‘difference’ in an insect. *Nature, 410*(6831), 930-933.

Gould, J. L. (1987). Honey bees store learned flower-landing behaviour according to time of day. *Animal Behaviour, 35*(5), 1579-1581.

Gould, J. L. (1988). A mirror-image ‘ambiguity’ in honey bee pattern matching. *Animal Behaviour, 36*, 487–492.

Gould, J. L. (2002). Learning instincts. In Gallistel, R. (Ed.), Stevens’ handbook of experimental psychology, 3rd ed. (pp. 239 – 257). New York, NY: J. Wiley.

Goulson, D. (2000). Why do pollinators visit proportionally fewer flowers in large patches?. *Oikos, 91*(3), 485-492.

Gumbert, A. (2000). Color choices by bumble bees (Bombus terrestris): innate preferences and generalization after learning. *Behavioral Ecology and Sociobiology, 48*(1), 36-43.

Hall, G. (1996). Learning about associatively activated stimulus representations: Implications for acquired equivalence and perceptual learning. *Animal Learning & Behavior, 24*, 233-255.

Hateren, J. V., Srinivasan, M. V., & Wait, P. B. (1990). Pattern recognition in bees: orientation discrimination. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 167*(5), 649-654.

Heinrich, B. (1979). Resource heterogeneity and patterns of movement in foraging bumblebees. *Oecologia, 40*(3), 235-245.

Heinrich, B. (2004). Bumblebee economics. Harvard University Press.

Herrnstein, R. J. (1979). Acquisition, generalization, and discrimination reversal of a natural concept. *Journal of Experimental Psychology*: *Animal Behavior Processes, 5*, 116-129.

Herrnstein, R.J. (1990). Levels of stimulus control: a functional approach. *Cognition, 37*, 133-166.

Herrnstein, R. J., & Loveland, D. H. (1964). Complex visual concept in the pigeon. *Science, 146*(3643), 549-551.

Herrnstein, R.J.. Loveland, D.H.. & Cable. C. (1976). Natural concepts in pigeons. *Journal of Experimental Psychology: Animal Behavior Processes, 2*. 285-311.

Hill, P. S., Wells, P. H., & Wells, H. (1997). Spontaneous flower constancy and learning in honey bees as a function of colour. *Animal Behaviour, 54*(3), 615-627.

Horridge, G. A. (1997). Pattern discrimination by the honeybee: Disruption as a cue. *Journal of Comparative Physiology A, 181*, 267-277.

Horridge, G. A., & Zhang, S. W. (1995). Pattern vision in honeybees (*Apis mellifera*): flower-like patterns with no predominant orientation. *Journal of Insect Physiology, 41*, 681-688.

Ings, T. C., Raine, N. E., & Chittka, L. (2009). A population comparison of the strength and persistence of innate colour preference and learning speed in the bumblebee *Bombus terrestris*. *Behavioral Ecology and Sociobiology, 63*(8), 1207-1218.

Ings, T. C., Wang, M. Y., & Chittka, L. (2012). Colour-independent shape recognition of cryptic predators by bumblebees. *Behavioral Ecology and Sociobiology, 66*(3), 487-496.

Jenkins, W. O., & Stanley Jr, J. C. (1950). Partial reinforcement: a review and critique. *Psychological bulletin*, *47*(3), 193.

Jitsumori, M. (1993). Category discrimination of artificial polymorphous stimuli based on feature learning. *Journal of Experimental Psychology*: *Animal Behavior Processes*, *19*(3), 244.

Jones, P. L., Ryan, M. J., & Chittka, L. (2015). The influence of past experience with flower reward quality on social learning in bumblebees. *Animal Behaviour, 101*, 11-18.

Kapustjansky, A., Chittka, L., & Spaethe, J. (2010). Bees use three-dimensional information to improve target detection. *Naturwissenschaften*, *97*(2), 229-233.

Katz, E., & Essenberg, C. J. (2018). The effect of the dispersion of rewarding and rewardless flowers on visitation and constancy by bumblebees (*Bombus impatiens*). *Journal of Pollination Ecology*, *23*(9-14). 119-126.

Kheradmand, B., Cassano, J., Gray, S., & Nieh, J. C. (2018). Influence of visual targets and landmarks on honey bee foraging and waggle dancing. *Insect Science,* 1-12.

Keller, F. S., & Schoenfeld, W. N. (1950). Principles of Psychology. New York, NY: Appleton-Century Crofts. Inc.

Kevan, P. G., Chittka, L., & Dyer, A. G. (2001). Limits to the salience of ultraviolet: lessons from colour vision in bees and birds. *Journal of Experimental Biology*, *204*(14), 2571-2580.

Korneluk, Y. G., & Plowright, C. M. S. (1995). Mirror image pattern matching by bumblebees. *Behaviour, 132*, 87–93.

Kunze, J., & Gumbert, A. (2001). The combined effect of color and odor on flower choice behavior of bumble bees in flower mimicry systems. *Behavioral Ecology, 12*(4), 447-456.

Laverty, T. M. (1994). Costs to foraging bumble bees of switching plant species. *Canadian Journal of Zoology, 72*(1), 43-47.

Lazareva, O. F., Freiburger, K. L., & Wasserman, E. A. (2004). Pigeons concurrently categorize photographs at both basic and superordinate levels. *Psychonomic Bulletin & Review*, *11*(6), 1111-1117.

Lea, S. E. G. (1984). In what sense do pigeons learn concepts. In Roitlblat, H. L., Bever, T. G. & Terrace, H. S. (Eds.), Animal cognition (pp. 263-276). Hillsdale, NJ: Lawrence Erlbaum Associates.

Lea, S. E. G., & Harrison, S. N. (1978). Discrimination of polymorphous stimulus sets by pigeons. *The Quarterly Journal of Experimental Psychology*, *30*(3), 521-537.

Leadbeater, E., & Chittka, L. (2009). Bumble-bees learn the value of social cues through experience. *Biology Letters*, *5*(3), 310-312.

Leadbeater, E., & Florent, C. (2014). Foraging bumblebees do not rate social information above personal experience. *Behavioral ecology and sociobiology*, *68*(7), 1145-1150.

Lehrer, M., & Campan, R. (2005). Generalization of convex shapes by bees: what are shapes made of?. *Journal of Experimental Biology*, *208*(17), 3233-3247.

Lehrer, M., Horridge, G. A., Zhang, S. W., & Gadagkar, R. (1995). Shape vision in bees: Innate preference for flower-like patterns. *Philosophical Transactions of the Royal Society B, 347*, 123–137.

Leonard, A. S., Dornhaus, A., & Papaj, D. R. (2011). Flowers help bees cope with uncertainty: signal detection and the function of floral complexity. *Journal of Experimental Biology, 214*(1), 113-121.

Leonhardt, S.D., Blüthgen, N. (2012). The same, but different: pollen foraging in honeybee and bumblebee colonies. *Apidologie, 43*, 449–464.

Looney, T. A. & Cohen, P. S. (1974). Pictorial target control of schedule-induced attack in white Carneaux pigeons. *Journal of the Experimental Analysis of Behavior, 21*, 571–584.

Mackintosh, N. J. (1965). Selective attention in animal discrimination learning. *Psychological Bulletin*, *64*(2), 124.

Marden, J. H., & Waddington, K. D. (1981). Floral choices by honeybees in relation to the relative distances to flowers. *Physiological Entomology*, *6*(4), 431-435.

Memmott, J., Craze, P. G., Waser, N. M., & Price, M. V. (2007). Global warming and the disruption of plant–pollinator interactions. *Ecology letters, 10*(8), 710-717.

Menzel, R. (1967). Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifica*). *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *56*(1), 22-62.

Menzel, R. (1969). Das gedächtnis der honigbiene für spektralfarben. *Zeitschrift für Vergleichende Physiologie*, *63*(3), 290-309.

Mirwan, H. B., & Kevan, P. G. (2014). Problem solving by worker bumblebees *Bombus impatiens* (Hymenoptera: Apoidea). *Animal cognition*, *17*(5), 1053-1061.

Mirwan, H. B., & Kevan, P. G. (2015). Maze navigation and route memorization by worker bumblebees (*Bombus impatiens Cresson*) (Hymenoptera: Apidae). *Journal of Insect Behavior*, *28*(3), 345-357.

Morawetz, L., & Spaethe, J. (2012). Visual attention in a complex search task differs between honeybees and bumblebees. *Journal of Experimental Biology, 215*(14), 2515-2523.

Morawetz, L., Svoboda, A., Spaethe, J., & Dyer, A. G. (2013). Blue colour preference in honeybees distracts visual attention for learning closed shapes. *Journal of Comparative Physiology A, 199*(10), 817-827.

Muth, F., Francis, J. S., & Leonard, A. S. (2016). Bees use the taste of pollen to determine which flowers to visit. *Biology Letters*, *12*(7), 20160356.

Nityananda, V. (2016). Attention-like processes in insects. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1842), 20161986.

Nityananda, V., & Pattrick, J. G. (2013). Bumblebee visual search for multiple learned target types. *Journal of Experimental Biology, 216*(22), 4154-4160.

Nityananda, V., Skorupski, P., & Chittka, L. (2014). Can bees see at a glance?. *Journal of Experimental Biology, 217*(11), 1933-1939.

Osgathorpe, L. M., Park, K., Goulson, D., Acs, S., & Hanley, N. (2011). The trade-off between agriculture and biodiversity in marginal areas: Can crofting and bumblebee conservation be reconciled?. *Ecological Economics*, *70*(6), 1162-1169.

Palottini, F., Estravis Barcala, M. C., & Farina, W. M. (2018). Odor learning and its experience-dependent modulation in the South American Native Bumblebee *Bombus atratus* (Hymenoptera: Apidae). *Frontiers in Psychology, 9*, 603.

Pastore, R. E. (1987). Categorical perception: Some psychophysical models. In S. Harnad (Ed.), Categorical perception: The groundwork of cognition (pp. 29-52). New York, NY, US: Cambridge University Press.

Penn, D. C., Holyoak, K. J., & Povinelli, D. J. (2008). Darwin's mistake: Explaining the discontinuity between human and nonhuman minds. *Behavioral and Brain Sciences, 31*(2), 109-130.

Pepperberg, I. M. (1987). Evidence for conceptual quantitative abilities in the African grey parrot: Labeling of cardinal sets. *Ethology, 75*, 37-61.

Perreault, M., & Plowright, C. M. S. (2009). Facilitation of learning of a simultaneous discrimination between rotated patterns by bumblebees. *Learning and Motivation, 40*, 234–256.

Perry, C. J., Baciadonna, L., & Chittka, L. (2016). Unexpected rewards induce dopamine-dependent positive emotion–like state changes in bumblebees. *Science, 353*(6307), 1529-1531.

Perry, C. J., & Barron, A. B. (2013). Honey bees selectively avoid difficult choices. *Proceedings of the National Academy of Sciences*, *110*(47), 19155-19159.

Plazas, E. A. & Cortés, D. (2017). Relation between exclusion and stimulus equivalence class formation in auditory-visual and visual-visual matching in preschoolers. *International Journal of Comparative Psychology, 30*, 1-30.

Plowright, C. M. S. (1997). Function and mechanism of mirror-image ambiguity in bumblebees. *Animal Behaviour, 53*, 1295–1303.

Plowright, C.M.S., Cohen-Salmon, D., Landry, F. & Simonds, F. (1999). Foraging for nectar and pollen on thistle flowers (*Cirsium vulgare*) and artificial flowers: How bumble bees (Hymenoptera: Apidae) respond to colony requirements. *Behaviour*, *136*, 951-963.

Plowright, C. M. S., Evans, S. A., Leung, J. C., & Collin, C. A. (2011). The preference of symmetry in flower-naïve and not-so-naïve bumblebees. *Learning and Motivation, 42,* 75–83.

Plowright, C. M. S., Ferguson, K. A., Jellen, S. L., Xu, V., Service, E. W., & Dookie, A. L. (2013). Bumblebees occupy: When foragers do and do not use the presence of others to first find food. *Insectes Sociaux, 60,* 517–524

Plowright, C. M. S., & Korneluk, Y. G. (1995). Mirror image pattern matching by bumble bees. *Behaviour, 132*(1-2), 87-93.

Plowright, R. C., Thomson, J. D., Lefkovitch, L. P., & Plowright, C. M. S. (1993). An experimental study of the effect of colony resource manipulation on foraging for pollen by worker bumble bees (Hymenoptera: Apidae). *Canadian Journal of Zoology*, *71*, 1393-1396.

Pyke, G. H. (1978). Optimal foraging: movement patterns of bumblebees between inflorescences. *Theoretical Population Biology, 13*(1), 72-98.

Pyke, G. H. (1991). What does it cost a plant to produce floral nectar?. *Nature, 350*(6313), 58-59.

Pywell, R. F., Warman, E. A., Hulmes, L., Hulmes, S., Nuttall, P., Sparks, T. H., Dicks, L. V., Bennett, D., Wright, A., Critchley, C. N. R., & Sherwood, A. (2006). Effectiveness of new agri-environment schemes in providing foraging resources for bumblebees in intensively farmed landscapes. *Biological Conservation*, *129*(2), 192-206.

Raine, N. E., & Chittka, L. (2005). Comparison of flower constancy and foraging performance in three bumblebee species (Hymenoptera: Apidae: Bombus). *Entomologia Generalis, 28*, 081–089.

Raine, N. E., & Chittka, L. (2007). Flower constancy and memory dynamics in bumblebees (Hymenoptera: Apidae: Bombus). *Entomologia Generalis, 29*, 179.

Raine, N. E., & Chittka, L. (2012). No trade-off between learning speed and associative flexibility in bumblebees: a reversal learning test with multiple colonies. *PLoS One, 7*(9), e45096.

Raine, N. E., Ings, T. C., Dornhaus, A., Saleh, N., & Chittka, L. (2006). Adaptation, genetic drift, pleiotropy, and history in the evolution of bee foraging behavior. *Advances in the Study of Behavior, 36*, 305-354.

Ribbands, C. R. (1949). The foraging method of individual honey-bees. *The Journal of Animal Ecology*, 47-66.

Riffell, J. A. (2011). The neuroecology of a pollinator's buffet: olfactory preferences and learning in insect pollinators. *Integrative and Comparative Biology, 51*(5), 781-793.

Riveros, A. J., & Gronenberg, W. (2012). Decision-making and associative color learning in harnessed bumblebees (Bombus impatiens). *Animal cognition, 15*(6), 1183-1193.

Roberts, W. A., & Mazmanian, D. S. (1988). Concept learning at different levels of abstraction by pigeons, monkeys, and people. *Journal of Experimental Psychology: Animal Behavior Processes, 14*(3), 247.

Rodríguez, I., Gumbert, A., Hempel de Ibarra, N., Kunze, J., & Giurfa, M. (2004). Symmetry is in the eye of the 'beeholder': Innate preference for bilateral symmetry in flower-naïve bumblebees. *Naturwissenschaften, 91*, 374–377.

Ronacher, B. (1992). Pattern recognition in honeybees: multidimensional scaling reveals a city-block metric. *Vision research, 32*(10), 1837-1843.

Ronacher, B. (1998). How do bees recognize visual patterns? *Biological Cybernetics, 79*, 477–485.

Rossi, N., Santos, E., Salvarrey, S., Arbulo, N., & Invernizzi, C. (2015). Determination of flower constancy in Bombus atratus Franklin and Bombus bellicosus Smith (Hymenoptera: Apidae) through palynological analysis of nectar and corbicular pollen loads. *Neotropical Entomology, 44*, 546–552.

Russell, A. L., Morrison, S. J., Moschonas, E. H., & Papaj, D. R. (2017). Patterns of pollen and nectar foraging specialization by bumblebees over multiple timescales using RFID. *Scientific Reports, 7*, 42448.

Saleh, N., Ohashi, K., Thomson, J. D., & Chittka, L. (2006). Facultative use of the repellent scent mark in foraging bumblebees: complex versus simple flowers. *Animal Behaviour, 71*(4), 847-854.

Salsa, A. M., & de Mendoza, O. P. (2007). Routes to symbolization: Intentionality and correspondence in early understanding of pictures. *Journal of Cognition and Development*, *8*, 79–92.

Savage-Rumbaugh, E. S., Rumbaugh, D. M., Smith, S. T., & Lawson, J. (1980). Reference: The linguistic essential. *Science, 210*(4472), 922-925.

Scheiner, R., Page, R. E., and Erber, J. (2001). Responsiveness to sucrose affects tactile and olfactory learning in preforaging honey bees of two genetic strains. *Behavioural Brain Research, 120*, 67–73.

Schusterman, R. J., & Kastak, D. (1993). A California Sea Lion "(*Zalophus californianus*)" is capable of forming equivalence relations. *The Psychological Record, 43*(4), 823.

Séguin, F. R., & Plowright, C. M. S. (2008). Assessment of pattern preferences by flower-naïve bumblebees. *Apidologie, 39*, 215–224.

Shettleworth, S. J. (2010). Cognition, evolution, and behavior. New York: Oxford University Press.

Shrestha, M., Dyer, A. G., & Burd, M. (2013). Evaluating the spectral discrimination capabilities of different pollinators and their effect on the evolution of flower colors. *Communicative & Integrative Biology*, *6*(3), 301-10.

Sidman, M. (1971). Reading and auditory-visual equivalences. *Journal of Speech and Hearing Research, 14*(1), 5-13.

Sokal, R. R., & Rohlf, F. J. (2012). Biometry (4th ed). New York: W. H. Freeman.

Southwick, E. E. (1984). Photosynthate allocation to floral nectar: a neglected energy investment. *Ecology, 65*(6), 1775-1779.

Spaethe, J., Tautz, J., & Chittka, L. (2006). Do honeybees detect colour targets using serial or parallel visual search?. *Journal of Experimental Biology, 209*(6), 987-993.

Spetch, M. L., & Friedman, A. (2006). Pigeons see correspondence between objects and their pictures. *Psychological Science, 17*, 966–972.

Stach, S., Benard, J., & Giurfa, M. (2004). Local-feature assembling in visual pattern recognition and generalization in honeybees. *Nature, 429*(6993), 758-761.

Benard, J., Stach, S., & Giurfa, M. (2006). Categorization of visual stimuli in the honeybee *Apis mellifera*. *Animal cognition*, *9*(4), 257-270.

Stach, S., & Giurfa, M. (2001). How honeybees generalize visual patterns to their mirror image and left-right transformation. *Animal Behaviour, 62*, 981–991.

Stach, S., & Giurfa, M. (2005). The influence of training length on generalization of visual feature assemblies in honeybees. *Behavioural brain research, 161*(1), 8-17.

Stephens, D. W., & Krebs, J. R. (1986). Foraging theory. Princeton University Press.

Strang, C. G., & Sherry, D. F. (2014). Serial reversal learning in bumblebees (*Bombus impatiens*). *Animal Cognition, 17*, 723-734.

Sturdy, C.B., Campbell, K.A., Congdon, J.V., Hahn, A.H., McMillan, N., & Scully, E.N. (2017). Moving from perceptual to functional categories in songbirds. *International Journal of Comparative Psychology*, *30*, 1-14.

Tedjakumala, S. R., & Giurfa, M. (2013). Rules and mechanisms of punishment learning in honey bees: the aversive conditioning of the sting extension response. *Journal of Experimental Biology, 216*(16), 2985-2997.

Thompson, E. L., & Plowright, C. M. S. (2014). How images may or may not represent flowers: Picture-object correspondence in bumblebees (*Bombus impatiens*)? *Animal Cognition, 17,* 1031–1043.

Townsend-Mehler, J. M., Dyer, F. C., & Maida, K. (2011). Deciding when to explore and when to persist: a comparison of honeybees and bumblebees in their response to downshifts in reward. *Behavioral Ecology and Sociobiology, 65*(2), 305-312.

Troje, N. F., Huber, L., Loidolt, M., Aust, U., & Fieder, M. (1999). Categorical learning in pigeons: the role of texture and shape in complex static stimuli. *Vision research, 39*(2), 353-366.

Tsutsui, K. I., Hosokawa, T., Yamada, M., & Iijima, T. (2016). Representation of functional category in the monkey prefrontal cortex and its rule-dependent use for behavioral selection. *Journal of Neuroscience, 36*(10), 3038-3048.

van Hateren, J. H., Srinivasan, M. V., & Wait, P. B. (1990). Pattern recognition in bees: orientation discrimination. *Journal of Comparative Physiology A., 197*, 649–54

Vauclair, J. (2002). Categorization and conceptual behavior in nonhuman primates. In The cognitive animal (pp. 239-245). Cambridge, MA: MIT Press.

Vaughan, W. (1988). Formation of equivalence sets in pigeons. *Journal of Experimental Psychology: Animal Behavior Processes, 14,* 36-42.

Vaughan, W., & Greene, S. L. (1984). Pigeon visual memory capacity. *Journal of Experimental Psychology: Animal Behavior Processes*, *10*(2), 256-271.

von Fersen, L., & Delius, J. D. (2000). Acquired equivalences between auditory stimuli in dolphins (*Tursiops truncatus*). *Animal Cognition*, *3*(2), 79-83.

Von Frisch, K. (1962). Dialects in the language of the bees. *Scientific American, 207*(2), 78-89.

Vonk, J. & Leete, J. A. (2017). Carnivore concepts: categorization in carnivores "bears" further study. *International Journal of Comparative Psychology, 30*, 1-22

Waser, N. M. (1986). Flower constancy: definition, cause, and measurement. The *American Naturalist, 127*(5), 593-603.

Watanabe, S. (1988). Failure of visual prototype learning in the pigeon. *Learning & Behavior*, *16*(2), 147-152.

Watanabe, S. (2000). How do pigeons see pictures? Recognition of the real world from its 2-D representation. In J. Fagot (Ed.), Picture perception in animals (pp. 71 – 90). Philadelphia, PA: Psychology Press Ltd.

Watanabe, M. E. (1994). Pollination worries rise as honey bees decline. *Science*, *265*(5176), 1170-1171.

Watanabe, S., Lea, S.E.G., Dittrich, W.H. (1993). What can we learn from experiments on pigeon concept discrimination? In: Bischof, H.J., & Zeigler, H.P. (Eds.), Avian Vision and Cognition (pp. 352–376). MIT Press, Cambridge.

Werner, A., Stürzl, W., & Zanker, J. (2016). Object recognition in flight: How do bees distinguish between 3D shapes?. *PloS one*, *11*(2), e0147106.

Wiegmann, D. D., Wiegmann, D. A., MacNeal, J., & Gafford, J. (2000). Transposition of flower height by bumble bee foragers (*Bombus impatiens*). *Animal Cognition*, *3*(2), 85-89.

Wiegmann, D. D., Wiegmann, D. A., & Waldron, F. A. (2003). Effects of a reward downshift on the consummatory behavior and flower choices of bumblebee foragers. *Physiology & behavior*, *79*(4-5), 561-566.

Weisman, R. G., & Spetch, M. L. (2010). Determining when birds perceive correspondence between pictures and objects: A critique. *Comparative Cognition & Behaviour Reviews, 5*, 117–131.

Wehner R, & Rossel, S. (1985) The bee’s celestial compass. A case study in behavioural neurobiology. In: Hölldobler B, & Lindauer, M. (Eds.), Experimental behavioral ecology and sociobiology (pp. 11-53). Fischer, Stuttgart.

Wells, H., & Rathore, R. R. (1994). Foraging ecology of the Asian hive bee, *Apis cerane indica*, within artificial flower patches. *Journal of Apicultural research*, *33*(4), 219-230.

Wells, H., & Wells, P. H. (1983). Honey bee foraging ecology: optimal diet, minimal uncertainty or individual constancy?. *The Journal of Animal Ecology*, 829-836.

Westphal, C., Steffan-Dewenter, & Tscharntke, T. (2006). Foraging trip duration of bumblebees in relation to landscape-wide resource availability. *Ecological Entomology*, *31*(4), 389-394.

Wilson, E. O. (1975). Sociobiology, the new synthesis Belknap Press. *Cambridge, Mass*.

Worden, B. D., & Papaj, D. R. (2005). Flower choice copying in bumblebees. *Biology Letters*, *1*(4), 504-507.

Wright, G. A., Mustard, J. A., Simcock, N. K., Ross-Taylor, A. A., McNicholas, L. D., Popescu, A., & Marion-Poll, F. (2010). Parallel reinforcement pathways for conditioned food aversions in the honeybee. *Current Biology*, *20*(24), 2234-2240.

Wu, W., Moreno, A. M., & Tangen, J. M. (2013). Honeybees can discriminate between Monet and Picasso paintings. *Journal of Comparative Physiology A, 199*, 45–55.

Xu, V., & Plowright, C. M. S. (2016). Different-but-similar judgments by bumblebees. *Animal Behavior and Cognition, 3*(3), 198–209. doi: 10.12966/abc.07.08.2016.

Xu, V., & Plowright, C. (2017). A transferrable change in preferences of floral patterns by bumblebees through reward reversal. *International Journal of Comparative Psychology*, *30*, 1-13.

Yamamoto, J. I., & Asano, T. (1995). Stimulus equivalence in a Chimpanzee"(*Pan troglodytes*)". *The Psychological Record, 45*(1), 3-22.

Zayan, R., & Vauclair, J. (1998). Categories as paradigms for comparative cognition. *Behavioural Processes, 42*(2), 87-99.

Zentall, T. R., Galizio, M., & Critchfield, T. S. (2002). Categorization, concept learning, and behavior analysis: An introduction. *Journal of the Experimental Analysis of Behavior, 78*(3), 237-248.

Zentall, T. R., Wasserman, E.A., Lazareva, O. F., Thompson, R. K. R., & Rattermann, M. J. (2008). Concept learning in animals. *Comparative Cognition & Behavior Reviews, 3*, 13-45.

Zhang, S. W., Bartsch, K. & Srinivasan, M. V. (1996). Maze learning by honeybees. *Neurobiology of Learning and Memory, 66*, 267-282.

Zhang, S., Srinivasan, M.V., Zhu, H., & Wong, J. (2004). Grouping of visual objects by honeybees. *Journal of Experimental Psychology, 207*, 3289-3298.

Table 2. Design for discrimination training and subsequent testing for Experiment 1.

Discrimination Training on Objects

	Colony 1		**Colony 2**	
Train (20 bees)	**Object X+**	**Object Y−**	**Object Y+**	**Object X−**
Test				
(10 bees)	Photo X	Object X	Photo Y	Object Y
(10 bees)	Photo X	Photo Y	Photo Y	Photo X

Discrimination Training on Photos

	Colony 3		**Colony 4**	
Train (20 bees)	**Photo X+**	**Photo Y−**	**Photo Y+**	**Photo X−**
Test				
(10 bees)	Object X	Photo X	Object Y	Photo Y
(10 bees)	Object X	Object Y	Object Y	Object X

Table 3. Design for training and testing procedures over eight conditions in Experiment 2. The number of bees that were tested is given for each colony.

Phase I Training with Ob. X+ Ph. X+ / Ob. Y– Ph. Y–

	Colony 1	**Colony 2**	**Colony 3**	**Colony 4**
	n = 10	**n = 10**	**n = 10**	**n = 9**
Phase II training	Ob. X– / Ob. Y+ (Switch)	Ob. X+ / Ob. Y– (Non-switch)	Ph. X– / Ph. Y+ (Switch)	Ph. X+ / Ph. Y– (Non-switch)
Test:	Photo X vs Photo Y		Object X vs Object Y	

Phase I Training with Ob. Y+ Ph. Y+ / Ob. X– Ph. X–

	Colony 5	**Colony 6**	**Colony 7**	**Colony 8**
	n = 9	**n = 8**	**n = 10**	**n = 10**
Phase II training	Ob. Y– / Ob. X+ (Switch)	Ob. Y+ / Ob. X– (Non-switch)	Ph. Y– / Ph. X+ (Switch)	Ph. Y+ / Ph. X– (Non-switch)
Test	Photo X vs Photo Y		Object X vs Object Y	

Table 4. Design for training and testing procedures over four conditions in Experiment 3. The number of bees that were tested is given for each colony.

Phase I Training with Ob. Y+ Ph. X+ / Ob. X– Ph. Y–

	Condition 1	**Condition 2**	**Condition 3**	**Condition 4**
	n = 10	**n = 10**	**n = 11**	**n = 9**
Phase II training	Ob. X+ / Ob. Y- (Switch)	Ob. Y+ / Ob. X- (Non-switch)	Ph. X– / Ph. Y+ (Switch)	Ph. X+ / Ph. Y– (Non-switch)
Test	Photo X vs Photo Y		Object X vs Object Y	

Table 5. Design for training and testing procedures over four conditions and eight colonies in Experiment 4. The number of bees that were tested is given for each condition.

	Colony 1+2	**Colony 3+4**	**Colony 5+6**	**Colony 7+8**
	n=30	**n=30**	**n=30**	**n=30**
Training	**Photos+/Objects-**	**X+ / Y-**	**Objects+/Photos-**	**Y+ / X-**
Test	Dimensionality test: Ob Z vs Ph Z			
	Flower type test: Ph X2 vs Ph. Y2			
	Similarity judgment test: Ob.X2 vs Ph. Z		Similarity judgement test: Ph. Y2 vs Ob. Z	

Table 6. Summary of statistics in Experiment 4

Test type				Cluster details	Statistics
Between-group analyses	Colony effects	Object +/ Photos - (col 1 v 2)	Test 1	Not applicable	χ^2 = .043, df=1, p=.836
			Test 2		χ^2=.16, df= 1, p=.69
			Test 3		χ^2=1.99, df=1, p=.157
		Y+ / X- (col 3 v 4)	Test 1		χ^2=.36, df=1, p=.548
			Test 2		χ^2=2.99, df=1, p=.084
			Test 3		χ^2=2.098, df=1, p=.148
		Photos +/ Object - (col 5 v 6)	Test 1		χ^2=.051, df=1, p=.822
			Test 2		χ^2=2.57, df=1, p=.108
			Test 3		χ^2=1.99, df=1, p=.157
		X+ /Y- (col 7 v 8)	Test 1		χ^2=.361, df=1, p=.548
			Test 2		χ^2=0, df=1, p=1
			Test 3		χ^2=.796, df=1, p=.372
Individual differences	Proportions*	Object +/ Photos -	Test 1	Group 1 (n = 3): proportion: >0.18 Group 2 (n = 7): proportion <0.14	t=1.20, df=8, p=.264
			Test 2	Group 1 (n = 7): proportion: >0.14 Group 2 (n = 3): proportion <0.08	t=.417, df=8, p=.687
			Test 3	Group 1 (n =3): proportion: >0.34 Group 2 (n = 7): proportion <0.24	t=.575, df=8, p=.581
		Y+ / X-	Test 1	Group 1 (n = 3): proportion: >0.18 Group 2 (n = 7): proportion <0.12	t=.735; df=8, p=.483
			Test 2	Group 1 (n = 7): proportion: >0.14 Group 2 (n = 3): proportion <0.08	t=-.434, df=8, p=.676
			Test 3	Group 1 (n = 3): proportion: >0.11 Group 2 (n = 7): proportion <0.08	t=-.549, df=8, p=.598
		Photos +/ Object -	Test 1	Group 1 (n = 2): proportion: >0.13 Group 2 (n = 8): proportion <0.11	t=-1.75, df=8, p=.118
			Test 2	Group 1 (n = 4): proportion: >0.15 Group 2 (n = 6): proportion <0.11	t=.368, df=8, p=.723
			Test 3	Group 1 (n = 5): proportion: >0.22 Group 2 (n = 5): proportion <0.17	t=.862, df=8, p=.414
		X+ / Y-	Test 1	Group 1 (n = 3): proportion: >0.23 Group 2 (n = 7): proportion <0.18	t=-.978, df=8, p=.357
			Test 2	Group 1 (n = 3): proportion: >0.25 Group 2 (n = 7): proportion <0.17	t=-.696, df=8, p=.506

			Test 3	Group 1 (n = 5): proportion: >0.12 Group 2 (n = 5): proportion <0.06	t=2.191, df=8, p=.06
	Days of training	Object +/ Photos -	Test 1	Group 1 (n = 4): training days >=4 Group 2 (n = 6): training days <4	t=.222, df=8, p=.83
			Test 2	Group 1 (n = 3): training days >=7 Group 2 (n = 7): training days <=5	t=2.31, df=8, p=.05
			Test 3	Group 1 (n = 3): training days =4 Group 2 (n = 7): training days =2	t=2.108, df=8, p=.68
		Y+/ X-	Test 1	Group 1 (n = 3): training days =3 Group 2 (n = 7): training days =2	t=.017, df=8, p=.196
			Test 2	Group 1 (n = 4): training days =3 Group 2 (n = 6): training days =2	t=-.434, df=8, p=.676
			Test 3**	Group 1 (n = 1): training days =9 Group 2 (n = 9): training days <=4	t=2.024, df=8, p=.078
		Photos+/ Objects -	Test 1	No clusters: all training days =2	
			Test 2	Group 1 (n = 4): training days >=4 Group 2 (n = 6): training days <4	t=.397, df=8, p=.723
			Test 3	Group 1 (n = 3): training days >=3 Group 2 (n = 7): training days =2	t=2.139, df=8, p=.065
		X+/ Y-	Test 1**	Group 1 (n = 1): training days =3 Group 2 (n = 9): training days =2	t=-2.145, df=8, p=.064
			Test 2	Group 1 (n = 3): training days >3 Group 2 (n = 7): training days <=3	t=.564, df=8, p=.588
			Test 3	Group 1 (n = 3): training days =3 Group 2 (n = 7): training days =2	t=-.381, df=8, p=.713
	Choices change over time ***	Objects+/ Photos-	Test 1		χ^2 = 2.78, df=1, p=.095
			Test 2		χ^2 = .04, df=1, p=.841
			Test 3		χ^2 =3.31, df=1, p=.069
		Y+/ X-	Test 1		χ^2 = 0, df=1, p=1
			Test 2		χ^2 =.332, df=1, p=.564

			Test 3		χ^2 =.233, df=1, p=.629
		Photos+/ Objects-	Test 1		χ^2 =.051, df=1, p=.822
			Test 2		χ^2 =.657, df=1, p=.418
			Test 3		χ^2 =.041, df=1, p=.84
		X+/ Y-	Test 1		χ^2 =.364, df=1, p=.546
			Test 2		χ^2 =.543, df=1, p=.461
			Test 3		χ^2 =.088, df=1, p=.766

*Choice proportions were calculated based on the bees' visits on either rewarding stimulus during training, so that a proportion value of 0 indicates a bee that had visited both rewarding stimuli equally and a proportion value of 1 indicates a bee that visited only one of the two rewarding stimuli during training

** Not valid given the group sizes

*** Comparisons are done between the first five and last five choices for each bee

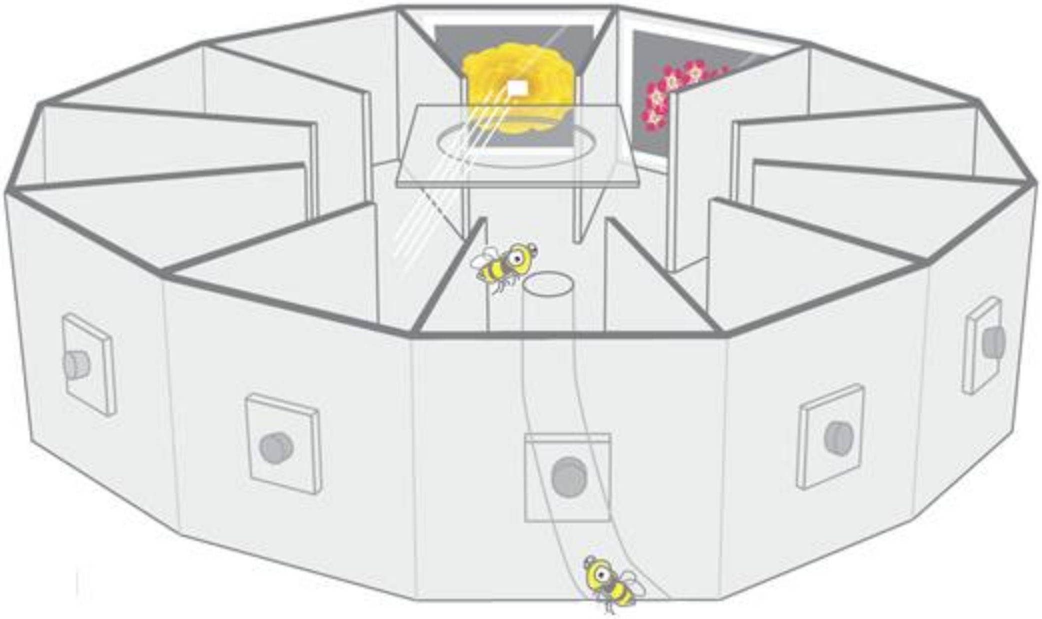


Figure 1. Diagram of bee entering maze through hole in the floor using wire mesh tunnel. Stimuli are mounted on the back walls of the corridors. Figure modified from an original (Plowright et al., 2013) with kind permission from Springer Science and Business Media.

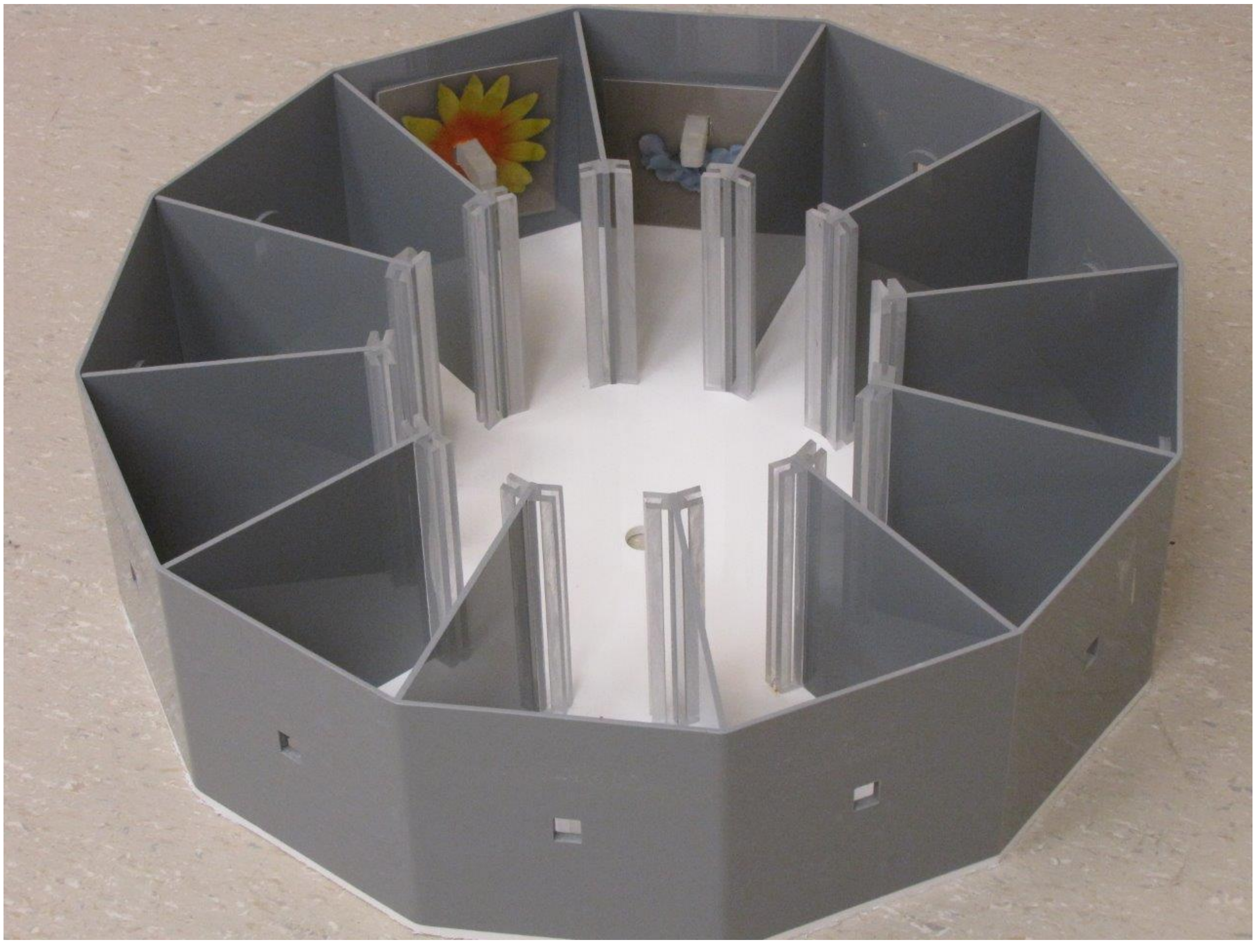


Figure 2. Photograph of radial arm maze with stimuli (Photo X and Photo Y) and feeders placed at the back walls.

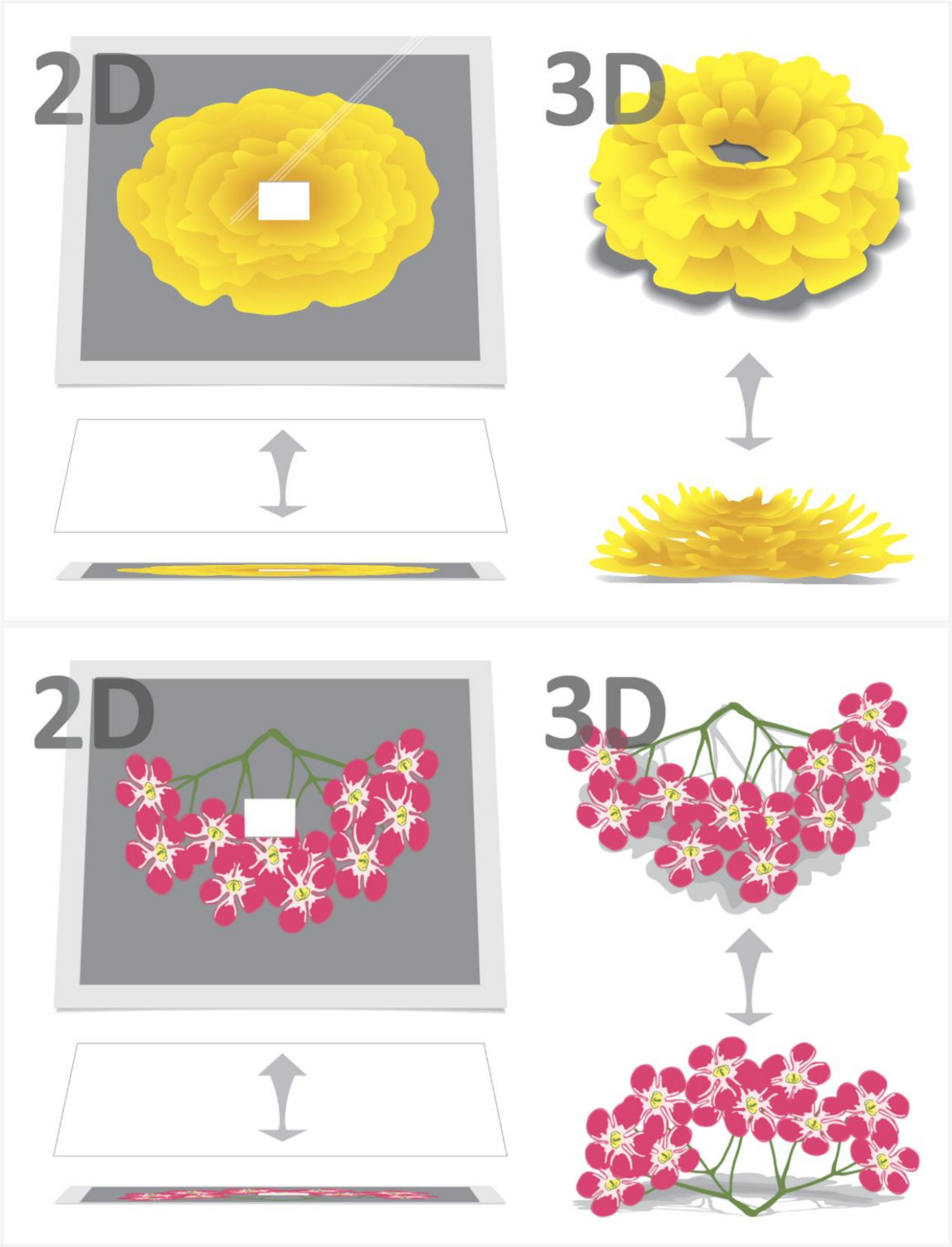


Figure 3. Images of Photo and Object X (on top) and Photo and Object Y (bottom) from Experiment 1. Photo and Object X2 used in Experiment 4 uses the same top stimuli. Object X reproduced from Thompson & Plowright (2014) with permission from Springer.

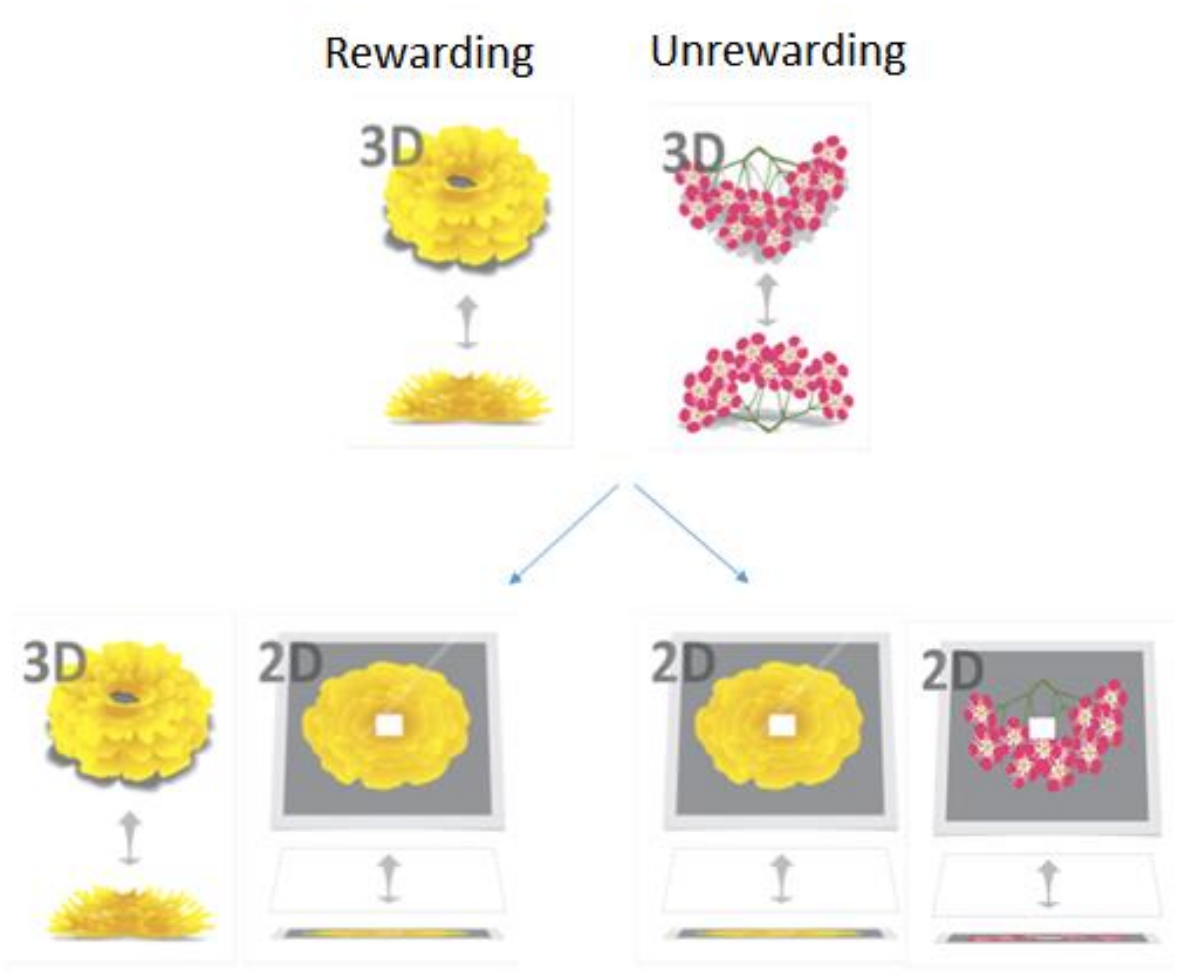
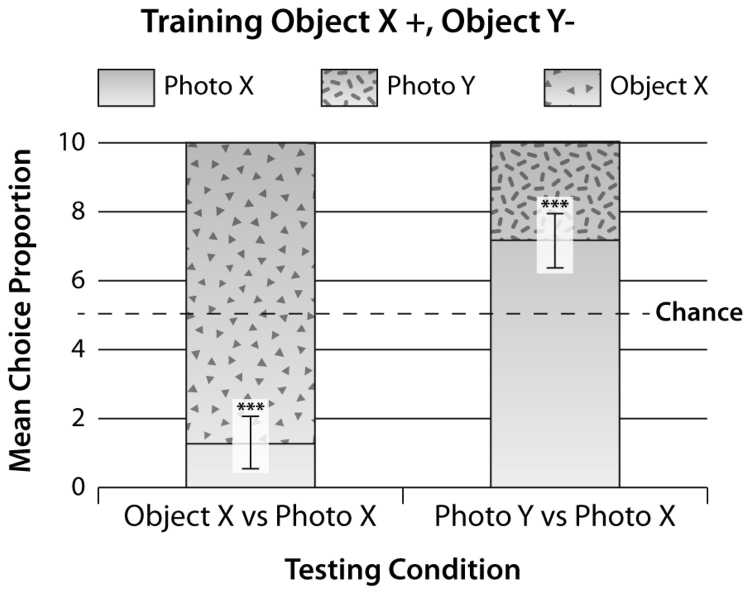
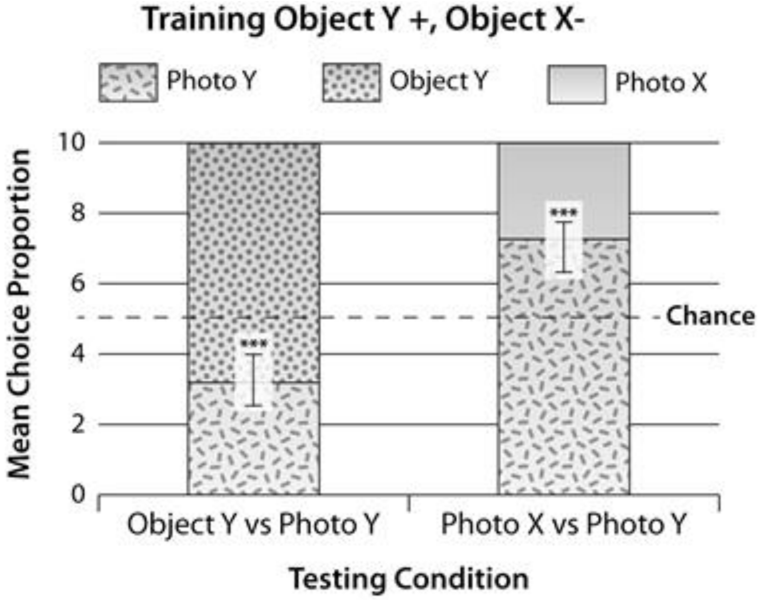


Figure 4. Example of a condition using the design from Experiment 1 with a rewarding Ob. X.

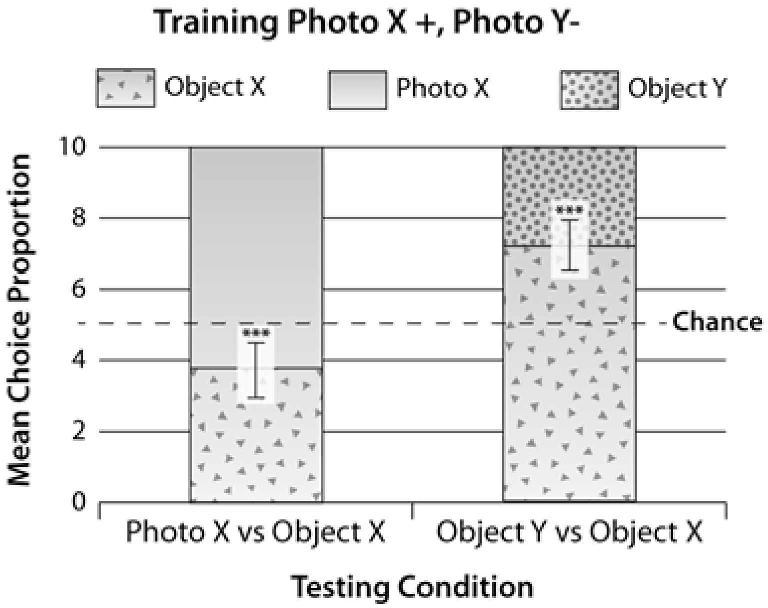


(a)

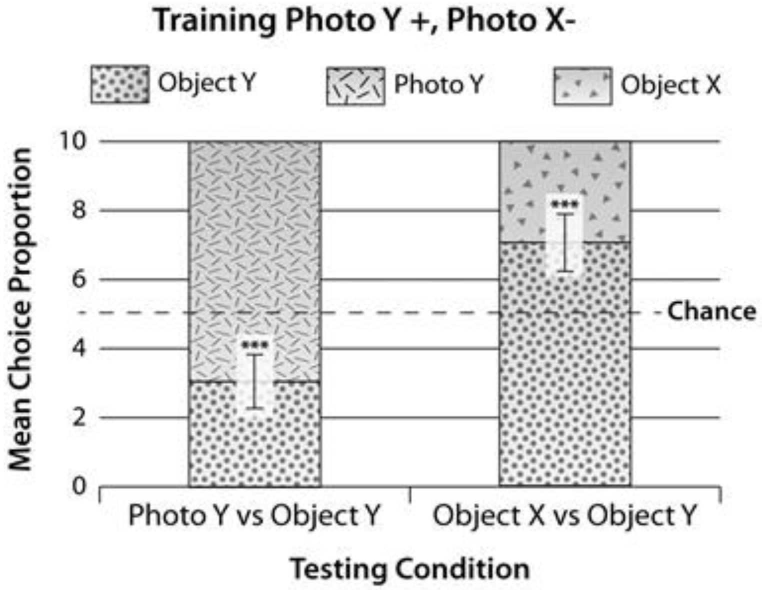


(b)

Figure 5. Choice proportions with standard error bars for tests shown in Table 2 for two groups trained to discriminate between two objects in Experiment 1. *** p <.001



(a)



(b)

Figure 6. Choice proportions with standard error bars for tests shown in Table 2 for two groups trained to discriminate between two photos of objects in Experiment 1. *** p <.001

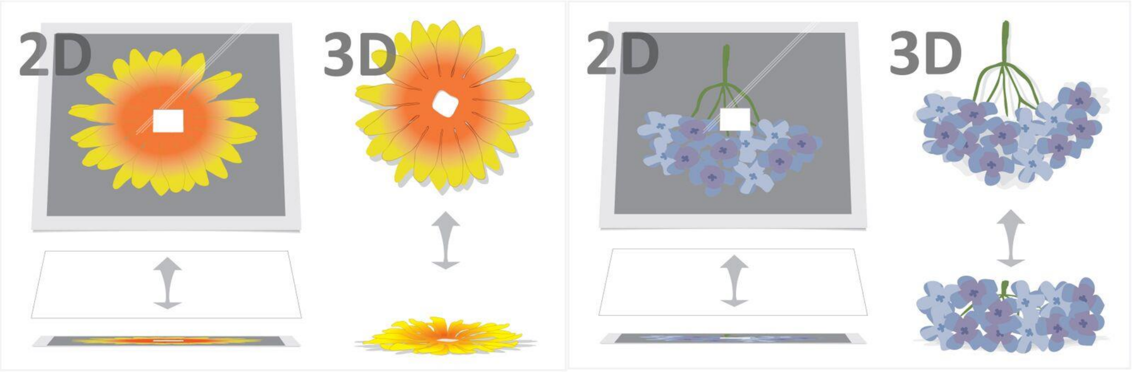


Figure 7. Images of Photo and Object X (on left) and Photo and Object Y (on right) for Experiments 2-4.

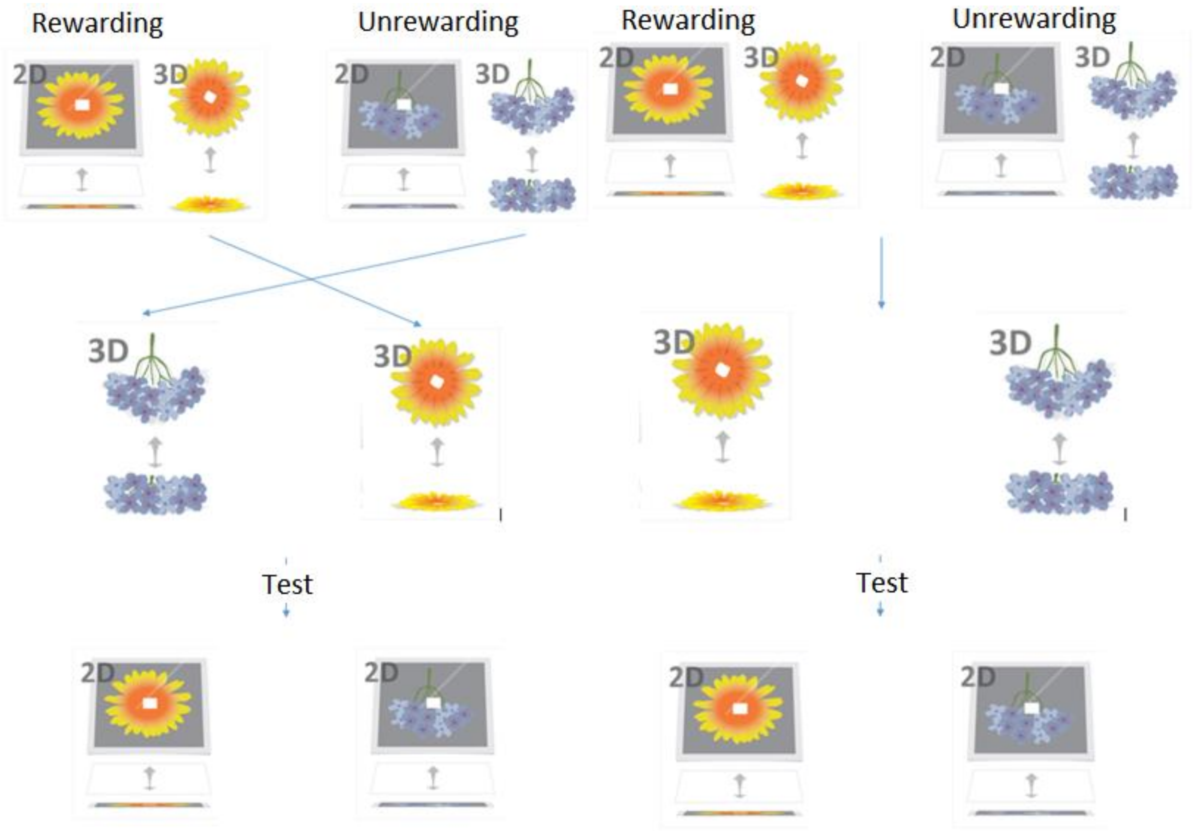


Figure 8. Example of a switched and non-switched condition with training on objects in Phase II using the design from Experiment 2

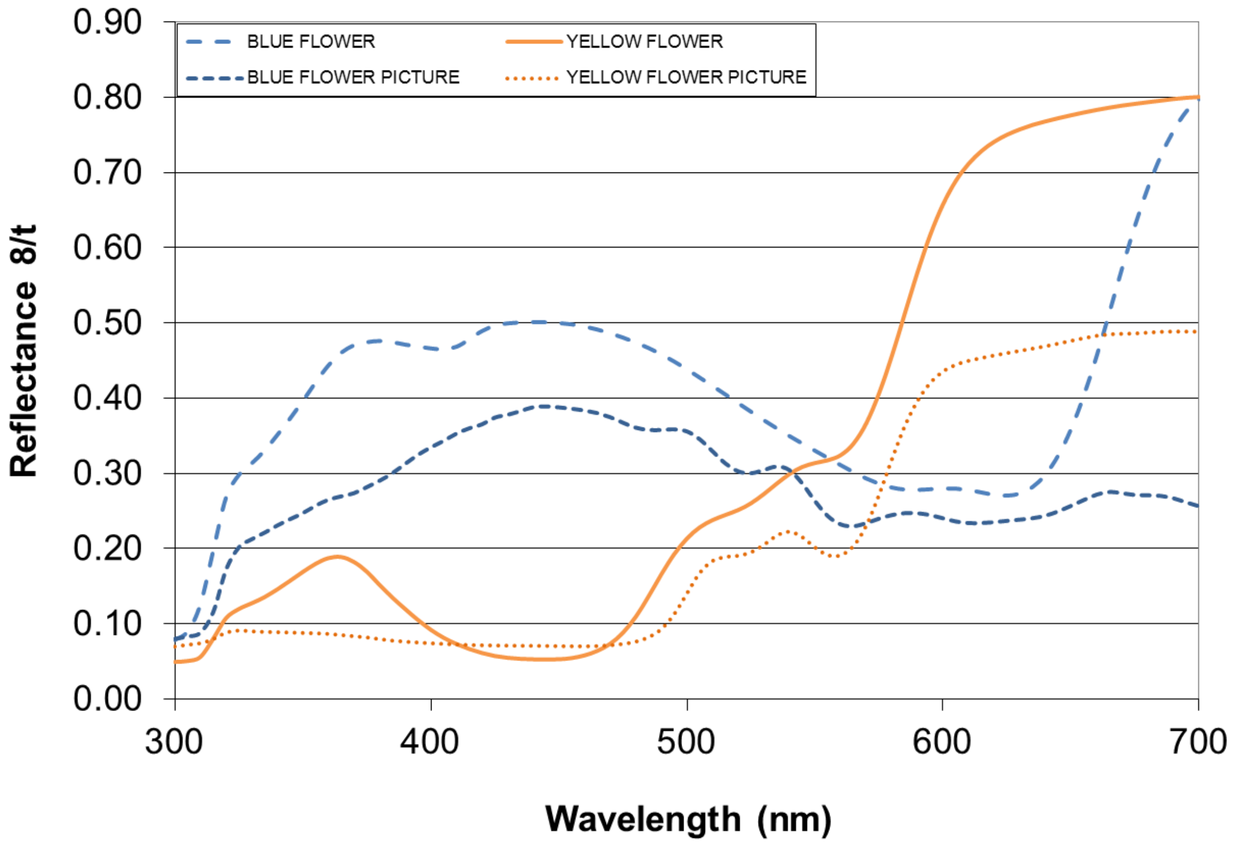
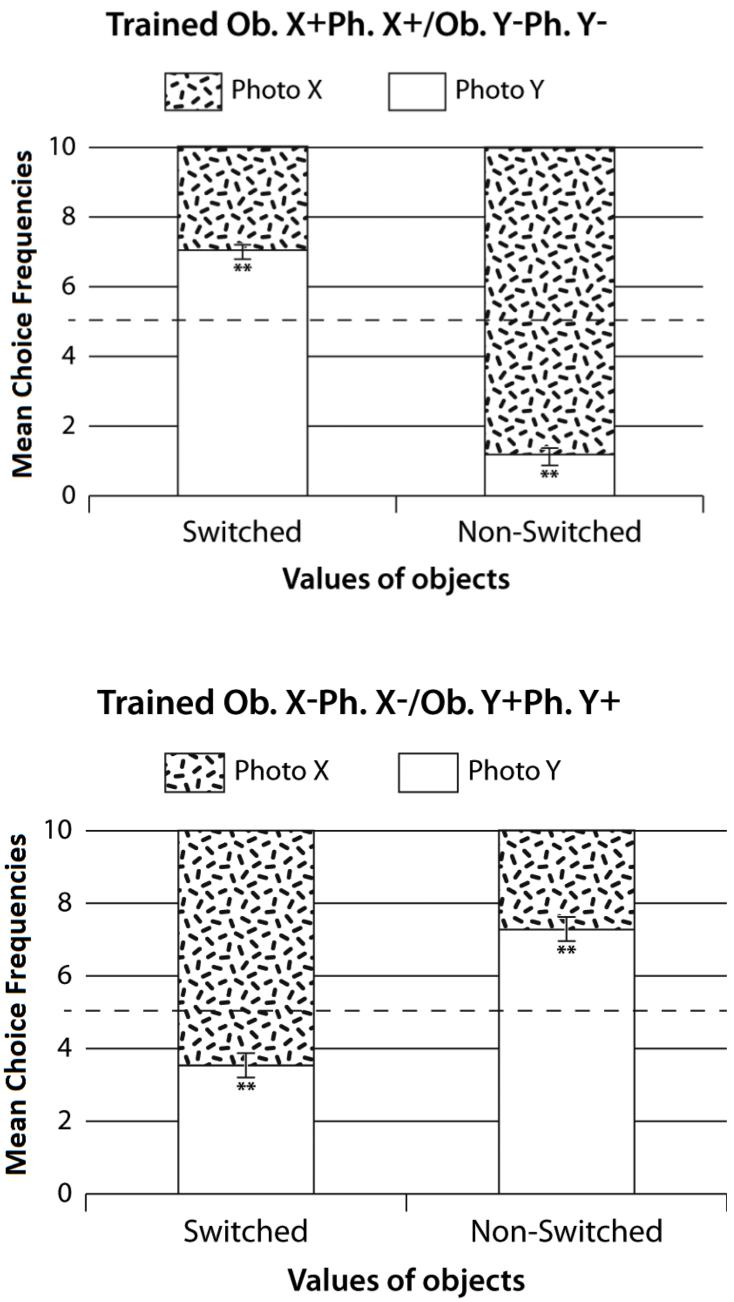


Figure 9. Total spectral diffuse reflectance factors (8°:t) for Yellow Flower and Yellow Flower Picture (Object and Photo X) and for Blue Flower and Blue Flower Picture (Object and Photo Y) in Experiments 2-4.



a)

b)

Figure 10. Mean frequencies, with standard error bars, of choices of photos in Experiment 2 after the corresponding objects have had the reward values that were experienced during initial training either switched or not. (a) Object and Photo X were initially rewarding while Object and Photo Y were unrewarding (b) the reverse contingency. The broken horizontal line indicates chance. ** the choice frequency deviates significantly from chance, $p < .001$

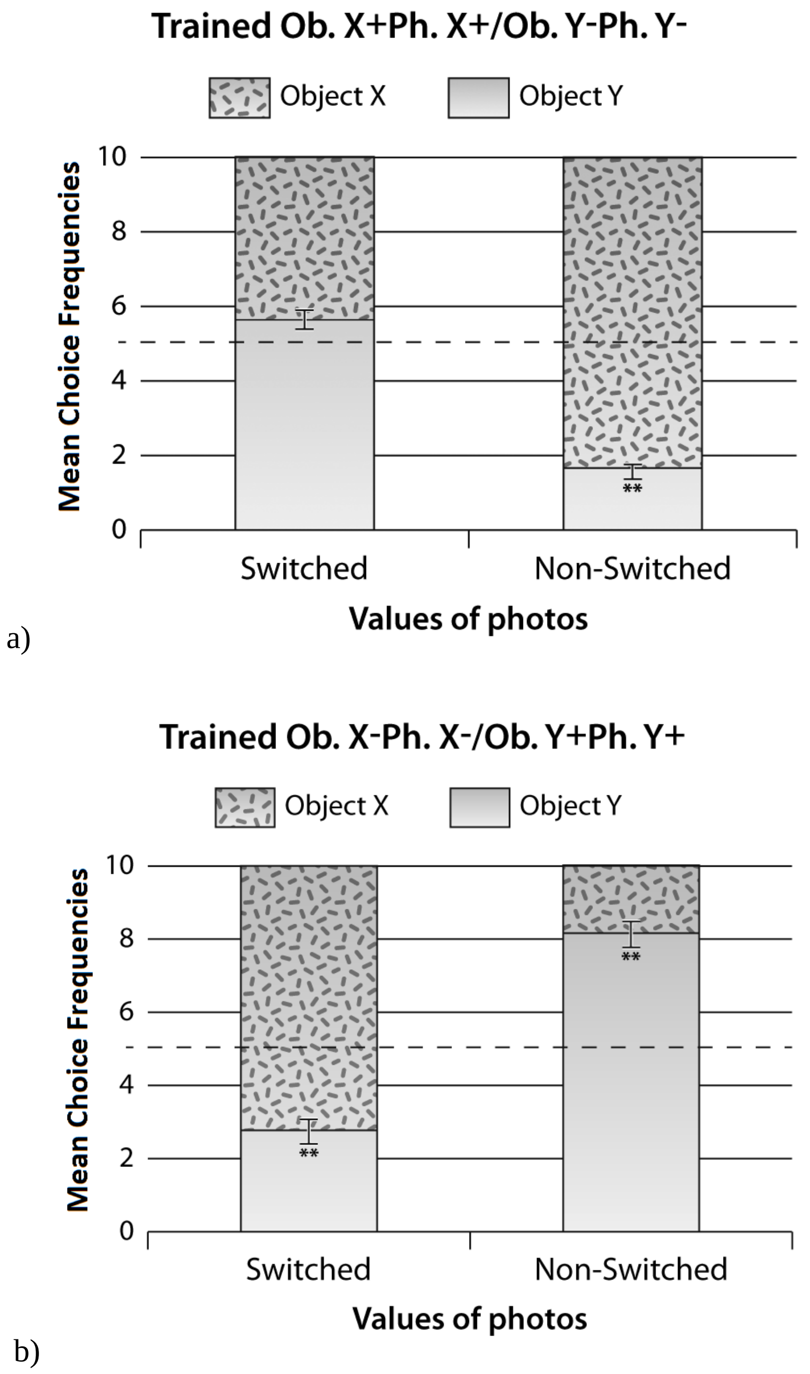


Figure 11. Mean frequencies, with standard error bars, of choices of objects in Experiment 2 after the corresponding photos have had the reward values that were experienced during initial training either switched or not. *(*a) Object and Photo X were initially rewarding while Object and Photo Y were unrewarding (b) the reverse contingency. The broken horizontal line indicates chance. ** the choice frequency deviates significantly from chance, $p < .001$

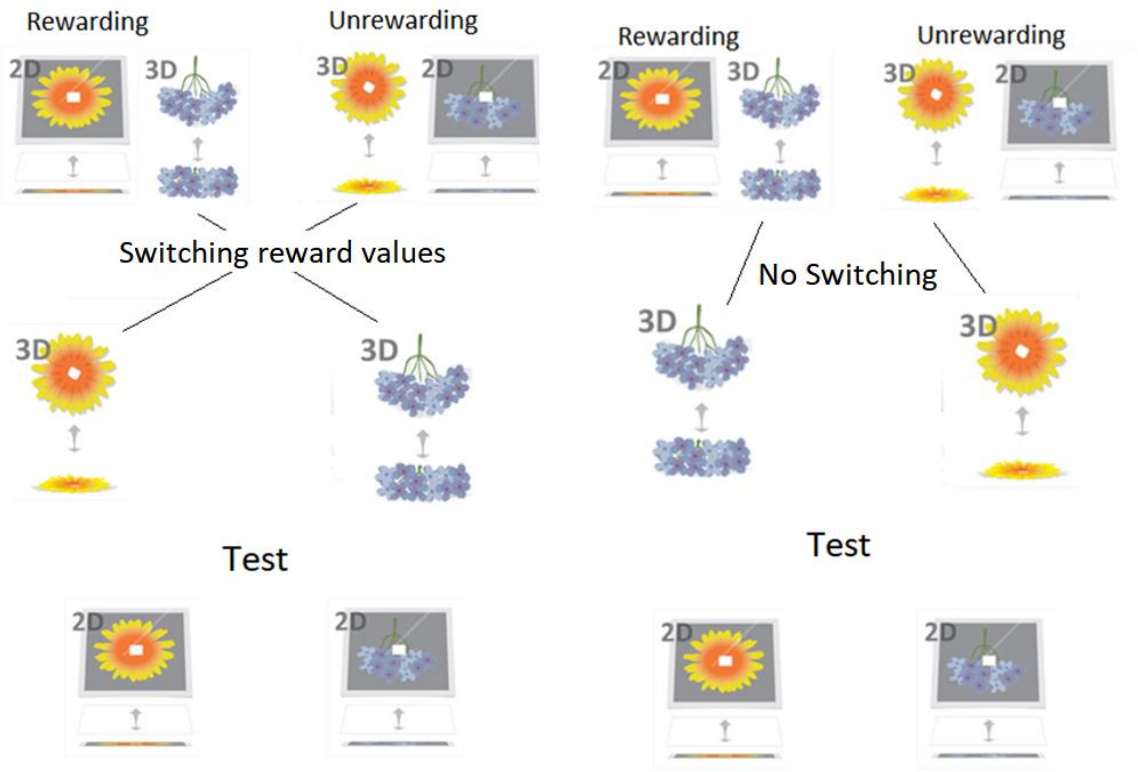
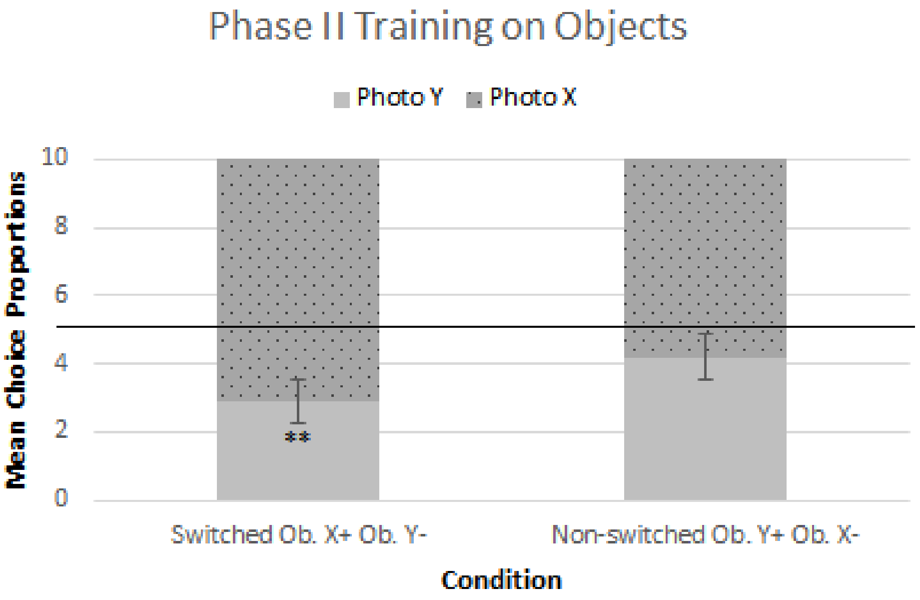
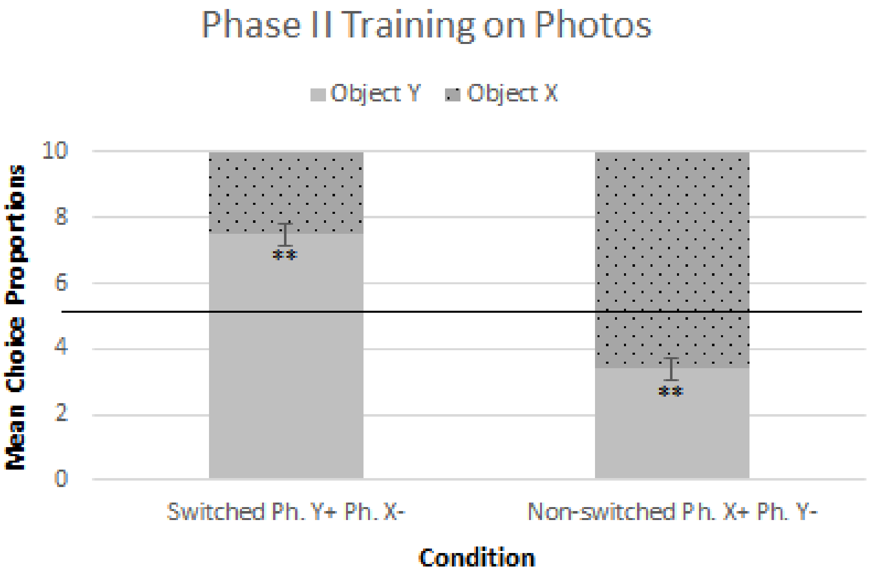


Figure 12. Example of a switched and non-switched condition with training on objects in Phase II using the design in Experiment 3



a)



b)

Figure 13. Mean frequencies, with standard error bars, of choices of photos and objects in Experiment 3, after the corresponding stimuli have had the reward values that were experienced during initial training either switched or not. Object Y and Photo X were initially rewarding while Object X and Photo Y were unrewarding for Phase I training. The horizontal line indicates chance. ** the choice frequency deviates significantly from chance, $p < .001$

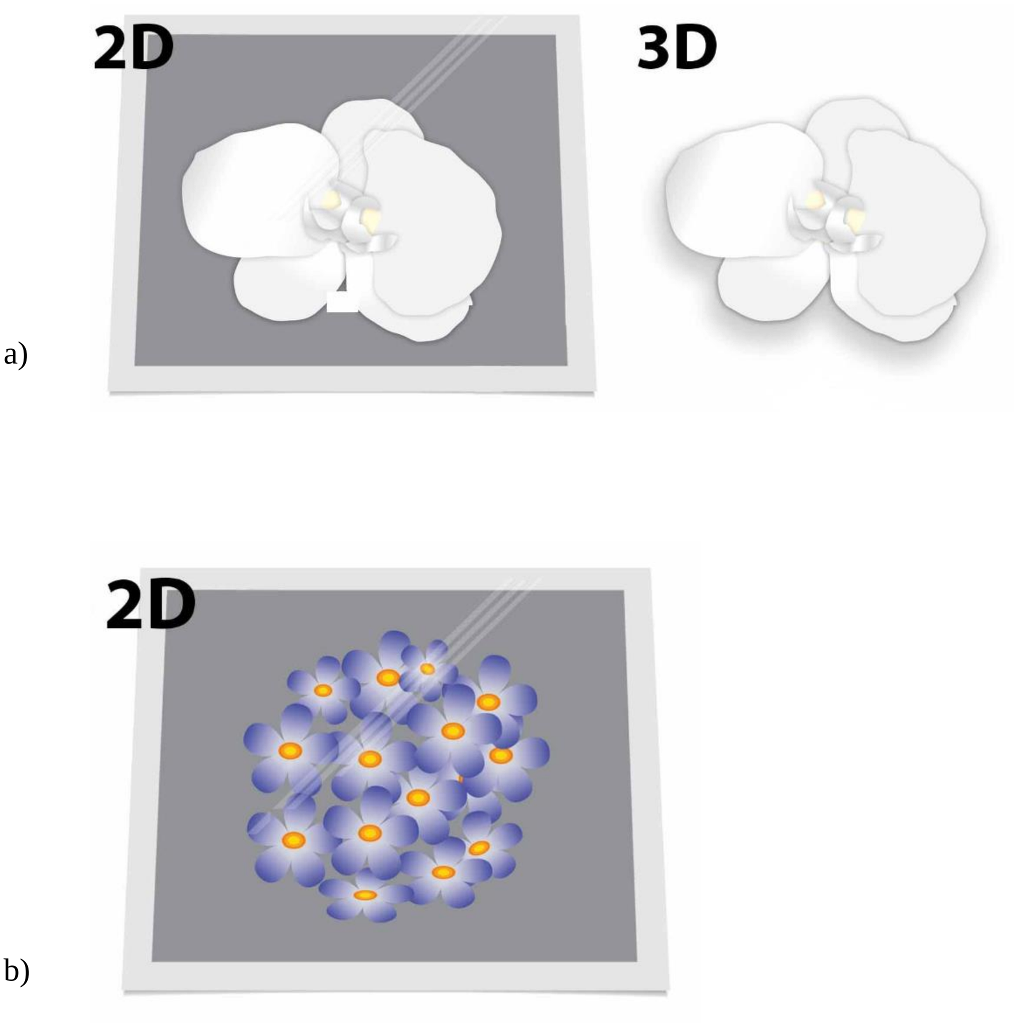


Figure 14. Images of Photo and Object Z (a) and Photo Y2 (b). Object and Photo X2 used in Experiment 4 are the same as Photo and Object X from Experiment 1.

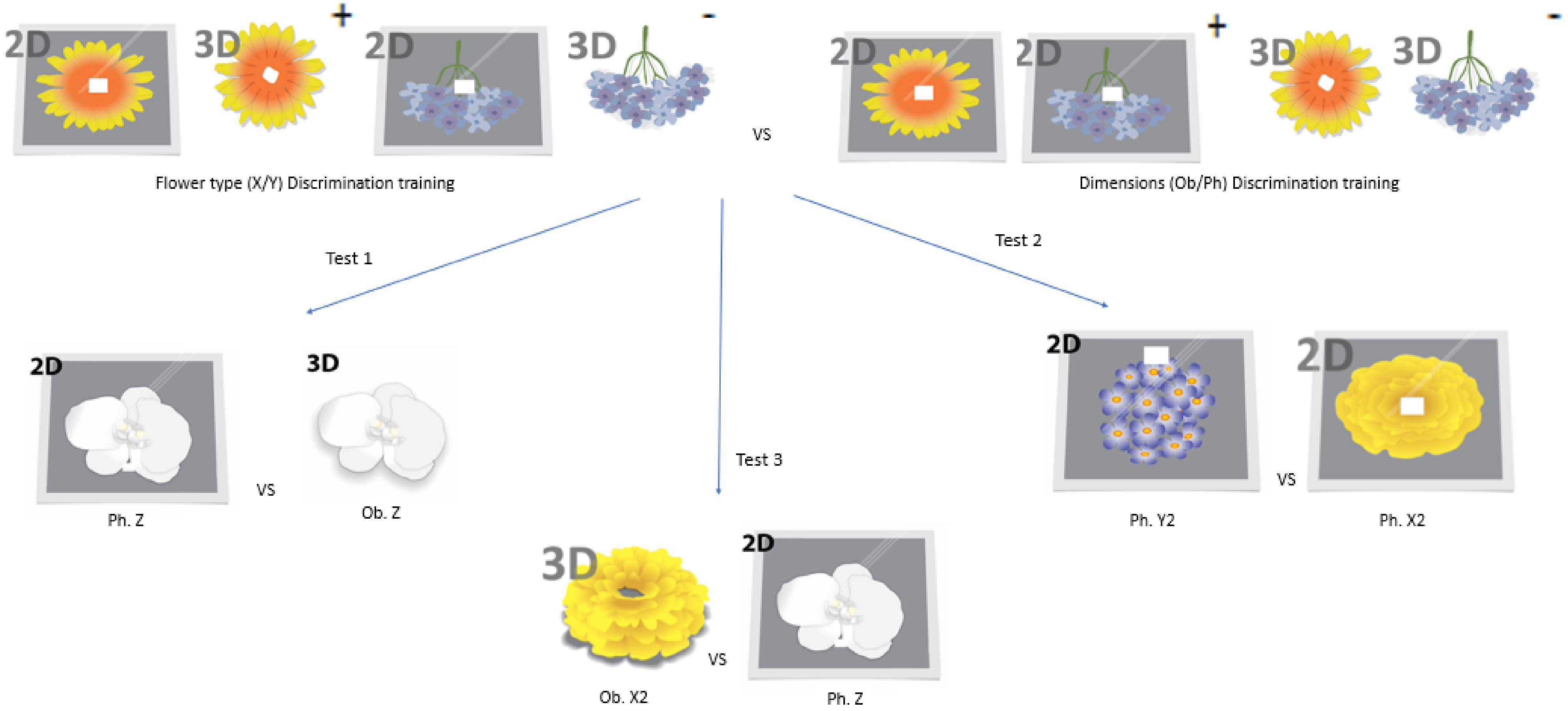


Figure 15. Example of design in Experiment 4 comparing the Ph+/Ob- to X+/Y- group.

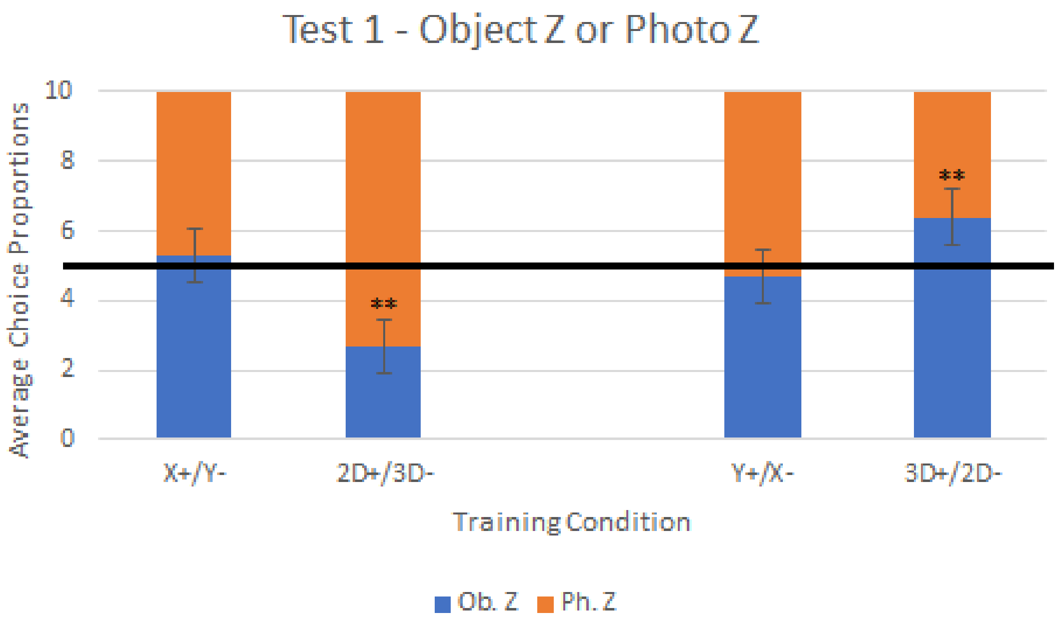


Figure 16. Mean frequencies, with standard error bars, of choices between photo and object Z following discrimination training on either X and Y or Objects and Photos in Experiment 4. The horizontal line indicates chance. ** the choice frequency deviates significantly from chance, $p < .001$

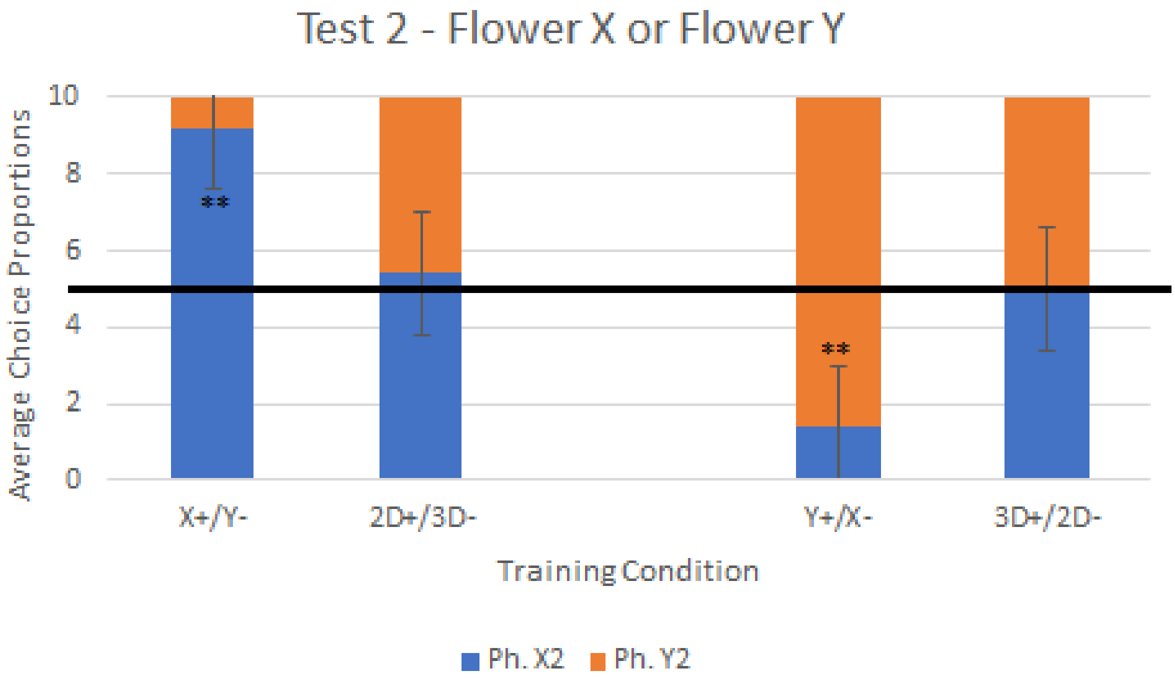


Figure 17. Mean frequencies, with standard error bars, of choices between X2 and Y2 after discrimination training with X vs Y or Photos vs Objects in Experiment 4. The horizontal line indicates chance. ** the choice frequency deviates significantly from chance, $p < .001$

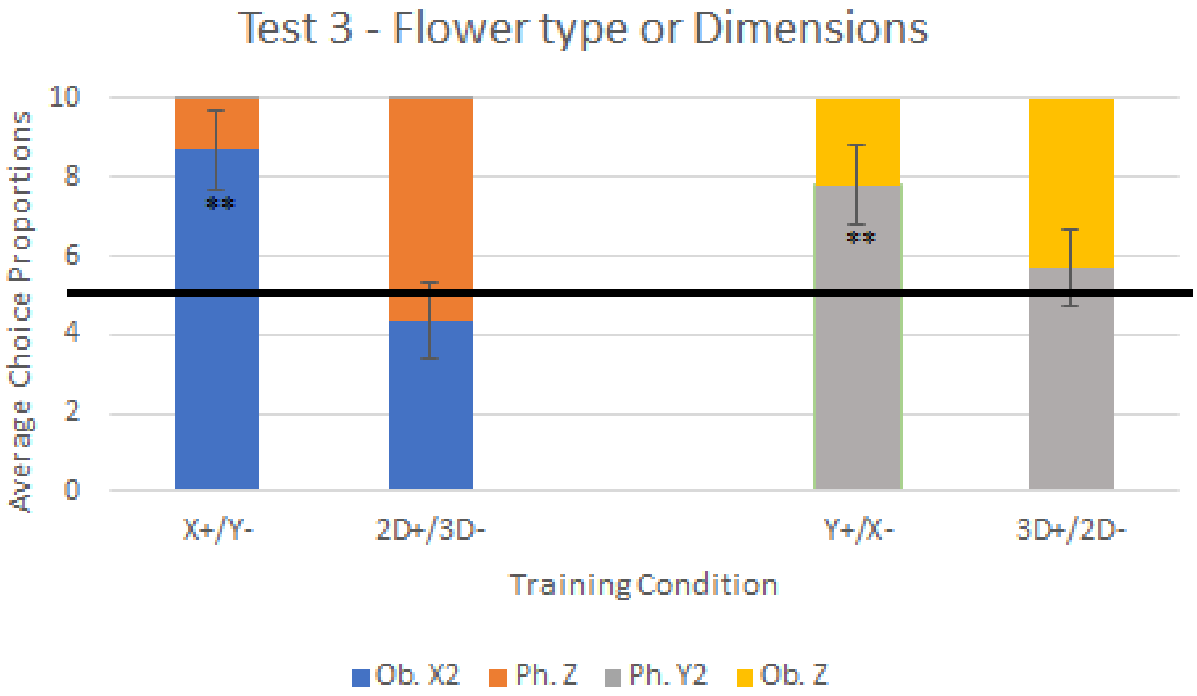


Figure 18. Mean frequencies, with standard error bars, of choices between Object X2 and Photo Z for the first two groups in the figure, or Photo Y2 and Object Z for the last two groups, after training with X vs Y or Photos vs Objects in Experiment 4. X2 and Y2 are depicted in blue, while Object and Photo Z are depicted in yellow. The horizontal line indicates chance. ** the choice frequency deviates significantly from chance, *p* < .001