Similar but different:

How foraging bumblebees (*Bombus impatiens*) treat flowers and pictures of flowers

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Abstract

Flowers, the sole natural source of pollen and nectar for bees, present many similar features, in colour, shape, size and scent, which facilitate pollinator attraction. This similarity among stimuli requires perception of commonality but also a capacity for differentiation between similar but different stimuli. While many flowers of a similar type will elicit approach and foraging, failure to access resources on any individual flower in an array (e.g. due to depletion) will not necessarily generalize and deter further foraging. Such conditions demand that bees respond to both the similarity and differences among stimuli which may share many common features but differ individually in available resources. Two questions are raised by this challenge and will herein be addressed: how do bees perceive and respond to 'similar but different' stimuli? And, how do bees use such cues to find rewarding flowers? Picture-object correspondence has not been previously specifically studied in invertebrates. The correspondence between picture-cue and object stimuli may offer a unique opportunity to trigger memory for corresponding targets while still retaining an important distinction between unrewarding cue and rewarding targets. Perception of pictures is not always perceived by animals as either the same as or equivalent to the intended subject. According to Fagot et al. (2000) the perceived relationship may result in *confusion*, *independence* or *equivalence* and is dependent upon experience. The objectives of this thesis are twofold: first, determine how bumblebees (*Bombus impatiens*) perceive the relationship between objects and corresponding pictures and secondly, to determine whether or not bees may be able to attend to and use pictures as cues while foraging. The correspondence of picture and object by bees was evaluated with four experiments of preference: (1) learned differentiation; spontaneous association to (2) colour, and (3) achromatic, impoverished images; and (4) learned picture cue use. Firstly, results show that bees do not confuse an object with a corresponding picture but nevertheless do perceive a relationship between them if colour cues are retained. Altered, achromatic images were not consistently treated as corresponding to coloured objects. Secondly, bees can learn to use a picture cue in a delayed matching foraging task. Results further suggest a role of three contributing factors in bumblebee picture cue use: (i) conditions of high inconsistency as to which target will be rewarding; (ii) stable target locations; and (iii) individual foraging experience. It appears that bumblebees can learn to use cues, in a delayed matching task, when the location of the corresponding target is known and stable, the individual bee has acquired some experience in successful foraging, and reward is otherwise unpredictable without the use of the cue. Bees may disregard secondary cues as noise under conditions of high target predictability whereby floral constancy or target perseveration may be most efficient, but attend to and learn such cues as signals if target reward is highly unpredictable. The conditions for this sensitivity may coincide with naturally occurring floral cycles.

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Overview

Cognitive ecology emphasizes the role of fundamental processes in animal cognition and behaviour in understanding and influencing environmental systems (Chittka & Thomson, 2001; Dukas, 1998 & 2009). A central challenge facing bumblebees is the perception of diverse floral stimuli as being both similar and different. Bees are often faced with a challenge of foraging amongst large groupings of very similar stimuli, such as the abundant spring bloom of fruit trees in an orchard or vast expanses of lavender. While the stimuli that make up these complex environments appear very similar they individually differ in available reward. Among these stimuli, some may offer plentiful reward and some may be depleted. Foraging bees face the challenge of perceiving both the common features of ‘floral’ stimuli and the differences between individual flowers and between types of flower.

Although flowers offer multiple and highly salient primary cues, or signals, in colour, size and shape, cues separated from the flower can aide choice from greater distances. Secondary cues can provide information about the target but, unlike primary cues, are in some way separate from the target, by physical location or time. Airborne scent offers one of the most salient secondary cues for foraging bees signalling approach to a flower before it can be perceived visually (Riffell, 2011). Additionally, some flowers offer hidden rewards, provisioning outward, visible cues (usually highly salient petals and petal patterning) corresponding to nectar located out of sight, deeper within the flower. Such a mechanism encourages pollinators to move across pollen on anthers to access nectar (Heinrich, 2004). In order for this adaptation to be successful bees must recognize that the visible cues they ‘see’ correspond to a reward available once they enter the flower. These types of secondary cues present a complementary challenge to the 'similar but different' problem, a challenge to perceive cues which may be different from the target but are still predictive of associated reward.

Pollinator-plant relationships are central to both the sustainability of many natural ecosystems and human agriculture. The impacts of human activity on both wild and domesticated colonies of bees are broad, including specific and direct habitat destruction, resource contamination and climate change. Bee populations are declining due to a complex interaction of resource and habitat depletion, range compression due to climate change, parasites and pathogens, fungicides and insecticides (Goulson et al., 2015; Kerr et al., 2015). Challenges with relatively sudden onset are not easily mitigated by species adaptation. Rising temperatures may be particularly harmful to bumblebees as they do not exhibit the compensatory northern migration observed among other insects (Kerr et al., 2015). Furthermore, neither honeybees nor bumblebees appear to avoid resources contaminated with neonicotinoids, a harmful and widely used insecticide, and in fact prefer contaminated over non-contaminated resources (Kessler et al., 2015). Many of the most harmful contributors to bee population decline result from modern agricultural practices: primarily the use of large areas of land for single crops, often in bloom for only a short period of time each year, with a heavy reliance on insecticides and other chemicals.

Agriculturally, supplying for a large inter-dependent human population has led to large scale monoculture farming with a heavy reliance on transitory commercial honeybees to pollinate many crops, such as clover, sunflowers, squash and many types of nut and fruit trees. Such practices are recognized as detrimental not only to bees, both commercial and wild (such as native bumblebees), but also the environment and consumer health (Woteki, 2013). Recent initiatives prioritize alternative methods of farming with the potential for pollination by resident, native bees, such as bumblebees, rather than transitory commercial honeybees. Increasing diversity of crops within a farm, and establishing bordering areas of wildflower or weed patches to provide alternative sources of food between crop blooms could facilitate a shift in pollinator reliance to resident colonies of native bees (Nicholls & Altieri, 2013). Although more environmentally sustainable and consistent with principles of local farming and consumption, the practicality of these methods requires that resident, native bees pollinate with a level of efficiency comparable to that of commercial honeybee colonies. Pollination of multiple, relatively small crops is better suited to bumblebee patterns of foraging behaviour, but less reliable than the more uniform patterns displayed by large colonies of honeybees. Honeybees display foraging behaviour traditionally preferable in agriculture, being inclined to visit only one flower type at a time as opposed to the more varied patterns of bumblebees (Heinrich, 2004).

The success of commercial colony pollination relies on a system of transporting very large populations of honeybees between farms as crops come into bloom and are therefore viable for pollination. The size of crops in large, industrial farming and the use of herbicides and other methods to minimize the presence of non-crop plants within farmland, increase the likelihood that commercial hives will forage exclusively on the intended crop, maximizing pollination. Alternative methods of small scale and diverse crop farming reliant on resident native bees, such as bumblebees, face unique challenges (Nicholls & Altieri, 2013). Firstly, resident colonies require resources throughout a season and not just at short periods of single crop bloom. Secondly, farmers must be able to ensure that multiple crops are pollinated reliably when blooming.

These problems, both the survival of native bee species and efficiency in agriculture, being dependent upon behaviour, could benefit from a cognitive-behavioural solution. The facilitation of foraging and movement in changing habitats and efficiency of pollination for agricultural purposes may be improved with some manipulation of naturally occurring foraging behaviour dependent on specific cognitive process of bee attention, memory, perception and learning. Potential for directed foraging may be found in the extent to which invertebrates show "picture-object correspondence" (treat representations of flowers as flowers), and can learn to use cues while foraging.

Pictures could provide a unique method of cued foraging, provisioning a cue which triggers memory for the corresponding object but limiting avoidance to a matching target following experience with an unrewarding cue. If associative cues, including pictures, can be used to direct foraging activity, difficulties currently undermining bee foraging and pollination may be diminished. First, it must be determined how bees perceive the correspondence between an object and picture. Secondly, can bumblebees learn to use a picture cue while foraging?

Foraging Behaviour: Environmental variability and complex targets

Highly social species of bees must maintain a high rate of foraging to acquire the significant supply of nectar and pollen required by an interdependent colony (Ydenberg, 1998). The obstacles to successful foraging by correctly identifying a target include, but are not limited to: significant colour, size, shape, scent and location variation; depletion, replenishment, deception (or mimicry); obstruction (by various other plants and natural features), growth, death and destruction (Benard, Stach, & Giurfa, 2006; Cnaani, Thomson, & Papaj, 2006; Raguso, 2004). As the relationship between pollinator and flower is often (but not always) reciprocal, a variety of signal mechanisms have evolved to increase the likelihood of mutual success, pollination for the flower and consumption for the bee (Heinrich, 2004).

The vast majority of flowers provide multiple, redundant identifying signals such as complex visual display, scent, time of bloom, taste and many more. The ‘efficacy backup’ hypothesis posits that multiple signals provided by the flower may prevent missed opportunity when foragers mistake signal for noise due to high variability (Cnaani et al., 2006; Kaczorowski, Leonard, Dornhaus, & Papaj, 2012; Raguso, 2004). It has also been suggested that multiple signals may act to ‘monopolize’ working memory in the bee, leading to higher than expected species fidelity due to limited capacity to memorize alternate targets (Leonard, Dornhaus, & Papaj, 2011). Although some studies point to a higher benefit to plant over pollinator, multiple cues nevertheless influence behavior (Kaczorowski et al., 2012). Multiple as compared with single cue stimuli have been found to reduce uncertainty, increase learning speed and provide serial priming cues, such as scent from a distance and visual guides at close range (Kulahci, Dornhaus, & Papaj, 2008; Leonard et al., 2011; Raguso, 2004). Additional cues can be used selectively to improve foraging performance under increasingly complex conditions, as Reinhard et al. (2006) found when bees were unable to use scent memory in foraging among more than two flower stimuli unless a colour discrimination was introduced. In general multiple cues provide a highly adaptive contingency to counter natural variation and ambiguity (Cnaani et al., 2006; Kaczorowski et al., 2012; Kulachi et al., 2008; Leonard et al., 2011). Although multiple cues increase the likelihood of signalling a potential pollinator, they also increase the differences which may be perceived among different types of flower. In other words, multiple cues may increase approach if those cues are perceived as similar to ‘known’ floral features or decrease approach if the features are unfamiliar and perceived as different from ‘known’ floral features.

Most complex or multiple cue floral displays correspond to target features (such as flower colour and shape) but one very prominent cue, airborne scent, offers a secondary cue that is separate from the target and detectable before the target itself is visible. This secondary cue can elicit foraging behaviour or prime specific target choice, including prompting specific foraging by nest mates after a scent is carried into the hive by another returning forager (Leonard et al., 2011). Dornhaus and Chittka (1999) suggest that the behaviour of returning foragers carrying floral scent reflects a simplistic form of communication, mirroring many of the excitatory actions, such as physical contact with nest mates, wing vibration and repetitive running, which comprise the waggle dance language of honeybees. Bumblebees are more likely to forage from the species of flower corresponding to the scent carried by the returning forager though no information about the location of the flower type is conveyed (Dornhaus & Chittka, 1999). Scent is a strong, naturally occurring cue which can influence bee foraging behaviour and target selection, and may suggest that other secondary cues may likewise be sufficient to elicit specific directed foraging.

Associative cues may be either general or specific. General cues, such as foliage being associated with flowers, do not correspond to any one stimulus but rather reflect a general trend whereby two or more types of stimuli are commonly associated. General cues can be learned and can be generalized to novel encounters (e.g. leaves in an area never before visited). Specific cues are always learned and reflect a correspondence or association formed between two or more specific stimuli based on experience and memory. Specific cues may serve as 'landmark' cues, a learned association between a stimulus (or stimuli) and a particular location or route (Collett 2005; Sherry & Strang, 2014; Zhang et al., 1999). Such cues would represent a learned secondary cue, dependent upon experience pairing the cue and target in memory.

Bees differentially exhibit various strategies of foraging behaviour, according to both environmental and colony resource conditions (Cartar & Dill, 1990; Weinberg & Plowright, 2006). Varying conditions are likely to favour different strategies of efficient foraging: perseveration, repeated approach to the same or similar targets, under conditions of consistent target reward; or flexibility and sampling, shifting attention and choice patterns among different types of targets, if a consistent resource is not found or begins to decrease (as a flower species transitions out of bloom). Efficient response to complex environmental factors which affect bee behaviour depend on cognitive processes of attention, memory and learning, as described in the following section.

Cognition in Bees: Attention, memory and learning

Floral constancy, the overrepresentation of a flower type during foraging to the exclusion of alternative resources, in bees has been discussed from various viewpoints (see Chittka, Thomson & Waser, 1999; Raine & Chittka, 2005; Raine & Chittka, 2007; Rossi et al., 2015) often centred on memory function or energy efficient behavior. Some propose that bees display floral constancy due to limited memory storage and retrieval for identifying features (Geagar, 2005). Chittka et al. (1999) emphasize efficient strategy over cognitive limitations, and the improved ability to identify and handle a single type of flower at a time as opposed to multiple types of flowers. Additionally, it may be beneficial to express behavioural floral constancy but simultaneously gather information about alternative potential resources (i.e. sampling, attention to and retention of information corresponding to multiple stimuli). Propositions of efficiency in floral constancy highlight the high cost which can be associated with highly variable and complex environments (many potential floral resources but not all are rewarding) (Chittka et al., 1999).

Memory constraints factor into the challenges associated with foraging, but emphasizing efficiency can better account for instances where constancy is not observed and explain why bees can learn cues which are otherwise redundant for ‘constant’ foraging. Learning multiple cues presented by the same flower independently would be unnecessary when foraging from that flower but useful when shifting between flower types which may share some but not all features (Cartar & Dill, 1990; Dyer, 2012; Ings, Wang, & Chittka, 2012). Bees also exhibit a preference for site constancy when foraging (Osborne & Williams, 2001) but despite the apparent behavioural rigidity of site and floral constancy, bees are capable of flexible foraging or sampling. Cartar and Dill (1990) found that bumblebees express both risk averse and risk prone foraging behaviour under different colony conditions: bees were more likely to forage from a highly rewarding but inconsistent patch when colony resources were strained but forage from a less rewarding but consistent patch when colony resources were plentiful. This flexibility in foraging behaviour benefits the bee in navigating a natural environment full of noise.

A fundamental cognitive challenge faced by all animals is how to most efficiently filter perceptual stimuli encountered in the natural environment. The relevance of any given type of incoming information varies according to, among other factors, motivation (e.g. foraging, escaping predation, returning to the hive etc.) and experience (e.g. past experience of reward). Animals differentially switch between strategies of selective attention, which focus activity but risk too narrow a search, or sampling strategies, which expand opportunities but risk too broad a search (Kamil & Bond, 2006). Each strategy may be more or less efficient under different conditions. Selective attention decreases latency in identifying a desired target, increases performance on difficult tasks related to acquisition (such as handling to acquire nectar) and lessens cognitive demand including memory resources recognizing identifying features (Dukas, 2002; Kamil & Bond, 2006). However, sampling behaviour outperforms selective strategies once a target type is depleted (by over predation or natural cycles) and new potential targets must be evaluated (Kamil & Bond, 2006). Successful foraging usually requires transition between these two strategies as environmental conditions favour either constancy, with plentiful, reliable reward, or flexibility, if reward becomes scarce or unpredictable.

Selective or filtered attention functions in part by processes of working memory, sorting the relevancy and retention of incoming perceptual stimuli by what is already known or experienced, long term memory (Dukas, 1998). The tendency to respond to limited cues or stimuli does not necessarily equate to actual memory constraints, but a ‘best case’ response which may vary according to environmental conditions. For example, bees can learn to navigate by both landmark memory and route memory but will disregard landmark memory unless the preferred reliance on route memory fails (Menzel, 2001). Although strategies of selective foraging and constancy may often be preferred, establishing a pattern of foraging does require multiple integrated, complementary experiences (Dukas & Real, 1993). Comparatively, memory in the bee in many ways resembles that of other animals: repeated experiences facilitate memory consolidation resulting in memories of differing strength and longevity (Menzel, 2001). Experience and memory form the basis for expressed foraging behaviour, evaluating encountered stimuli by what is known or not known about the target, whether or not a reliable target resource has been repeatedly encountered and, the state of the colony (size, reserves etc.).

Generalization is an adaptive cognitive method of integrating past experience with novel stimuli, enabling evaluation of a new stimulus according to similarities it may share with stimuli previously encountered. Flexible responding to common features or cues allows for a shift in reliance from more salient, highly predictive elements (such as target colour and shape) to less salient but still potentially informative cues (such as likely location) when preferred cues are unavailable, altered or otherwise ambiguous (Dyer, 2012; Fauria et al., 2002). Generalization among common features primarily refers to target cues (common elements of colour, shape, size) which may allow a forager to recognize potentially rewarding targets among a wide variety of stimuli but can extend to secondary information such as context or location (Dale, Harland, Manning-Jones, & Collett, 2005). Although the relative strength of learned, associative cues is likely to be overshadowed by common target cues, variable conditions may alter expected learning patterns. In other words, bees may be more likely to ignore secondary cues such as foliage if flowers themselves are plentiful and easily recognized, such as would be the case in large flowering patches, but attend to foliage when flowers are sparse or difficult to find, such as rocky or sandy environments.

The experimental study of bee cognition and behaviour tests limits of flexibility and learning. Past assumptions of inflexible, innate behaviour patterns among insects are being increasingly challenged (Dukas, 2008). Bees have been found to express latent knowledge of cues usually disregarded, most notably by learning a discrimination based upon illumination. Illumination is most often discounted among animals, including the bee, as not predictive due to frequently changing light conditions, in favour of colour constancy (Clarke & Lotto, 2009; Lotto & Chittka, 2005). It is suggested that learning illumination cues, even if such information is most often discounted in favour of more reliable cues, may provide a benefit due to the high degree of natural ambiguity (Lotto & Chittka, 2005). Not only does this illustrate that the bees are able to learn and use a cue which is ordinarily disadvantageous, but will disregard target cues (the actual colour and location of the rewarding stimuli) if these are unreliable and instead use learned cues of illumination.

Further examples of complex learning in bees can be found in use of contextual and configural cues. Bees exhibit a learned response to concepts of ‘same and different’ (Brown & Sayde, 2013; Gould 1988) and even express this knowledge in novel cross modal tests when trained to a visual ‘same-different’ task but tested to an olfactory discrimination (Giurfa, Zhang, Jenett, Menzel, & Srinivasan, 2001; Giurfa, 2013). Bumblebees are also able to learn that two stimuli are alternately rewarding according to location (Dale et al., 2005). Interference effects can limit the capacity of bees to respond to multiple tasks if the required response is similar (Cheng & Wignall, 2005). However, bees have also been found to overcome interference, one element being common both to positive and negative stimuli, provided there are multiple, configural global cues that differentiate the two (Fauria et al., 2002). Additionally, honeybees master non-elemental learning, separating cues presented in isolation as opposed to being presented in combination (Giurfa, 2007). The capacity of bees to learn and respond to complex cues and context while foraging has been well established but is often only elicited under high experimental control.

When faced with conditions or tasks of particular difficulty, bees have been found to implement strategies of ‘top-down’ processing or benefit from facilitated learning, whereby a task presented to the bee without prior, gradual training, is not solved but can be following training to a parallel but simplified version of the task, in other words an “easy to hard” effect. This ‘easy to hard effect’ also highlights the need to test not just spontaneous response to task or environmental stimuli but also learned response. Zhang and Srinivasan (1994) found that honeybees were incapable of initially recognizing and responding to camouflaged patterns but following facilitated pre-training to the non-camouflaged equivalent patterns were later able to perform the camouflaged detection task. Honeybees have also been found to learn to associate various novel views of a stimulus but again only following gradual training (Dyer & Vuong, 2008). Bumblebees require facilitated learning in a rotated pattern discrimination (Perreault & Plowright, 2009) and gradual training for complex problem solving in the movement of a physical obstruction (Mirwan & Kevan, 2015).

The practical application of cognitive-behavioural bee research to agricultural practice and ecological projects promoting sustainability, including facilitated or directed foraging, requires that results obtained in highly controlled laboratory settings be replicable under low control field settings with minimal and easily implemented training procedures. Fundamental principles of learning and conditioning can illuminate why such replication is difficult under natural conditions. The strength of target cues (flower colour, shape etc.) is likely to be such that secondary cue learning (e.g. to a picture cue) is difficult to establish (Rescorla & Wagner, 1972). However, cues may be more likely to be attended to and learned under conditions of high unpredictability and the ‘surprise’ of inconsistent reward (Kamin, 1969). Repeated visits to a consistently rewarding target comprised of highly salient cues (colour, shape) increase the strength of the target and decrease the potential to learn a secondary cue. Even if a secondary cue is learned, perseveration is likely to continue despite cue change (i.e. the secondary cue association, if learned, is relatively weak and cannot override the strength of the target cue). However, as consistency of reward paired with a target decreases so does the strength and thereby the potential learning of a secondary cue and the comparative strength of this association may increase. For example, if a large patch of purple flowers, thyme and lavender, are both rewarding a bee may disregard the scent of lavender and rely heavily upon the highly predictable and salient cue of colour. However, if thyme flowers stop producing nectar and pollen before lavender does, the bee may learn that purple has become an unreliable cue but the scent of lavender is highly predictive of reward. The associated strength of the cue of colour will decrease and the strength of the cue of scent will increase. Regarding multimodal signal integration, Munoz and Blumstein (2012) theorize that multisensory information may be processed and elicit behaviour differentially under variable conditions including environmental uncertainty whereby costs and benefits of time and energy may correspond to wasted time attending to alternative signals when uncertainty is low but vice versa when uncertainty is high.

These fundamental principles of conditioning for secondary cues appear, intuitively, to coincide with natural periods of transition in plant blooming cycles and bee foraging behaviour. Bees may exhibit high floral constancy when a single plant species offers consistent and plentiful reward but shift foraging strategy to one of sampling and increased attention and learning for alternate signals when a food source is depleted or no one consistent source is available. Timing of cue presentation with the natural depletion of one crop and blooming of another (or supplementary wild food source) may allow for directed foraging by coinciding cue presentation with natural tendencies to shift foraging strategy based on changing experience.

Effective cueing in directed foraging requires a type of cue that can trigger memory for a corresponding target but remain easily discriminated from the target, lessening the risk that an unrewarding cue will lead to avoidance of the ‘matching’ target. Picture cues, if spontaneously perceived by a subject as equivalent, offer a unique opportunity to pair a cue perceived as ‘similar but different’.

Picture-Object Correspondence: Comparative

Pictures or images are frequently presented in studies of pattern recognition in humans and other animals in place of actual corresponding objects. This substitution can facilitate ease of presentation, changes to stimuli and control of features (Watanabe, 2000). However, perception of images as equivalent to the corresponding object is complex. Study of picture-object correspondence in invertebrates has yet to be specifically investigated, with the exception of video image predation response among spiders (Clark & Uetz, 1990), even though the use of two dimensional patterned stimuli are frequently used to study perception and cognition among bees and other insects. Furthermore, images and corresponding objects offer a more general opportunity to investigate perceptions of how animals respond to stimuli that share some but not all features, including tests of novel views (Spetch & Friedman, 2006). Extensive vertebrate study, primarily with birds and mammals, has established a framework for how pictures may be perceived and corresponded to objects. Fagot, Martin-Malivel and Depy (2000) suggest that pictures may be perceived with *confusion*- failure to differentiate an object from the corresponding image (for example attempting to eat an image of a food item); *independence*- differentiation between the two but no perceived relation; or *equivalence*- the ability to differentiate but also intuit some relationship, either based on shared featural qualities (such as colour or shape) or a learned symbolic association (Fagot et al., 2000; Leighty, Menzel, & Fragaszy, 2008). Furthermore, pictures may be viewed by either a naïve subject, and thereby reliant on spontaneous recognition, or by an experienced subject, with acquired knowledge (Bovet & Vauclair, 2000). Not only will the experience of the subject with pictures influence perception but also experience with the intended subject matter (Watanabe, 2000). In summary, response to, and perception of, a picture is known to vary by degree of differentiation (confusion, independence or equivalence) and experience (spontaneous or learned). This complex interaction is most often disregarded in experimentation, in which confusion or equivalence is often implicitly assumed and results based on response to images are generalized by researchers to expected responses to naturally occurring stimuli.

Correspondence between an image and object as either *confusion* or *featural equivalence* represents a form of generalization not unexpected among many animal species. Most animals face the challenge of high natural variability in both potential reward and threat. The ability to generalize among stimuli that share some common features is expected and despite a loss of depth information specifically, picture-object combinations share many features. However, perception of image content is uniquely complicated by an indirect and unpredictable relationship between realism and recognition. The apparent realism of high quality colour photographs does not necessarily correspond to ease of recognition. Among humans, often an image lacking realism but reflective of key elements associated with the subject is most easily recognized (see Deregowski, 2000). The use of colour photograph presentation to non-human animals is even more subject to misinterpretation as the colour correspondence most often reflects human visual perception and not that of the animal of study (Delius, Emmerton, Horster, Jager, & Ostheim, 2000). Some studies, particularly those with pigeons, have shown better picture-object correspondence with black and white rather than colour images and with fewer or simplified representational elements, such as transfer to silhouette images (Bovet & Vauclair, 2000; Delius et al., 2000). However, the relationship between recognition and amount of detail or feature exaggeration is not clear. Drawn images, although they appear to represent key features of the subject matter to experienced human observers, are often not easily perceived or learned by animal subjects (Delius et al., 2000; Watanabe, 2000). Furthermore, the reduction and recombining of features (such as colour or configuration) have failed to lead to the discovery of any consistent key component elements in recognition (Cook, Katz, & Kelly, 2000). Such results are in keeping with the highly flexible nature of generalization among animals in the identification of stimuli despite great variety and apparent inconsistency among common features, especially when faced with forced choice.

Generalization among various stimuli by animals is common but a 2D image corresponding to an object is relatively unnatural with the exception of water reflections and cast shadows (Casati, 2008). Interestingly, parallels may be seen between object-shadow and object-picture correspondence problems. Although used rarely, shadows are not dismissed as visual noise but believed to be primarily processed at a low, coarse level of object to shadow matching (Dee & Santos, 2011). Mammasian (2004) has identified some of the challenges in the shadow-object correspondence problem: correspondence is often uncertain, obstruction and deformation of the shadow is frequent and correspondence is time consuming and usually unnecessary. It is suggested that animals most often resort to very quick, simplistic, coarse matching and rarely engage in further interpretation of shadows in order to avoid possible confusion (Braje, Legge, & Kersten, 2000; Casati, 2006; Dee & Santos, 2011; Mammasian, 2004). However, under certain circumstances shadows can provide useful information, best exemplified when the shadow casting object cannot itself be seen. If the casting object cannot by seen but a cast shadow is observed, this may provide cues concerning the presence of a predator, prey or other relevant object otherwise undetectable (Dee & Santos, 2011). This specific scenario of a possibly useful image-like cue in the natural world provides some interesting clues as to how such information may be processed in relation to the contrived problem of picture-object correspondence. Furthermore, images viewed as similar but different from corresponding objects may also provide insight into how similar but different stimuli are perceived in general. For example, how bees respond to the similarities and differences among flowers of the same type but differing slightly in appearance or resource (full or depleted).

Although pictures and other representational images offer unique potential in highly controlled stimulus presentation in experimentation, it must be established how a specific species perceives the relationship between object and picture. Appropriate interpretation of results pertaining to response to picture stimuli requires a basic understanding of how bees see pictures spontaneously, to what features they attend (patterning without depth) and how experience or exposure to both picture and object may alter perception.

Plan of Study

This thesis will seek to both investigate (1) picture object correspondence in bumblebees and (2) their learned use of picture cues while foraging in a delayed matching task. A test of picture object-correspondence would serve to both evaluate the methodological validity of using 2D stimuli in bee cognitive and perceptual research and address the possibility that pictures, if perceived as equivalent to objects, may offer a unique opportunity to cue target matching and potentially influence foraging behaviour.

Picture object correspondence has been extensively studied in vertebrates but not in invertebrates, despite increased attention to bee cognition in particular. The first three studies in this thesis test how bees perceive the correspondence between picture and object: (experiment 1) *confusion*, can bees differentiate an object from a picture of that object?; (experiment 2) *independence*, do bees perceive only the difference between an object and picture but not the similarity?; or, (experiment 3) *equivalence,* can bees respond to a picture as being different from but similar to a corresponding object?

If bees can perceive equivalence between a picture and object, it may be possible to use pictures to cue foraging behaviour. Learned directed foraging by picture cue will be tested in a delayed matching to sample task (DMTS) (experiment 4). Experiment 4 will test the possibility for trained directed foraging by picture cue.

To facilitate comparison of results across the four experiments in this thesis, methods will be retained with as much consistency as possible. All experiments will take place within a radial arm maze. During each experiment bees will be trained in groups but tested individually. Training criteria will be observed to establish consistent levels of training and experience among tested bees. Testing will consist of at least the first 5 consecutive ‘choices’ of each tested bee.

‘Choices’ will always be defined as physical contact with either the stimuli in any one of the open corridors or the end of the corridor itself. The fourth experiment will present the most complex task of the four here reported: a delayed matching to sample task. In this task, a picture cue will be presented prior to the bee entering the center of the radial arm maze. The training will consist of this cue corresponding to the matching, rewarded object stimuli in the maze; all other stimuli will remain unrewarded. This cue-rewarded target match will be randomly switched between all the presented stimuli. All spontaneous and learned responses will be assessed for significance with a comparison to a chance value of either .5 or .25 according to the number of stimuli presented.

Preface to Part I: Picture-Object Correspondence in Bumblebees

Images are frequently used in the study of bee cognition and perception; such studies are commonly discussed as if results can be generalized to responses within the natural world, primarily to flowers. However, picture-object correspondence has yet to be specifically evaluated in bees. Furthermore, the correspondence perceived between an object and corresponding picture will change with exposure and experience. For example a subject which may at first confuse a picture and object may be able to learn to differentiate the two. Alternately, a subject which does not perceive a relation between a picture and object may be able to learn an association.

The first part of this investigation (experiments 1, 2 & 3), picture-object correspondence in bumblebees (*Bombus impatiens*), has been published in *Animal Cognition*.

Part I: The following manuscript was published in *Animal Cognition*:

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How images may or may not represent flowers:

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Abstract Studies of bee cognition frequently use two-dimensional stimuli referred to as floral patterns, and yet how bees perceive pictorial representations is not known. An investigation of bumblebee (*Bombus impatiens*) picture-object correspondence was undertaken according to the theory of Fagot et al. (2000) that pictures and objects may be confused, perceived as independent or equivalent. In three experiments, bumblebees were given discrimination training and unrewarded testing in a radial maze. In the first experiment, preferences between artificial flowers *versus* photos of those flowers revealed a significant learned preference for the stimulus rewarded during training: no confusion following training. In the second experiment, bees did transfer learning from artificial flowers to photographs: some commonality between an object and photograph was perceived. In the third experiment, bees spontaneously generalized a learned preference for one artificial flower to its silhouette, but only for one of two flowers used in training. No generalization was obtained to drawn images. Some transfer between image and object is exhibited, likely by low level feature matching but transfer is poor with degraded images, cautioning against extrapolation of picture based responding to natural correspondents. Despite evidence that bees exhibit some transfer while retaining discrimination, it is likely that the observed response is due to generalization more akin to confusion than true equivalence. Furthermore, although 2D patterning cues (line, edge and shade) provide discriminable cues for bees between 2D stimuli, it is not here supported that such features are perceived as equivalent to the intended floral structures.

Keywords: picture-object correspondence, generalization, bumblebee, *Bombus*

Part I: General Introduction

Compromise between naturalistic validity and experimental control is a challenge for any area of comparative cognition. Concessions often result in the use of less than desirable but carefully contrived stimuli, such as pictorial, two-dimensional (2D) images in lieu of the natural objects they represent. Although simple 2D patterns may seem unambiguous, even among humans, accurate content recognition of representational images is a learned ability (Delius et al. 2000; Deregowski 2000). A presumption of spontaneous, generalized norms of perception by animals as intended by the designer is largely unfounded as responses of animals tested depend on a history of exposure and vary greatly across species (Aust and Huber 2010; Bovet and Vauclair 2000; Fagot et al. 2000; Weisman and Spetch 2010).

Though invertebrates have been historically underrepresented in psychological research (Bitterman 1996), cognition in honeybees and bumblebees has in recent years become topical (see Avarguès-Weber et al. 2011a; Cheng 2000; Chittka and Thomson 2001; Gould 1990 for reviews). Not surprisingly, this emerging field often relies upon picture presentation in place of actual flowers. Common designs of black and white patterning allow for purposeful isolation of ‘floral-like’ features, such as petal arrangement (Lehrer et al. 1995; Srinivasan 2010), spatial frequency (Horridge 1997) and symmetry (e.g. Plowright et al. 2011; Rodríguez et al. 2004). However, perception of a pictorial image is rarely spontaneously equivalent to that of an object (Fagot et al. 2000). Not only are many cues, such as depth, absent in picture form but designs inevitably reflect human bias in equivalency representation, such as the use of white to represent ‘background’ or ‘not the object’, and colour, shade or lines as physical boundaries. Behavioural response to pictures is frequently thought to predict response of the bee to a flower but simply combining a cue and reward does not necessitate that the bees perceive the cue as ‘floral’ (Gould 2002).

Accurate perception of content in representational images requires some prior history of exposure (Delius et al. 2000; Deregowski 2000). Both experience with images and familiarity with the intended corresponding subject matter enhance picture recognition (Bovet and Vauclair 2000; Watanabe 2000). As such, problems with picture use are two-fold in many laboratories, as animals often lack prior exposure to either images or corresponding objects; especially likely among short lived, easily confined species such as honeybees and bumblebees. Picture perception can be discussed as being viewed either by an experienced subject, expressing acquired knowledge, or by an inexperienced subject, responding spontaneously (Bovet and Vauclair 2000).

Further parameters for picture perception are outlined by Fagot et al. (2000) who theorize that pictures may be perceived in one of three ways: *confusion*, the failure to differentiate an object from a corresponding picture (responses to objects, such as food items, and to pictures of the objects are indistinguishable); *independence,* the failure to perceive a relation between the two; and *equivalence*, the ability to perceive both the difference and the similarity between an object and a picture of that object, by feature matching (e.g. colour) or symbolic association (see Fagot et al. 2000). Although well researched among vertebrates, including primates, birds, reptiles and fish, wherein transfer is common but not universal and degree of perceived equivalence varies (for reviews see Bovet and Vauclair 2000; Fagot et al. 2000), insect picture-object correspondence has received little if any attention. Given the prevalence of 2D stimuli use in the study of bee cognition, behavioural evaluation is needed of what a bee may perceive spontaneously, what may be dependent upon learning and what may be the perceived relationship between image and corresponding object.

The ability of the bee to generalize between an object and picture based upon shared features is not unexpected. Despite the contrived nature of representational images, cognitive flexibility by generalization is necessary in the navigation of a highly variable and complex natural environment (Horridge 1999; Huber 2000; Peissig et al. 2000; Stach and Giurfa 2001). Adaptive response to environmental cues must persist despite vast visual inconsistency due in part to distance, direction of approach, obstruction, natural change and variation (Benard et al. 2006; Chittka and Raine 2006; Dyer 2012; Peissig et al. 2000). Although the 2D presentation of pictures is relatively unnatural, with the exception of cast shadows (Casati 2008), flexible recognition and generalization according to shared elements (e.g. colour or shape) is not. Bees must be able to recognize important features even from a distance which, due to limited visual acuity, may degrade detail and colour cues (Chittka and Raine 2006). Benard et al. (2006) propose that animals may deal with natural variability by either stimulus generalization (i.e. responding to common features) or categorization (i.e. conceptual knowledge of group membership). It is therefore reasonable to assume that pictorial representations can capture elements of a flower and be recognized by the bee as ‘floral’. Indeed, Chittka and Walker (2006) found that bees do approach and land more often on painted images of flowers (Van Gogh’s *Sunflowers*) as opposed to non-flower images (Caulfield’s *Pottery*). The findings suggest that images may well capture ‘floral’ cues preferred by bees. Recently Wu et al (2013) extended these findings to non-floral discrimination of painting style (impressionist and cubist).

Despite a lack of direct study of picture-object correspondence in bees, some indirect evidence already exists in the study of pattern recognition (for a review see Avarguès-Weber et al. 2011a). Bees show discriminations among 2D patterns based on relative spatial frequency, radial patterning and symmetry (Lehrer et al. 1995). Additionally, Gould (1988) found that honeybees were able to differentiate between a pattern and its mirror image but chose the mirror image over a novel stimulus. Gould concluded that "…they perceive there is a difference, but also that the two patterns have something important in common" (Gould 1990; pg 87). Similar studies with bumblebees also found evidence of such "similar but different" judgments (Korneluk and Plowright 1995; Plowright 1997). Further evidence that suggests that bees may have at least the building blocks to support picture-object correspondence is found in a variety of cognitive analyses of behaviour. Working memory underlies the ability to evaluate and integrate novel stimuli (Brown and Demas 1994). Categorization and concept responding, like picture-object correspondence, require a perceived similarity or relation between stimuli which may differ in appearance, configuration or context (Avarguès-Weber et al. 2011b; Benard and Giurfa 2008; Zhang 2004), including 'same-different' concept learning (Brown and Sayde 2013), cross modal rule transfer (Giurfa et al. 2001), rule abstraction (Giurfa 2007), contextual cue use (Lotto and Chittka 2005), interpolation (Dyer and Vuong 2008), and non-elemental learning (Giurfa 2003). It remains to be determined whether these processes predict bee response in a picture-object correspondence task and the capacity to perceive both a similarity and difference between the two.

Herein, an initial investigation into the perceived relationship between an object and picture by the bumblebee (*Bombus impatiens*) is presented. According to the previously established account of Fagot et al. (2000) we tested bees for evidence of confusion, independence and featural equivalence in relation to picture-object correspondence. In all three studies, bees given different discrimination training experiences were subsequently given common tests on unrewarding patterns. Study 1 tested a learned differentiation between an object and corresponding photograph. If bees could learn this differentiation, then confusion could be ruled out. Study 2 tested spontaneous transfer from an object to a photograph of the object. If bees were able to differentiate an object from a photograph (Study 1) but also exhibit a preference for a previously unseen photograph of an object that had been rewarded during training (Study 2), then independence could also be ruled out and some level of equivalence supported. Feature equivalence was evaluated in Study 3, which tested spontaneous transfer from an object to an altered image representing a silhouette and drawn version of the object. This test of transfer to altered images was aimed at isolating the role of colour in feature equivalence processing. A result of independence would detract from generalizations made from bee behaviour towards pictures to natural correspondents. Conversely results supportive of either confusion or equivalence could bolster picture use in research on bee cognition.

Part I: General Methods

Much of the methodology was consistent among experiments 1, 2 and 3 to allow for direct comparison of results. Elements of the method unique to each experiments are outlined separately.

Subjects

A total of six colonies, two colonies per study, of commercially raised bumblebees *(Bombus impatiens)* were donated by Koppert Canada. The bees had no experience outside their colony prior to the experiments. The colonies were fed pollen *ad libitum* but required to forage for sugar solution (1:2 sugar to water by volume) within a radial maze during daily group training sessions. Workers were individually labelled with coloured number tags glued to the thorax to allow for individual monitoring of training.

Apparatus

Bees were housed within a plastic nest box. The nest box was attached via a wooden corridor and screen tube to a radial maze consisting of 12 corridors (14 cm long and 15 cm high) that open to a central area (17 cm diameter) from the entrance of each corridor (6 cm wide). Bees entered the maze from a central entry hole in the bottom (as in Plowright et al. 2013), of equal distance to every corridor (Fig. 1). Feeder troughs were located at the end of eight out of twelve corridors (randomly selected and alternated), filled with sugar solution for rewarded stimuli and left empty for unrewarded stimuli. The remaining four corridors were made unavailable by blocking them off at their entrance.

Artificial flowers or photographs, mounted flat against the wall at the end of the corridor using Velcro, were located at the end of eight of twelve corridors in the maze (four rewarded and four unrewarded) containing a feeder trough located in the middle of each stimulus (protruding through the center). The bees could obtain sensori-motor information from the stimuli when they landed on them, but they could not fly around them. The location of stimuli was randomly rearranged every day and the maze was rotated multiple times throughout the day to deter place learning. Two types of synthetic fabric and plastic artificial flowers were used: a yellow single flower and a pink cluster of flowers (i.e. inflorescence). The corresponding pictures of each flower were unaltered colour photographs, photographs altered to black silhouette images and drawn images (Fig. 2). Photographs were taken with a Panasonic DMC-FZ20 camera and printed on a Canon MP560 ink jet printer. Normal photographic printing was used and colours of object and picture were not precisely matched. This decision was made to specifically test the correspondence perceived by bees between a coloured object and printed colour photograph rather than colour vision in bees which has been elsewhere investigated (Briscoe and Chittka 2001). Although artificial flowers are the object of choice in this study the nature of the object is not important, as correspondence is testing only the ability of the subject to perceive the relation between any object and corresponding image.

High frequency (> 40 KHz) light ballasts (Sylvania Quicktronic T8 QHE4x32T8/112) were used to minimize the risk of disruption of behaviour due to the perception of flicker (Srinivasan and Lehrer 1984). The fluorescent light bulbs (Sylvania model FO32/841/XP/SS/EC03) were located directly above the maze.

Procedure

Training

Bees were allowed to freely forage as a group within the maze for approximately five hours every day. The identity of each foraging bee was recorded by the observer and a tally kept in real time for subsequent visits up to the predetermined number of visits required by the training criteria. Training criteria, as follows, had to be met prior to testing for each individual bee. First, an individual had to be observed foraging for a minimum of two days, visiting the rewarding stimulus at least three times per day. After this criterion was satisfied the bee proceeded to testing once it made 8 choices of the rewarding stimuli within 10 consecutive choices, or 10 out of 13, whichever came first.

Testing

Upon meeting training criteria, bees were tested the following day. Next day testing lessened the effect of fatigue following training (bees exhibit variable patterns of activity and may go long periods without emerging to forage following a training session). Comparatively, memory in the bee in many ways resembles that of other animals: repeated experiences facilitate memory consolidation resulting in memories of differing strength and longevity sufficient for next day testing (including early long term memory of 1-2 day duration and late long term memory of 3+ days duration (see review on honeybee memory by Menzel (2001)). The maze was cleaned with water to dissolve sugar residue and left to dry overnight. Scent marks left by bumblebees have been found to no longer influence behaviour 24 hours after being left and so testing was completed a day after cleaning (Stout and Goulson 2001). Test stimuli were placed in the maze but without any reward and again with random placement of stimuli to deter place learning. Trained bees were released one at a time into the maze. The first ten choices of each bee were recorded. A 'choice' was defined as physical contact made by the bee with the stimulus (object or photograph) at the end of the corridor, the feeding trough or the end wall of the corridor.

Statistical Analysis

Because the data were binary choice proportions and there was replication within bees (10 choices per bee), a replicated Goodness-of-Fit test using the G-statistic was used. The G_P value tests for whether the pooled or group proportion differs from a theoretical value of chance and G_H tests for heterogeneity or individual differences. Where individual differences were significant, the total G value was partitioned into individual contributions. In tests of significance, the G test statistics are compared to a χ^2 value (Sokal and Rohlf 2012).

Design

In all three studies training was conducted with four of eight open corridors containing rewarding stimuli and four unrewarding stimuli.

Tests were run with two different colonies for each study (Fig. 2). All bees in a colony experienced the same contingency. The rewarding and unrewarding stimuli were switched for the second colony for each study.

Experiment 1

Do bees confuse an object with the corresponding picture?

Common features between images and corresponding objects may lead to confusion, or an inability to differentiate the two stimuli. Furthermore, confusion may occur spontaneously to novel stimuli but develop into differentiation with experience (Fagot et al., 2000). Experiment 1 tested bumblebees for a learned ability to differentiate objects and corresponding pictures.

Methods

In the first study, either the object (artificial flower) or the corresponding colour photograph was rewarded during training with sugar solution and the other was left empty (group O+/P– : object was rewarding and picture unrewarding; group P+/O– : vice versa). Testing was conducted on the same stimuli, four of each, which were all unrewarding (Fig. 2).

Results

Figure 3 illustrates the choice proportions of the picture or object for individual bees during testing, depending on whether the object (group O+/P–) or the picture (group P+/O–) was rewarding during training. Bees learned to differentiate the objects from corresponding pictures; preference for the previously rewarded stimuli during testing was 95% for group O+/P–, which was significantly greater than a chance value of 50:50 ($G_P=195.29$, $p<0.0005$, df=1). For group P+/O– the choice proportion was 92%, which was also significantly greater than chance ($G_P=165.75$, $p<0.0005$, df=1). The first five choices were comparable to the second five choices: for group O+/P– (excluding bee #13 that did not complete 10 choices), the mean choice frequency for the object was 4.84 in the first five choices and 4.68 in the second five; for group P+/O–, the mean choice frequency for the picture was 4.8 in the first five choices and 4.4 in the second five. Individual differences were not significant ($G_H=23.64$, $p=0.21$, df=19 (group O+/P–) and $G_H=23.75$, $p=0.21$, df=19 (group P+/O–)).

Main Finding

Results show that bumblebees can easily learn to differentiate an object from a corresponding picture. This signifies that bees perceive images and objects as either unrelated (independent) or similar but different (equivalent) (Fagot et al., 2000).

Experiment 2

Do bees spontaneously transfer learning from an object to a corresponding picture?

Given that bees can learn the difference between an object and image (Experiment 1), the second study sought to test whether or not bees spontaneously perceived a correspondence between the two or not (independence). Bumblebees were tested on a spontaneous transfer to images of objects previously rewarded during training.

Methods

In the second study, bees were trained to one of two artificial flowers: objects A and B. For group OA+/OB– : single yellow artificial flower was rewarding and pink inflorescence was unrewarding; group OB+/OA– , vice versa. Testing was conducted using unrewarding corresponding unaltered photographs of both stimuli (four of each).

Results

Figure 4 illustrates the proportions of choices for the picture corresponding to the object that was rewarded during training for individual bees in each group. Preference during testing for the unrewarding colour photograph corresponding to the previously rewarded object was 83% for group OA+/OB– , which was significantly greater than chance ($G_P = 83.78$, $p < 0.001$, df=1). For group OB+/OA– the choice proportion was 73%, which was also significantly greater than chance ($G_P = 43.96$, $p < 0.001$, df=1). Again, the first five choices were comparable to the second five choices: for group OA+/OB– (excluding bee #12 that did not complete 10 choices), the mean frequency of choices for Object A was 4.11 in the first five choices and 4.05 in the second five; for group OB+/OA–, the mean frequency of choices for Object B was 3.8 in the first five choices and 3.5 in the second five. No significant individual differences were found for either group ($G_H = 23.94$, $p = 0.20$, df=19 (Group OA+/OB–) and $G_H = 21.33$, $p = 0.32$, df=19 (Group OB+/OA–)).

Main Finding

Bees do spontaneously prefer novel images of previously rewarding objects, supporting an interpretation of equivalence processing for picture-object correspondence in bumblebees (Fagot et al. 2000). The combined results of the first two experiments show that bumblebees can easily differentiate an object from an image of that object but also, spontaneously respond to a similarity between the two. Lastly, it remains to be tested if this equivalence will be expressed when the prominent common feature of colour is removed.

Experiment 3

Do bees spontaneously transfer learning from an object to a degraded but corresponding picture (silhouette or drawn)?

Bees respond strongly to cues of colour, it is likely that objects and pictures are both easily differentiated and corresponded based upon colour similarities. Achromatic images are presented in this third study to determine if the equivalence processing supported by experiments 1 and 2 will transfer to altered, black and white images of coloured objects.

Methods

For the third study, bees were again trained to either the yellow or pink object as in Study 2 but tested on altered images, both silhouette versions of each flower and drawn corresponding images (two silhouettes of each and two drawings of each).

Results

Whether the yellow artificial flower or the pink inflorescence was rewarding during training, there was no preference for their images (silhouette and drawn, combined). For neither group did the choice proportions differ from a chance value of 50:50 (group OA+/OB– G_P =0.02, *p*=0.89, df= 1 and group OB+/OA– G_P =2.66, *p*=0.10, df= 1). This failure to find a preference for pictures, however, concealed a difference between the silhouette and the drawn image for group OA+/OB–. Figure 5 illustrates the proportions of choices by individual bees for the two altered pictures corresponding to object A, which was rewarded during training (a and b) and images corresponding to object B, which was unrewarded during training (c and d). In this initial test of featural equivalence bees in group OA+/OB– , the choice proportion for the silhouette of object A was 34%, which was significantly above a chance level of 25:75 (G_P =8.07, *p*=0.005, df= 1) while the choice proportion for the drawn image was 16.5%, which was significantly below chance (G_P = 8.43, *p*=0.004, df=1) . Note that the preference for one image did not necessarily entail avoidance of the other because there were four patterns available. Neither choice proportion for images corresponding to the S- was significant: the silhouette of object B (S-) was 20.5% (G_P = 1.65, *p*=0.2, df=1) and the drawn image 29% (G_P = 2.26, *p*=0.133, df=1). Again no individual differences were detected for any of the images.

While object A and its silhouette were apparently perceived as similar, the same was not true of object B. Figure 6 shows the proportions of choices by individual bees for the two altered images corresponding to object B, which was rewarded during training (a and b) and to object A, which was unrewarded during training (c and d). The results for this group (OB+/OA–) did not parallel those of group OA+/OB– : neither the preference (28%) for the silhouette of object B (G_P = 1.02, *p*=0.31, df = 1) nor the drawing (also 28%) of object B (G_P =

0.72, p=0.40, df = 1) was significantly different from a chance value of .25. Likewise, neither image of the S- was chosen at significantly high or low levels: 23.5% for the silhouette of the S- (G_P = 0.21, p=0.647, df=1) and 20.5% for the drawn image of the S- (G_P = 2.14, p=0.144, df=1). The individual differences were significant for the silhouette version of object B (G_H =37.14, p=0.008, df= 19); three of twenty bees (#9, #15 and #20) tested never chose silhouette B (G >=5.18, p=0.02, df= 1). Individual differences were also found to be significant for the drawn image of object A (S-) (G_H =34.05, p=0.018, df= 19). One bee (#15) chose the drawn image of A (S-) eight out of ten times (G=13.32, p=0.0003, df= 1) and two bees (#16 and #19) never chose the drawn image of A (G=5.75, p=0.016, df= 1).

Main Finding

Spontaneous picture-object equivalence exhibited by bumblebees occurs to some but not all altered, achromatic images of coloured objects. It appears that colour best facilitates spontaneous equivalence perception in bee picture-object correspondence but some achromatic images may retain sufficient featural commonality to be perceived as ‘similar’.

Part I: General Discussion

The first experiment in this series established a learned differentiation; the bees were trained to either a picture or object and tested for learned preference for the formerly rewarded stimulus. Bees easily learned to respond to both object and 2D stimuli, which justified the use of our materials in the subsequent experiments. While there are undoubtedly limits in generalizability of these results to 3D *vs* 2D discrimination in general, the results of this first study do show that bees are able, under these conditions, to differentiate an object from its corresponding picture.

The second study in this series tested for spontaneous independence in the response of the bee to unaltered photographs of previously rewarded or unrewarded objects. A response of independence was not supported as bees did transfer learning from the previously rewarded object to the corresponding photograph. This correspondence may reflect low level feature matching (e.g. colour), even though the spectral characteristics of the photographs and objects may have appeared as more different to the bees than they do to humans. The successful correspondence of colour photograph and object suggests that normal photographic printing of the objects used in study 2 did retain some similarity to the original object colour which is perceived by bees. Colour photographs have been found to convey sufficient correspondence for matching by bees but the results of both successful differentiation (experiment 1) and generalization (experiment 2) may be based on sensitivity to colour which can be differentiated by bees but also matched. One caveat is that different groups of bees were tested in experiment 1 and experiment 2, and so it remains to be determined whether the same individuals can both process similarities and differences.

Bumblebees do appear to perceive both the difference and some commonality between an object and picture, much as Gould (1990) found for mirror images. Degree of picture-object correspondence may be variably expressed by the same individual or species under varying training conditions. In general, discrimination training leads to sharpened generalization gradients, and indeed, confusion or independence may be obtained with inexperienced subjects who may later express equivalence following exposure and training (Fagot et al 2010). The results of our first two studies in combination suggest a process of feature equivalence, a result obtained following discrimination training either between object and picture (experiment 1) or between two types of objects (experiment 2). The observed feature equivalence may be based only on matching of low level feature cues (such as colour). However, when faced with a forced choice between only images and not the objects (experiment 2), bees may perceive the pictures as similar enough to prompt significant preference, as if the image were the object, suggestive of possible cognitive, if not perceptual, confusion of the two stimuli (Kapustjansky et al. 2010). Further testing of spontaneous response is needed, but learned differentiation was highly significant, thereby it is known that confusion can, at least, be eliminated with training. Learned differentiation (experiment 1) and spontaneous correspondence (experiment 2) have here been tested but further research is required to determine if bees exhibit spontaneous confusion, the inability to differentiate object from picture in the absence of training.

The third study found significant spontaneous equivalence transfer of learning in group OA+/OB– to a silhouette image but not a drawn image. The second group, trained to the pink inflorescence object (B) did not, as a group, show significant transfer to either the corresponding silhouette or drawn image (Figure 2) of the former S+, but significant individual variation was expressed in the choice proportion for the silhouette. Earlier results suggest that bees are capable of some level of featural equivalence between object and picture but mixed results from experiment 3 point to a threshold need for common features between the two. Not all images correspond to a given object equally well even though they may be derived with the same method (i.e. silhouette or drawn). Some difference in the ability to transfer from object to representation will vary based upon those features which may or may not be adequately represented by the image (Deregowski 2000). This is evidenced by the strong transfer observed in study 2 by group OB+/OA–, where bees preferred the colour photograph of object B, but the absence of transfer to the silhouette or drawn image in study 3 by group OB+/OA– trained in the same way. Future research might profitably include a test between identical patterns coloured in the same way as the objects. In addition, using the logic of Brodbeck and Shettleworth (1995), a test in which shape and colour were pitted against each other (e.g. the shape of A with the colour of B vs the shape of B with the colour of A) might begin to reveal the importance of various elements.

The difference in successful transfer between objects A or B and their images may arise from individual subject variability, insufficient feature representation by the abstraction and lack of lower visual field cues. Individual variability in picture-object correspondence is widely exhibited among animals, ranging from experienced humans to pigeons, and now possibly, bees (Deregowski 2000; Fagot et al. 2000). The experience levels of each bee tested did inevitably vary due to the freedom of group training that required a minimum but not maximum level of exposure.

Individual variation in response may have been exaggerated by increased ambiguity in the abstract picture of the B stimulus. The stimuli (A and B) were chosen to reflect characteristics (in colour, shape and number) found to be easily differentiated by bees (Srinivasan 2010). The features of each stimulus are not uniformly transferable to, or represented by, a silhouette or drawn image. Most likely the image did not adequately represent multiple flowers in object B, the inflorescence, relying upon shade (silhouette) or lines (drawn) to represent the physical separation of clustered flowers. Similarly, increased complexity of the inflorescence (stimulus B) over the single flower (stimulus A) may also be implicated, as increased complexity of a stimulus may lead to learning of isolated features only, to the exclusion of others (Enquist and Arak 1998). Although bees can learn multiple cues simultaneously (Ronacher 1998), the complexity of the inflorescence may have reduced this tendency, instead resulting in learning by a single highly salient feature (e.g. colour), which when lost in abstraction resulted in an inability to respond to alternate cues (e.g. similar shape). Past research has repeatedly found that bees respond to 2D patterning cues such shade, line and edge (Horridge 2007; Lehrer et al. 1995) but the current study does not evidence a perceived correspondence between such 2D pattern features and actual physical correspondents (such as flower petals or individual flowers in a cluster).

The lack of feature cues in the lower portion or below the feeder for the second stimuli may also have hindered transfer. Giurfa et al. (1999) found that the method of conditioning alters degree of learning in honeybees. Absolute conditioning (training with only a rewarded stimulus) resulted in better learning of the entire stimulus, whereas differential conditioning (training to both rewarded and unrewarded stimuli) resulted in bees attending more to the lower portions, which were significantly lacking in the B stimulus. However, it is not clear why such an effect would be present in the third but not second experiment. One possible post-hoc explanation may again be the presence of colour. Although bees are able to respond to either colour or shape in isolation (Benard and Giurfa 2008; Dukas and Waser 1994; Gumbert 2000; Ings et al. 2012), it may be that colour was sufficiently represented by the photograph (study 2) but that shape was not well depicted by the abstract images (study 3), allowing transfer to the coloured but not uncoloured images, even under differential conditioning.

The failed spontaneous correspondence may be resolved in the future using an approach of ‘easy-to-hard’ training, testing the potential for learned correspondence. Gradual or facilitation training such as that used by Perrault and Plowright (2009) for pattern rotation or Zhang (1994) for camouflaged stimuli imperceptible without (non-camouflaged) training, could determine whether the inability to transfer learning was reflective of a difficult but potentially learnable task or one that was insurmountable. A learned differentiation could be established by training bees in a match to sample task, whereby they learn that a given image (silhouette or drawn) predicts reward in a corresponding object. Lines, edges and other features commonly used to depict physical definition in images may be perceived and learned by bees as components of 2D patterns but it is not here supported that bees respond to this patterning as if such elements correspond to features of an object (i.e. shade definition, lines or edges in an image are not corresponded to petals). It may be that although bees do not spontaneously exhibit correspondence between 2D pattern cues of degraded images or patterned stimuli with physical features, they may be able to learn to perform this task. Further testing of the potential for learned correspondence between non-colour pattern cues and object is needed to determine whether or not bees cannot perceive the correspondence between line, shade and edge cues and physical elements or just do not exhibit this correspondence spontaneously.

Conclusion

Bumblebees, like many mammals and birds, do not perceive a picture and corresponding object as one and the same. Behavioural responses in our studies indicate that bees treat images of objects as similar but not identical to a corresponding object. Bees neither confuse pictures and objects, nor treat the two as unrelated. The most likely conclusion of such results is that bees apply a common practice of feature generalization or categorization (Benard et al. 2006) in evaluating similarity between an object and corresponding picture, much as they would any two stimuli. This type of correspondence is likely, due to a spontaneous transfer to degraded images, based on low level feature matching (such as colour). Fagot et al. (2000) suggest that true equivalence among animals is rare and indeed it may be argued that although perceptual confusion or the inability to differentiate an object from picture does not occur among bees, the tendency to generalize learning between the two is more likely to represent a level of generalization more akin to confusion than true equivalence.

This study was primarily undertaken to evaluate the validity of using 2D or picture stimuli in research on bee cognition. Additionally, correspondence between one stimulus (an object) and another, sharing some common but degraded features (an image), reflects a natural correspondence challenge bees face in matching a distant perception of floral features, lacking colour and detail due to visual acuity, to a memory. The loss of significant cues, such as colour, may occur often as a bee navigates either a novel or changed environment and must match memories to incomplete visual perceptions. Present results caution against assumptions that bee learning and response to patterned stimuli can be extrapolated to perception of natural correspondents (flowers). The bee is not likely to interpret, unaided, complex two dimensional representational pattern cues as intended by the researcher. For example, our results offered little support for the notion that white contrast reliably represents ‘background’ or that shading represents depth or lines boundary. Despite common use of pattern cues to represent ‘floral features’ in research it has not been supported that bees see such features as representative of actual physical features. The emphasis in contemporary research on bee cognition is on overlooked similarities between the achievements of vertebrates and invertebrates (e.g. Bateson et al. 2011; Gould 1990; Giurfa et al. 2001). While our research on an invertebrate is very much in keeping with the conclusions regarding picture-object correspondence in vertebrates (Bovet and Vauclair 2000), further similarities and differences remain to be tabulated in a comparative analysis.

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Preface to Part II: Picture-object correspondence as a method of cued, directed foraging

The three expressions of picture-object correspondence, confusion, independence and feature equivalence, may be variably expressed by the same individual or species under varying conditions (such as confusion or independence by an inexperienced subject who may later express equivalence following exposure and training). So too, symbolic or associative picture-object equivalence is expected only under specific circumstances as a learned correspondence. Following an initial investigation of bee picture-object correspondence (experiments 1-3) picture cue use in foraging bumblebees could be addressed in experiment 4.

Pictures offer a unique method of cueing target choice. As bees perceive and respond to pictures and corresponding objects as different but related (experiments 1 and 2), pictures may function as unique associative, secondary cues in a delayed matching task. First, delayed matching tasks, wherein the cue and target are identical, risk a subject exhibiting avoidance rather than preference when the cue is unrewarded. The subject may associate the stimulus with the absence of reward and thereby avoid the matching target. Secondly, a picture, perceived as similar to a target object, should more easily trigger memory for the target than would an arbitrary cue. Lastly, a picture, which is spontaneously recognized as related to a corresponding target, may allow for cued response to stimuli pairs never before seen and therefore without specific prior training. In order to test the possibility for directed foraging by picture cue a fourth experiment was undertaken, pairing picture and object in a DMTS task. While pictures are also uniquely contrived stimuli, they do correspond to natural cues of visual repetition. For example, the structure of some flowers present visual cues (such as petals) which correspond to reward deeper inside the flower but often these different parts of the flower share common colour or sometimes patterning (such as stripes).

DMTS tasks have been used successfully to investigate various cognitive questions in bees, including: numerical matching, symbolic colour association, ‘same-different’ discrimination and sequential context (Cooke, Couvillon, & Bitterman, 2007; Dale et al., 2005; Gross, Pahl, Si, Zhu, Tautz & Zhang, 2009; Moreno, das Graças de Souza, Reinhard, 2012; Zhang et al., 2005). Although DMTS tasks have resulted in significant discoveries of bee cognition, some studies have failed to successfully elicit a cued response, resulting in perseveration (persistent choice patterns despite disadvantage in reward acquisition) rather than learning (Brown, McKeon, Curley, Weston, Lambert & Lebowitz, 1998; Dale et al., 2005; Isnec, Couvillon & Bitterman, 1997). Various explanations are possible but one may be the pattern of exposure. If the difficulty in differentiating stimuli was relatively low (different colour targets) alternating trials resulted in successful learning (Cooke et al., 2007). Frequent change to stimuli reduces the likelihood of perseveration even to highly salient, easily differentiated cues by limiting repeated exposure to associated reward. However, if the difficulty of differentiating stimuli was increased (e.g. a numerical discrimination) bees were more likely to be trained by blocked trials (Gross et al., 2009; Moreno et al., 2012; Zhang, Bock, Si, Tautz & Srinivasan, 2005). The performance of a difficult task, such as numerical discrimination, is less likely to result in perseveration even following repeated exposure, due to the difficulty of discrimination and identification. The fourth experiment in this dissertation was undertaken to test the use of picture cues in directing bumblebee choice for matching targets.

Part II: The following manuscript will be submitted for publication. The formatting of references has been altered in this section to meet journal requirements.

Author contributions:

Emma L. Thompson: Experiment design, data collection, data analysis and written manuscript.

Catherine M.S. Plowright: Experimental design and editing.

Learned use of picture cues by bumblebees (*Bombus impatiens*) while foraging in a delayed matching task

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Abstract

Picture-object correspondence provides an alternate method of investigating delayed matching by providing a cue (picture) which may be spontaneously perceived as similar but different from a corresponding target. Memory for, and corresponding choice of, a target corresponding to a cue could be facilitated by the use of a picture. Bumblebees have been found to both easily differentiate images from corresponding objects but also spontaneously perceive a similarity between the two. Herein, an approach was designed to test the possible use of picture cues to signal reward in a delayed matching task. Target choice preference corresponding to picture cues was tested among three bumblebee (*Bombus impatiens*) colonies using photograph cues (presented prior to target stimuli) corresponding to one of four target stimuli. Photograph cues were the only predictor of corresponding target reward, presented in stable locations. Rewarded and unrewarded tests show a choice preference significantly higher than chance for targets matching the cue but not transfer to novel cue-target combinations. Results suggest that bumblebees can learn to use picture cues in a delayed matching task. Furthermore, experience, conditions of reward inconsistency and location, are discussed as possible contributing factors to learning in a delayed matching task.

Keywords: bumblebee, *Bombus***,** delayed matching, picture-object correspondence

Introduction

In a dynamic and noisy world it is best to attend to reliable cues and when possible disregard those that may be irrelevant, misleading, or at times redundant (Dukas 1998). The more distantly removed, in space or time, a secondary cue is from a corresponding target the less likely that cue will reliably convey information about the target. In other words, it is best to rely relatively more on direct target cues and relatively less on indirect cues. However, flexibly attending and responding to different sources of information can provide coping mechanisms in complex environments (Dukas 2009; Kendal, Coolen and Laland 2009). Bees depend upon floral resources in constant flux of bloom, depletion and expiration, which provide multiple signal cues, such as colour, shape, taste and size. Additionally, the environment provides many potential indirect or secondary indicators of nearby targets, including: foliage and vegetation; conspecifics and other animals; and, airborne scent. Any one of these cues may signal nectar or pollen, but in redundant combinations not all may elicit equal attention.

Secondary cues may be learned as being generally associated with certain types of stimuli or specifically associated with a specific target. General correspondents provide flexible information about common associations among similar types of stimuli, such as the higher likelihood of flowers being located in areas with foliage than areas without. Specific correspondents provide information about individual stimuli, such as the learning of particular landmarks along a route to a known flower patch (Chittka and Geiger 1995). Delayed matching tasks test the capacity to see a cue, separated from a target, and then from multiple stimuli select a target or location corresponding to that cue directly or symbolically. Bumblebees can match-to-sample (cue remains visible when choosing among stimuli) in a ‘same-different’ task (Brown and Sayde 2013). They can also learn to respond to contextual priming colour cues when choosing between two subsequently presented stimuli as long as the pairs of colours were presented at two different places (Dale et al. 2005). Honeybees can learn to solve both symbolic (Cooke et al. 2007) and non-symbolic delayed matching to sample (DMTS) tasks including categorization (Zhang et al. 1999). Honeybees are further able to learn to disregard an additional irrelevant cue in a DMTS task, and transfer this learning to a novel cue but only when the relevant cue is provided in a consistent place or position in a sequence (Zhang et al. 2005). DMTS among bumblebees has remained elusive and evidenced a difficulty which may correspond to differing foraging strategies employed by honeybees and bumblebees (for a review see Sherry and Strang 2014), but hints of success, usually by one or few individuals, suggest that this capacity or variations may yet be elicited in bumblebees under some circumstances.

One significant challenge for bumblebees in tests of DMTS may be the location variation needed to test ‘true’ matching to sample instead of the positional learning, similar to landmark cue use. However, this condition is relatively unnatural; while bees may face challenges of flower growth, depletion and expiration, flowers do not move. In a confined laboratory setting the constantly changing location of known stimuli may undermine tests of delayed matching. Context can provide necessary discriminating information for bumblebees when faced with a delayed priming task (Dale et al 2005) and tasks requiring matching of one cue to a reward and another to their nest (Avarguès-Weber et al 2011; Fauria et al 2002). Stable location of target stimuli may facilitate cue to target correspondence in a variation of traditional DMTS whereby a non-spatial cue can correspond to a predictable location (van Hest and Steckler 1996).

The use of a picture cue in a delayed matching task may further facilitate bumblebee performance. Previous findings have determined that bumblebees can both easily differentiate an object from a corresponding picture but also perceive a relation between them (Thompson and

Plowright 2014). As such, photographs can play a unique role in cue learning: (i) potentially being more likely to trigger memory for, and therefore approach to, the corresponding target; (ii) and lessen the risk that experience with an unrewarding cue will diminish the strength of rewarded training to the target, as may occur when the cue and target are exactly the same. In other words, the subject can learn that the picture of the object (the cue) is unrewarding but the object itself (target) is rewarding, as opposed to the object (cue) seen first being unrewarding but that same object seen later (target) being rewarding. It has been suggested that honeybee performance on DMTS may improve by a 'win-stay' approach, whereby honeybees are more likely to approach more familiar stimuli (Chittka and Jensen 2011; Sherry and Strang 2014). Honeybees and bumblebees display significant differences in foraging strategy and behaviour, and it is possible that among bumblebees, a familiar but unrewarding stimulus (cue) could reduce rather than increase the likelihood of approaching the same type of stimulus again (target).

In the present study, photographic cues corresponding to a selection of target stimuli test cue use by bumblebees in an alternative DMTS task with stable target location whereby a cue signals a rewarding target among four stimuli but all four stimuli remain consistently positioned (van Hest and Steckler, 1996). The photograph cue-target combination was chosen to also facilitate association between cue and target and potential novel transfer to never before seen photograph and object stimuli during testing. Cue-target matching was defined as approaching the object corresponding to a photographic cue immediately following a cue-target combination change. Bees displaying matching at levels above chance during training were subsequently tested for rewarded, unrewarded, and novel preference for targets matching picture cues.

Methods

Subjects

Three colonies of commercially raised bumblebees (*Bombus impatiens*) were donated for this study by Koppert Canada. A total of N= 20 bees were tested, 10 bees from colony 1, and 10 bees from colonies 2 and 3. The bees did not have any experience outside of the colony before the experiment began. The colonies were fed pollen *ad libitum* and sugar solution (1:2 sugar to water by volume) was available by foraging during experimental training sessions within a radial arm maze each day. Coloured number tags were glued to the thorax of each worker to allow for individual identification and monitoring.

Materials

The bees were housed in a plastic nest box attached via a wooden corridor and screen tube to a 12-arm radial maze (corridors were 14 cm long and 15 cm high opening to a central area 17 cm high and 6 cm wide). Bees entered the maze through one corridor, in which a picture-cue was mounted on the gated entry to the central opening of the maze (see figure 7). The entry gate provided a small hole through which bees entered the maze, randomly flipped so that the entry hole was positioned either in the top or bottom half of the gate. Picture cues were unaltered photographs of the artificial flower stimuli (see figure 8) presented in the maze. Photographs were taken with a Panasonic DMC-FZ20 camera and printed on a Canon MP560 ink jet printer (see Thompson and Plowright 2014). Only four corridors and the entry were accessible within the radial maze, all remaining corridors were closed with a solid gate. The open corridors contained one of four synthetic fabric ‘flowers’ (see figure 8) located on the back wall of the corridor surrounding a feeder trough.

High frequency (>40kHz) light ballasts (Sylvania Quicktronic T8 QHE4x32T8/112) were used to minimize disrupting behaviour due to flicker (Srinivasan and Lehrer 1984). The fluorescent bulbs (Sylvania model FO32/841/XP/SS/EC03) hung above the maze.

Procedure

Design

Bees were trained to a delayed matching task wherein a photograph of one of four artificial flower target stimuli was presented prior to entering the center of the maze. A training trial consisted of a bee entering the maze, foraging from sugar solution reward at one of the four stimuli and returning to the hive. Movement back and forth between the maze and hive was unhindered for bees undergoing training. Additionally, within the maze bees could continue foraging even if they made an incorrect initial choice. A picture cue signalled reward available at the target stimulus corresponding to the picture. The stimuli were positioned at the end of each of the four open corridors, on or near a feeder trough protruding into the maze through the outer wall. During rewarded trials the feeder trough contained sugar solution for the object corresponding to the picture cue but the remaining three stimuli troughs were empty. The cue-target combinations presented were always chosen randomly. The location of each target stimulus within the maze was kept consistent during training and all testing.

Training

Colony 1: It was hypothesized that repeated exposure would be needed for bees to learn the delayed matching task and so training sessions presented cue-target combinations for multiple foraging visits for 20 minutes before the combination was changed for one of the other cue-target combinations, hereafter referred to as ‘blocked’ trials. Training sessions were conducted for approximately 6 hours a day, during which the four cue-target combinations were randomly presented in blocked trials. Bees received training in small groups of approximately five. Training ranged from 3 to 11 days for each bee before individually meeting training criterion for testing. The training criterion used to assess learning of the task required bees to first approach the object corresponding to the picture cue 3 out of 5 times immediately following a cue-rewarded target change. When bees repeatedly failed to meet the training criterion the number of successive exposures to each cue-target combination was gradually decreased. Exposure was reduced until each cue-target combination was presented for only one foraging visit for every bee. Different cue-target combinations presented for every subsequent trial are hereafter referred to as ‘alternating’ trials. As the time between cue-target changes diminished bees began to pass the training criteria prompting testing.

Colonies 2 & 3: Given that bees from colony 1 only exhibited matching during alternating trials this training method was implemented for both colonies 2 and 3. However, not only did bees not exhibit learning, the activity level of foragers was very low and so the exposure time was stretched into blocked trials. Activity level increased but again bees failed to meet the criterion for delayed matching. Once again, the exposure to each combination was reduced to alternating trials, as with colony 1, and the bees began to meet the training criterion allowing testing for rewarded, unrewarded and novel matching.

Testing

Colony 1: Bees from this colony were only tested under rewarded conditions upon meeting the training criteria. Bees were individually observed for up to 10 test trials (or until activity ceased), during which the picture cue and corresponding reward with the matching object was changed for every visit (as it had been under randomly alternating training trials). The first choice of the bee was recorded, defined as contact made with the object or feeder trough at the end of any open corridor. If the first choice corresponded to the picture cue, this was considered to be a 'match' and if not a 'non-match'. All picture cue and object combinations were familiar to the bees (the same as those used during training: A, B, D, & E).

Colonies 2 & 3: Bees from colonies 2 and 3 were tested for unrewarded matching and novel matching in addition to the original test of rewarded matching. Once the training criterion was met bees were again observed for between 4 and 10 subsequent trials, during which reward remained present and the first choice preference was recorded. The nature of a delayed matching task restricts the number of unrewarded tasks which may be presented; unrewarded trials presented too frequently risk the subject learning that the contingency has become unreliable. Given that a matching task cannot make use of unrewarded testing often, unrewarded tests were conducted approximately every 10 rewarded trials. All picture-cue and object stimuli combinations for rewarded and unrewarded tests were familiar stimuli (A,B,C & D) used in training. Given the differing activity level of the individual bees, each bee completed 2, 3 or 4 unrewarded tests. One novel unrewarded test was also conducted for eight of the ten bees (a drop in activity for the two remaining bees prevented novel testing). The novel test was similar to the familiar unrewarded test but none of the stimuli had previously been exposed to the bees. This novel test could only be presented once per bee, before experience may influence subsequent choice, and was also presented following 10 rewarded trials.

Statistical Analysis

A replicated goodness-of-fit test using the *G*-statistic was used to analyze the binary choice proportion data replicated within bees (10 rewarded test choices, 2 to 4 unrewarded test choices and 1 novel test choice each). G_P value tested whether or not pooled or group proportion differed from a theoretical value of chance (1/4), and G_H for heterogeneity or individual differences. Tests of significance compared the *G* test statistic to a χ^2value (Sokal and Rohlf 2012). A logistic model, using SPSS22, was fit to the choice proportions to determine whether cue-target matching depended on which of the four flowers was rewarding.

Results

Can bees learn to use a picture cue, associated with the corresponding object target, in a delayed matching task?

Colony 1: *Rewarded tests of delayed matching.*

Following training 10 bees exhibited a significant preference to match a photograph cue to a target in a foraging task (Fig. 9). Upon meeting the training criterion (see methods) the choice proportions of 10 bees were observed for up to 10 rewarded trials each. These results show a preference for the object corresponding to the picture cue at rates significantly higher than chance ($G_{P=}$ 13.8, p=.0002, df= 1). Individual differences were not significant (G_H=8.07, p=.53, df=9).

A significant difference was found in matching preference among the stimuli types (A, B, D and E) (χ^2= 11.96, p=.008, df=3), with the 'pink inflorescence' stimulus B being matched at significantly lower levels than the other 3 stimuli (χ^2=4.72, p=.03, df=1) (Fig. 10).

Colonies 2 & 3: Rewarded, unrewarded and novel tests of delayed matching.

Another 10 bees from two colonies (n=4 and n= 6) again exhibited higher than chance proportion preference for objects corresponding to a matching picture cue during rewarded testing (G_P= 11.14, *p*=.0008, *df*=1) (Fig. 11). Again, no significant individual differences were found (G_H=3.71, *p*=.93, *df*=9).

No significant difference in matching among the four stimuli types (A,B,C & D) was found during rewarded testing (χ^2= .201, p=.977, df=3) (Fig. 12).

Interspersed between every 10 rewarded trials of the DMTS task an unrewarded test was presented as often as possible for each bee. Unrewarding test trials also showed a choice proportion for object stimuli corresponding to the picture cue significantly higher than chance (G_P= 11.97, *p*=.0005, *df*=1) (Fig. 13). No significant individual variation was found (G_H=13.73, *p*=.132, *df*=9).

Unrewarded novel testing of picture cue matching to objects never before experienced by the bees did not evidence transfer. Eight out of ten bees were each tested once on a novel DMTS task but the choice proportions did not differ from chance, only 2 out of 8 (25%) chose the novel object corresponding to the picture cue.

Discussion

Bumblebees can learn to use a picture cue to find rewarding stimuli while foraging in a delayed matching task when the stimuli remain in consistent positions. Both rewarded and unrewarded testing showed significant preference for object stimuli matching picture cues. This matching did not appear to transfer to novel cue-target combinations but the number of choices measured was limited. Without evidence of novel transfer or location change, the present results of delayed matching may correspond to delayed cue-target matching, cue-location matching, or a combination whereby the cue became associated with the location facilitated by picture to target correspondence. In the present study, only the cue predicted which target would be rewarding. Although floral constancy, the tendency to approach relatively more familiar stimuli, could also explain the choice pattern of ‘matching’ corresponding objects to picture stimuli, it is unlikely given that the image cues were never rewarding. Positioning of the cue was random and provided no information about reward. Unrewarded testing of picture cue-target matching was consistent with DMTS proportions found for honeybees (Lind et al. 2014). However, the bumblebees in the present study may have been using the picture cues as route cues, signalling the location of reward and not necessarily matching the cue and object. The delayed matching task employed in this study retained unchanging position for target stimuli within the maze to reduce the previously suggested difficultly reflected by constantly changing ‘flower’ locations within a small, familiar environment.

Past research had determined that bumblebees perceive both a difference and similarity between pictures and corresponding object targets (Thompson and Plowright 2014). The results of the current study further show that pictures can also be used by bumblebees in a delayed matching task. It is possible that the picture cue became associated with both a known location and the corresponding target therein. Multimodal floral signalling can increase learning speed, persistence, and facilitate memory among foragers (Kulahci, Dornhaus and Papaj 2008; Leonard et al. 2011). Secondary or associative cues can further provision serial priming cues to facilitate detection before a target becomes visible (Raguso 2004). Airborne scent in particular is believed to trigger memory and foraging for the corresponding, but out of sight, flower (Dornhaus and Chittka 1999). While naturally learned secondary cues, specific to a route or location, would not necessarily resemble the target, directed foraging may benefit from using cues similar to the target itself, by triggering memory for the similar, corresponding stimulus.

Observations made throughout this study highlight some additional components that may contribute to delayed matching by bumblebees but require further exploration. The present results, in combination with past difficulty training bumblebees to DMTS (Sherry and Strang 2014), may suggest additional conditions required for bumblebee learning in a delayed matching task: (i) a history of foraging experience; (ii) high reward inconsistency, (iii) and stationary location of familiar stimuli.

(i) Experience

Training in the current study required days (from 3 to 11) of exposure before bees exhibited a preference for stimuli matching the photograph cue. In a meta-analysis of DMTS among many animals including honeybees, Lind et al (2014) suggest that amount of training improves signal to noise differentiation and recall for memories of cues. Other studies have shown that facilitation or pre-training can influence learning of an otherwise impossible task or shift strategic or attentional processes. Experience or facilitated learning has been found to be beneficial or essential in the performance of bumblebee: reversal learning; use of global over local cues in navigation and rotated image discrimination (Jin et al. 2014; Perreault and Plowright 2009; Sherry and Strang 2014). Facilitated learning may well have contributed in this study to experienced bees later exhibiting matching but it cannot be ruled out that any form of foraging experience may facilitate delayed matching. A general history of foraging experience may simply encourage bees to remain active despite high levels of inconsistency and difficulty finding reward.

(ii) Reward inconsistency

Selective attention best facilitates foraging or hunting for consistent resources but when resources change, either declining or increasing in availability, broadening of attention becomes advantageous (Kamil and Bond 2006). Changing conditions can alter the relative worth of various resources as well as the corresponding sources of information signaling those resources by re-directing attention and reinforcing memories or recognition of alternatives (Leonard et al. 2011; Reinhard et al. 2004).

Successful delayed matching by bumblebees in this study may have required conditions of high reward inconsistency. Bumblebees do show flexibility under inconsistent or changing circumstances, and outperform the more persistent honeybee on tasks of reversal learning with repeated experience (see Sherry and Strang 2014). In the current study, when the same target was rewarded twice or more in a row (blocked trials), bees exhibited a strong preference for the previously rewarded stimulus, disregarding the cue. However, when the cue and rewarding target combination were changed following every foraging visit for each bee (alternating trials), matching was observed and evidenced by both rewarded and unrewarded testing. Foraging animals often rely on selective attention to best attend to relevant over irrelevant stimuli but still gather information broadly when needed due to resource change, loss or depletion (Kamil and

Bond 2006). McLinn and Stephens (2006) found that blue jays used a predictive signal when choosing between two stimuli but, as with the bumblebees in the present study, only when the signal was reliable and under inconsistent environmental conditions. The signal was ignored if unpredictable and the environment was consistent. The possible effect of inconsistent resources influencing delayed matching has also been suggested for the spider *Misumena vatia* (Defrize, Llandres and Casas 2014).

While floral constancy may represent a more energy efficient and focused strategy when compared with sampling, information about alternate resources may still be retained for later use when resource state changes and sampling becomes necessary (Chittka et al. 1999). In this study, during blocked trials, bees could rely on a relatively successful perseverating strategy because change occurred only following repeated reward with the same stimulus. Only when perseverating was never successful, during alternating trials, did the bees begin to exhibit matching and use of the picture cue.

Lastly, interference may be reduced with increased number of cues and reward variability among cues. Repeated or prolonged exposure to a cue can interfere with acquisition of alternate cues. Greater numbers of stimuli are known to reduce interference in DMTS (van Hest and Steckler 1996), and picture stimuli changed more frequently may further reduce interference, reducing prolonged exposure to any one stimulus. In other words, the use of four stimuli and corresponding cues for which reward was changed frequently (alternating trials) may have reduced the potential for interference.

(iii) *Location*

As mentioned, the delayed matching evidenced in this study may correspond to either a cue-target match or a cue-location match. Changing target stimuli location previously resulted in significantly decreased foraging activity, but this does not necessarily suggest that bees were learning a cue-location match. In a confined, laboratory space the movement of stimuli within a familiar environment corresponds poorly with natural challenges of floral cycle. Field study may be better able to replicate stimuli change and thereby test for DMTS without stable location of target stimuli. However, it is also likely that the bees were learning to use the picture cues as spatial cues to direct location or route choice rather than matching the picture to the corresponding object (Collett 2005; Dale et al. 2005). While secondary, environmental cues may have limited value predicting rewarding resources, spatial cues used along a route to rewarding resources are commonly used in bee navigation (Collett 2005; Dale et al. 2005; Sherry and Strang 2014; Zhang et al. 1999). Airborne scent cues likewise rely on directing attention to the corresponding flower of a known location to elicit foraging for a specific flower (Reinhard et al. 2004).

Conclusion

The results of the present study do evidence a capacity among bumblebees for learned picture cue use when foraging in a delayed matching task, under conditions of high environmental inconsistency without variation in target location. Experience is likely needed for foraging bumblebees to persist with a highly frustrating, low reward task, whereby only one stimulus is rewarding at a time and the rewarding stimulus changes constantly. This likely corresponds to a preferred reliance on primary cues, those directly presented by the potentially rewarding stimulus (flower colour, size, location etc.), instead of relying on secondary or associated cues, likely to be less predictive or reliable (e.g. presence of foliage). Switching from a preferred to a less preferred strategy has been observed in bee navigation, whereby bees will disregard landmark cues in favour of route memory unless that route memory is unreliable (Menzel 2001). Similarly, although illumination is most often disregarded as a misleading feature (which changes throughout the day), bees can learn to attend to and use this cue when it is the only predictive cue for reward (Lotto and Chittka 2005). The results of the current study likewise suggest that bumblebees prefer alternative strategies to delayed matching but can learn to use cues when foraging if preferred behaviour patterns fail in a highly inconsistent environment. Perseveration is a relatively less risky and costly method of foraging when conditions favor repeated visits to the same or similar flowers, but under conditions when perseveration is unsuccessful alternative methods, such as the use of associative cues, could be beneficial.

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General Discussion

In this two part thesis, it has been found that bumblebees (*Bombus impatiens*) can perceive both the difference and similarity between an object and corresponding image and learn to use picture cues to forage from corresponding object targets in familiar locations in a delayed matching task. It has been evidenced that bees exhibit equivalence in perception of a picture and object: (i) easily learning to differentiate an object from a corresponding picture; but also (ii) spontaneously preferring a picture of a previously rewarding object over a picture of a previously unrewarding object including (iii) exhibiting some transfer to altered, achromatic images. Furthermore, it was determined that (iv) pictures can be used as cues to corresponding targets in a delayed matching task. These results support a classification of equivalence in bumblebee picture-object correspondence (Fagot et al. 2000).

An investigation of picture-object correspondence in bees had not previously been conducted despite frequent use of 2D images and patterns in bee cognitive and perceptual research. The results of the present study do support the use of images in bee research but not the over generalization of bee response with achromatic, abstracted patterned imagery to natural, 3D floral correspondents. While a result of confusion between image and object would support generalizable results from studies using 2D stimuli to 3D correspondents, a result of equivalence is more complex. Equivalence in picture-object correspondence, perception of both a similarity and difference between image and object, is problematic for generalizing responses obtained with images to objects (flowers). It cannot be assumed that a response obtained in relation to an image, intended to represent a flower, will be the same as a response to a flower. While images may serve to investigate mechanisms of bee perception and cognition, field studies can provide an important comparison for how these mechanisms influence behaviour in a natural setting.

Investigation of bumblebee delayed matching had been previously attempted but results were inconclusive (see Sherry and Strang, 2014). The current study made use of the unique properties of a picture cue in testing delayed matching: (i) a picture provides a close correspondent to a target potentially facilitating memory and therefore matching; and (ii) a picture cue, perceived as similar but different from the target, lessens the risk of avoidance to a target following unrewarded experience with the cue. Delayed matching was found in experiment 4 and may have depended on three possible contributing factors, which differentiate the current study from other attempts to train bumblebees to a DMTS task (Dale, 2005; see review by Sherry and Strang, 2014): a history of individual foraging experience; stable stimuli location; and an environment of highly inconsistent and rare reward (see part II discussion).

Results in relation to foraging behaviour

The presented results show that a learned, specific secondary cue (an image of a target) can influence bumblebee choice when foraging in a delayed matching task. With experience a picture cue will significantly influence approach for a corresponding target in a known location. Target complexity, including the number of associated cues (multiple signals), can improve memory and recall for targets by increasing the length of exposure to a target and types of experiences associated with that target (Leonard et al., 2011). However, primary target cues may inhibit learning of more complex cues when reward is common or easily found. This inhibitory effect may be diminished when reward among multiple targets is unpredictable and relatively rare (randomly swapped between 1 of 4 targets). Under such conditions the benefit to learning and remembering a cue may out-weigh the cost.

Both the results from experiment 3 and 4 showed a diminished response in relation to ‘pink’ floral stimuli as compared with ‘yellow’. Differences in learning acquisition or response based on stimuli colour has recently been found by Jaworski et al. (2015) when despite an absence of pre-training preference by bumblebees observed to either yellow or magenta flower stimuli, following training preference only increased for yellow but not magenta flowers.

Secondary signals, separated from a target in space or time, can trigger choice patterns before the target itself is visible (Dornhaus & Chittka, 1999; Leonard et al., 2011). Perception of time can also offer a naturally occurring secondary, contextual cue to signal reward in relation to known targets. Honeybees can use the context of time as a cue for changing reward, allowing them to effectively forage among flowers which may only produce nectar at a particular time of day (Gould, 1988;Pahl et al., 2007; Zhang et al., 2007). Both scent and timing cues can signal that a target, which may not always being rewarding, is producing nectar. Likewise, images in the present study provided the only cue to reward among multiple targets frequently shifting between a state of plentiful reward to no reward.

Although secondary cues may often be disregarded in favour of primary cues when reliable and available, bumblebees are able to use secondary cues when perseveration fails. Observations made during experiment 4 coincide with behaviour patterns observed under conditions favouring sampling. The use of picture cues in a delayed matching task appeared to be limited to conditions whereby constancy was not effective due to frequent shifts in reward among the four targets. Strategies of constancy are known to outperform sampling behaviour if conditions for reward are relatively stable (Kamil & Bond, 2006). However, should strategies of perseveration fail and result in failure to obtain reward alternate strategies should be attempted (Carter & Dill, 1990) and attention broadened (Kamil & Bond, 2006). Flowers in bloom are neither always rewarding not always unrewarding. When flowers sometimes produce reward and sometimes do not, based on cycles of timing or depletion and replenishment, bees may be more likely to learn to rely on secondary cues of reward such as timing (Pahl et al., 2007; Zhang et al 2007), airborne scent (Leonard et al., 2011), the presence of other pollinators (Dawson et al. 2013; Leadbeater & Chittka, 2007 & 2009), or, in the case of the current study a picture cue.

Results in relation to cognition

As mentioned, the current results suggest that bumblebees prefer to rely on primary cues and past experience with rewarding targets over the use of secondary cues by delayed matching, but can and will learn to use such cues if preferred methods are unreliable. Switching away from a preferred to a less preferred strategy has also been observed in bee navigation, whereby bees will disregard landmark cues in favour of route memory unless that memory is unreliable (Menzel, 2001). Additionally, bees: show a preference for use of local cues (within a maze) over panoramic cues (within a room) in walking maze navigation but will use panoramic cues if local cues are removed (Jin et al., 2014); learn to navigate a maze devoid of navigational cues by memory of motor sequence only (Mirwan & Kevan, 2015); and, will use commonly disregarded cues of illumination when such cues are the only reliable predictors of reward (Clarke & Lotto, 2009; Lotto & Chittka, 2005). Changes in strategy preference can further be elicited by relative reward, while bees do not prefer complexity over simplicity when it comes to acquiring nectar, they will show a preference for complex flowers if reward is relatively greater among those flowers (Muth et al., 2015).

The role of experience observed in the current study is also in keeping with past research on bees and complex task completion or problem solving. Bees require facilitated learning or experience to master tasks of illumination discrimination, camouflaged detection, novel view transfer, rotated pattern perception, and obstruction manipulation (Clarke & Lotto, 2009; Lotto & Chittka, 2005). Although bumblebees were able to learn to use picture cues at levels significantly higher than chance in a delayed matching task, this response was only exhibited by experienced bees under conditions whereby a single rewarding target among four was changed frequently (alternating trials). Bees otherwise displayed a perseveration response pattern, when the targets were presented as successively rewarding (blocked trials).

Results in relation to picture-object correspondence

With the exception of a study of video perception in spiders (Clark & Uetz, 1990), picture-object correspondence had not previously been specifically studied among invertebrates. Reasons for the extension of this research to bees is twofold: (i) to provide comparative information about invertebrate picture-object correspondence to complement extensive vertebrate study; and (ii) evaluate common methodological practice of using 2D patterned images to test bee perception and cognition in relation to natural correspondents (flowers). In keeping with the outline of Fagot et al. (2000) bumblebees were tested for picture-object confusion, independence and equivalence, further differentiated as being spontaneous or learned (Bovet & Vauclair, 2000). Bumblebees exhibit equivalence processing, perceiving both the similarity and difference between an image and corresponding object under both spontaneous and experienced conditions. Furthermore, bumblebees appear to rely heavily on colour for this equivalence, at least for spontaneous transfer, given the diminished equivalence processing observed with achromatic images. The observed inability to spontaneously correspond objects to altered, achromatic images does not necessarily suggest that bees could not learn to associate the two stimuli. Spontaneous responses in picture perception change with experience and most instances of picture-object correspondence require some experience with both image and object (Fagot et al., 2000; Watanabe, 2000). Sea lions (*Zalophus californianus*) have recently been found able to use mirrors to locate corresponding objects in both familiar and novel locations but this ability is dependent upon experience with the task (Hill et al., 2015). Even human beings, from societies and environments without representational imagery are unable to spontaneously decipher the content of representational images, including photographs, of familiar people (Deregowski, 2000).

The use of pictures as cues in a delayed matching task also coincided with related theories and research on perception of cast shadows (Dee & Santos, 2011). Use of a picture cue during foraging appeared to be disregarded until reward was highly inconsistent and rare. As with cast shadows, bees may disregard a picture cue as ‘noise’ while perseveration and constancy successfully resulted in frequent reward but attend to the picture cue under rapidly changing conditions, whereby the picture cue provided the only reliable information about reward location.

Future Directions and Significance

Secondary cue use by bumblebees in delayed matching has been further clarified by the successful picture cue matching elicited in experiment 4. It remains to be resolved if bumblebees can learn to match a cue to a target with a changing location in a DMTS task. The confined space of a radial arm maze may not suitably represent natural variation in flower placement and growth, but instead disorient bees with an unnatural challenge of moving familiar stimuli within a familiar environment. It is suggested that field experiments with delayed matching to sample may benefit from a more natural test of cue to target match controlling for location. A field study of DMTS could test cue to target matching while controlling for location by mimicking the natural growth and death of similar flowers within a complex and large environment. Secondly, although it appears that a picture can trigger an associated memory for, and therefore approach to, a corresponding target, it has not yet been determined if this influence occurs prior to or after the target comes into view. Given the role of stable location, it is likely that the trigger begins to influence approach behaviour before the bee 'sees' the target, if not changing location should have a limited effect on 'matching'.

Most importantly it remains to be tested, in field application, if cues may be used to significantly influence foraging behaviour within an agricultural or natural setting. The current results suggest that cues may be used to direct experienced foragers towards known locations but not necessarily novel floral targets or crops specifically. The ability to cue location, as crops or resources change, could significantly improve pollinator efficiency in agriculture or colony resource collection in at-risk areas. In fact, cueing location can allow for greater influence than novel cue-target matching. A bee that has learned that cue signals location (ie. cue A signals reward in patch A) is likely to outperform a bee that learns to match a cue with only a particular target (i.e. a picture of cue A signals reward with flower A). The latter form of cued matching would require that the corresponding target be in sight, whereas the former can cue location from potentially large distances (similar to a landmark cue) and without the need for specific cue-target experience with new flowers. Such a method may prove useful given the fluctuation in bloom cycles, different areas of a farm or natural environment may be signalled as different flowers come into and out of bloom.

Addressing challenges, such as those posed by climate change or the artificial demands of agricultural pollination, with a relatively sudden onset and frequently changing conditions, may be aided by cognitive behavioural solutions. The effects of climate change, chemical pollution and habitat destruction, may occur too quickly to be overcome by typical adaptation, resulting in the loss of vulnerable species, including some species of bumblebees (Kerr et al., 2015). While evidence shows that bees may be failing to adapt to warming temperatures (Kerr et al., 2015) or avoid harmful chemicals (Goulson, 2015; Kessler et al., 2015), a wealth of research has also exposed a vast capacity for learning and flexible behaviour. Facilitation of bee survival and a continued role as a domesticated pollinator could benefit from cognitive behavioural solutions, such as the use of secondary cues to influence foraging. Cues may be used to facilitate locating of scarce resources, avoidance of harmful resources (such as those laced with pesticides and fungicides), and improve efficiency of more sustainable, multi-crop farms.

References

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

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. *London B: Biological Sciences, 282*, 20142384.

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

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

Bateson, M., Desire, S., Gartside, S.E., & Writing, G.A. (2011). Agitated honeybees exhibit pessimistic cognitive biases. *Current Biology, 21*, 1070-1073.

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*, 257-270.

Bitterman, M.E. (1996). Comparative analysis of learning in honeybees. *Animal Learning and Behavaviour, 24*, 123-141.

Bovet, D., & Vauclair, J. (2000). Picture recognition in animals and humans. *Behavioral Brain Research, 109*, 143-165.

Braje, W.L., Legge, G.E., & Kersten, D. (2000). Invariant recognition of natural objects in the presence of shadows. *Perception, 29*, 383-398.

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

Brodbeck, D.R. & Shettleworth, S.J. (1995). Matching location and color of a compound stimulus: Comparison of a food-storing and a nonstoring bird species. *Journal of Experimental Psychology: Animal Behavior Processes*, *21*(1), 64-77.

Brown, M.F., & Demas, G.E. (1994). Evidence for spatial working memory in honeybees (*Apis mellifera*). *Journal of Comparative Psychology, 108*, 344-352.

Brown , M.F., McKeon, D., Curley, T., Weston, B., Lambert, C. & Lebowitz, B. (1998). Working memory for color in honeybees. *Animal Learning and Behavior, 26* (3), 264-271.

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

Cartar, R.V., & Dill, L.M. (1990). Why are bumble bees risk sensitive foragers? *Behavioral Ecology and Sociobiology*, *26*, 121-127.

Casati, R. (2006). The cognitive science of holes and cast shadows. *TRENDS in Cognitive Sciences*, *10*(2), 54-55.

Casati, R. (2008). The copycat solution to the shadow correspondence problem. *Perception, 37*, 495-503.

Cheng, K. (2000). How honeybees find a place: Lessons from a simple mind. *Animal Learning and Behavior, 28*, 1-15.

Cheng, K. & Wignall, A.E. (2005). Honeybees (*Apis mellifera*) holding on to memories: response competition causes retroactive interference effects. *Animal Cognition, doi 10.1007/s10071-005-0012-5.*

Chittka, L. & Geiger, K. (1995). Can honey bees count landmarks? *Animal Behavior, 49*, 159-

164.

Chittka, L.& Jensen, K. (2011). Animal cognition: Concepts from apes to bees. *Current Biology, 21*(3), R116-R119.

Chittka, L., & Raine, N.E. (2006). Recognition of flowers by pollinators. *Current Opinion in Plant Biology, 9*, 428-435.

Chittka, L., Thomson, J.D. & Waser, N.M. (1999). Flower constancy, insect psychology, and plant evolution. *Naturwissenchaften, 86*(8), 361-377.

Chittka, L., & Thomson, J.D. (2001). *Cognitive ecology of pollination*. Cambridge: Cambridge University Press.

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

Clarke, R., & Lotto, R.B. (2009). Visual processing of the bee innately encodes higher-order image statistics when the information is consistent with natural ecology. *Vision Research, 49*, 1455-1464.

Clark, D.L. & Uetz, G.W. (1990). Video image recognition by the jumping spider, *Maevia inclemens (*Araneae: Salticidae). *Animal Behaviour*, *40*(5), 884-890.

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,* 278-285.

Collett, T.S. (2005). Invertebrate Memory: Honeybees with a sense of déjà vu. *Current Biology, 15(11),* R419-R421.

Cook, R.G., Katz, J.S., & Kelly, D.M. (2000). Pictorial same-different categorical learning and discrimination in pigeons. In Fagot, J. (Ed.), *Picture perception in animals*. (pp . 181-218). East Sussex, UK. Psychology Press Ltd.

Cooke, M.H.Y., Couvillon, P.A. & Bitterman, M.E. (2007). Delayed symbolic matching in Honeybees (*Apis mellifera*). *Journal of Comparative Psychology, 121*(1), 106-108.

Dale, K., Harland, D.P., Manning-Jones, A., & Collett, T.S. (2005). Weak and strong priming cues in bumblebee contextual learning. *Journal of Experimental Biology, 208*, 65-74.

Dawson, E.H., Avarguès-Weber, A., Chittka, L. & Leadbeater, E. (2013). Learning by observation emerges from simple associations in an insect model. *Current Biology, 23*, 727-730.

Dee, H. M., & Santos, P.E. (2011). The perception and content of cast shadows: An interdisciplinary review. *Spatial Cognition & Computation, 11*, 226-253.

Defrize, J., Llandres, A.L.& Casas, J. (2014). Indirect cues in selecting a hunting site in a sit-and-wait predator. *Physiological Entomology*, *39*, 53-59.

Delius, J.D., Emmerton, J., Horster, W., Jager, R., & Ostheim, J. (2000). Picture-object recognition in pigeons. In: Fagot, J. (Ed.), *Picture perception in animals*. Psychology Press Ltd, East Sussex, UK, pp 1-36.

Deregowski, J.B. (2000). Pictorial perception: individual and group differences within the human species. In: Fagot, J. (Ed.), *Picture perception in animals*. Psychology Press Ltd, East Sussex, UK, pp 397-429.

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

Dukas, R. (1998). Constraints on information processing and their effects on behavior. In: Dukas, R. (Ed.), *Cognitive ecology: The evolutionary ecology of information processing and decision making*. The University of Chicago Press, Chicago and London, pp 89-119.

Dukas, R. (2002). Behavioural and ecological consequences of limited attention. *Philosophical Transactions of the Royal Society of Biological Sciences, 357*, 1539-1547.

Dukas, R. (2008). Evolutionary biology of insect learning. *Annual Review of Entomology, 53,* 145-160.

Dukas R (2009) Learning: Mechanisms, ecology, and evolution. In: Dukas R and Ratcliffe JM (eds) *Cognitive ecology II*. The University of Chicago Press, Chicago and London, pp 7-26.

Dukas, R. & Real, L.A. (1993). Effects of recent experience on foraging decisions by bumble bees. *Oecologia, 94*, 244-246.

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

Dyer, A.G. (2012). The mysterious cognitive abilities of bees: why models of visual processing need to consider experience and individual differences in animal performance. *Journal of Experimental Biology, 215,* 387-395.

Dyer, A.G., & Vuong, Q.C. (2008). Insect brains use image interpolation mechanisms to recognise rotated objects. *PLOS One, 3*: e4086.

Enquist, M., & Arak, A. (1998). Neural representation and the evolution of signal form. In: Dukas, R. (Ed.), *Cognitive ecology: The evolutionary ecology of information processing and decision making*. The University of Chicago Press, Chicago, USA, pp 21-88.

Fagot, J., Martin-Malivel, J., & Depy, D. (2000). What is the evidence for an equivalence between objects and pictures in birds and non-human primates? In: Fagot, J. (Ed.), *Picture perception in animals*. Psychology Press Ltd, East Sussex, UK, pp 295-320.

Fagot, J., Thompson, R.K.R. & Parron, C. (2010). How to read a picture: Lessons from nonhuman primates, *PNAS*, *107*(2), 519-520.

Fauria, K., Dale, K., Colborn, M., & Collett, T.S. (2002). Learning speed and contextual isolation in bumblebees. *The Journal of Experimental Biology*, *205,* 1009-1018.

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

Giurfa, M. (2003). Cognitive neuroethology: dissecting non-elemental learning in a honeybee brain. *Current Opinion in Neurobiology, 13*, 726-735.

Giurfa, M. (2007). Behavioral and neural analysis of delayed learning in the honeybee: a taste from the magic well. *Journal of Comparative Physiology, 193*, 801-824.

Giurfa, M. (2013). Cognition with few neurons: Higher order learning in insects. *Trends in Neurosciences,36*(5), 285-294.

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

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

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

Gould, J.L. (1988). Timing of landmark learning by honey bees. *Journal of Insect Behavior, 1*(4), 373-377.

Gould, J.L. (1990). Honey bee cognition. *Cognition, 37*, 83-103.

Gould, J.L. (2002). Can honey bees create “Cognitive Maps”? In: Bekoff, M. Allen, C. & Burghardt, M. (Eds.). *The Cognitive Animal: Empirical and Theoretical Perspectives on Animal Cognition*. MIT Press, Cambridge, MA, pp. 41–45.

Goulson, D., Nicholls, E., Botías, C. & Rotheray, E.L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science, 346* (6229), 1255957-1-9.

Gross, H.J., Pahl, M., Si, A., Zhu, H., Tautz, J. & Zhang, S. (2009). Number-based visual generalisation in the Honeybee. *Plos one, 4*(1), e4263.

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

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

Hill, H. M., Webber, K., Kemery, A., Garcia, M. & Kuczaj, S.A. (2015). Can sea lions' (*Zalophus Californianus*) use mirrors to locate an object? *International Journal of Comparative Psychology, 28*, 1-12.

Horridge, A. (1997). Spatial and non-spatial coding of patterns by the honey-bee. In: *From living eyes to seeing machines*. Oxford University Press, Oxford, pp. 52-79.

Horridge, A. (1999). Two-dimensional pattern discrimination by the honeybee. *Physiological Entomology, 24*, 197-212.

Horridge, A. (2007). The preferences of the honeybee (*Apis mellifera)* for different visual cues during the learning process. *Journal of Insect Physiology, 53*, 877-889.

Huber, L. (2000). Generic perception: open-ended categorization of natural classes. In: Fagot, J (Ed.), *Picture perception in animals*. Psychology Press Ltd, East Sussex, UK, pp 219-262.

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

Isnec, M.R., Couvillon, P.A. & Bitterman, M.E. (1997). Short-term spatial memory in honeybees. *Animal Learning & Behaviour*, *25*(2), 165-170.

Jaworski, C.C., Andalo, C., Raynaud, C., Simon, V., Thébaud, C. & Chave, J. (2015). The influence of prior learning experience on pollinator choice: An experiment using bumblebees on two wild floral types of *Antirrhinum majus. PLoS ONE, 10*(8), e0130225. doi: 10.1371/journal.pone.0130225

Jin, N., Landgraf, T., Klein, S. & Menzel, R. (2014). Walking bumblebees memorize panorama and local cues in a laboratory test of navigation. *Animal Behavior, 97,* 13-23.

Kamil, A.C., & Bond, A.B. (2006). Selective attention, priming and foraging behavior. In: Wasserman, EA and Zentall, TR (Eds.) *Comparative cognition: Experimental explorations of animal intelligence*. Oxford University Press, Oxford, pp 106-126.

Kamin, L.J. (1969). Predictability, surprise, attention, and conditioning. In: Campbell, BA and Church, RM (Eds.), *Punishment and aversive behavior*. Meredith Corporation, New York, pp 279-296.

Kaczorowski, R.L., Leonard, A.S., Dornhaus, A., & Papaj, D.R. (2012). Floral signal complexity as a possible adaptation to environmental variability: a test using nectar-foraging bumblebees, *Bombus impatiens*. *Animal Behaviour, 83,* 905-913.

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

Kendal RL, Coolen I, Laland KN (2009) Adaptive trade-offs in the use of social and personal information. In: Dukas R (ed) *Cognitive Ecology II*. The University of Chicago Press, Chicago and London, pp 249-271

Kerr, J.T., Pindar, A., Galpern, P., Packer, L., Potts, S.G., Roberts, S.M., Rasmont, P., Schweiger, O., Colla, S.R., Richardson, L.L., Wagner, D.L., Gall, L.F., Sikes, D.S. & Pantoja, A. (2015). Climate change impacts on bumblebees converge across continents. *Science, 349* (6244), 177-180.

Kessler, S.C., Tiedeken, E.J., Simcock, K.L., Derveau, S., Mitchell, J., Softley, S., Stout, J.C. & Wright, G.A. (2015). Bees prefer foods containing neonicotinoid pesticides. *Nature, 521,* 74-78.

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

Kulahci, I.G., Dornhaus, A., & Papaj, D.R. (2008). Multimodal signals enhance decision making in foraging bumble-bees. *Proceedings of the Royal Society: Biological Sciences, 275,* 797-802.

Leadbeater, E. & Chittka, L. (2007). The dynamics of social learning in an insect model, the bumblebee (*Bombus terrestris*). *Behavioral Ecology and Sociobiology, 61*, 1789-1796.

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

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

Leighty, K.A., Menzel, C.R., & Fragaszy, D.M. (2008). How young children and chimpanzees (*Pan troglodytes*) perceive objects in a 2D display: putting an assumption to the test. *Developmental Science*, *11*(5), 778-792.

Leonard, A.S., Dornhaus, A., & Papaj, D.R. (2011). Forget-me-not: Complex floral displays, inter-signal interactions, and pollinator cognition. *Current Zoology, 57*(2), 215-224.

Lind, J., Enquist, M., & Ghirlanda, S. (2014). Animal memory: A review of delayed matching-to-sample data. *Behavioural Processes,* http://dx.doi.org/10.1016/j.beproc.2014.11.019

Lotto, R.B., & Chittka, L. (2005). Seeing the light: Illumination as a contextual cue to color choice behavior in bumblebees. *PNAS, 102*: 3852-3856.

Mammasian, P. (2004). Impossible shadows and the shadow correspondence problem. *Perception, 33*, 1279-1290.

McLinn, C.M., & Stephens, D.W. (2006). What makes information valuable: signal reliability and environmental uncertainty. *Animal Behaviour, 71,* 1119-1129.

Menzel, R. (2001). Searching for the memory trace in a mini-brain, the honeybee. *Learning and Memory, 8,* 53-62.

Mirwan, H.B. & Kevan, P.G. (2014). Problem solving by worker bumblebees *Bombus impatiens* (Hymenoptera: Apidae). *Animal Cognition, 17,* 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,* 345-357.

Moreno, A.M., das Graças de Souza, & D. Reinhard, J. (2012). A comparative study of relational learning capacity in honeybees (*Apis mellifera*) and stingless bees (*Melipona rufiventris*). *Plos one*, *7*(12), e51467.

Munoz, N.E., & Blumstein, D.T. (2012). Multisensory perception in uncertain environments. *Behavioral Ecology, 23,* 457-462.

Muth, F., Keasar, T. & Dornhaus, A. (2015). Trading off short-term costs for the long-term gains: how do bumblebees decide to learn morphologically complex flowers? *Animal Behaviour*, *101*, 191-199.

Nicholls, C.I., & Altieri, M.A. (2013). Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. *Agronomy for sustainable development, 33,* 257-274.

Osborne, J.L., & Williams, I.H. (2001). Site constancy of bumble bees in an experimentally patchy habitat. *Agriculture, Ecosystems and Environment, 83*, 129-141.

Pahl, M., Zhu, H., Pix, W., Tautz, J. & Zhang, S. (2007). Circadian timed episodic-like memory- a bee knows what to do when, and also where. *Journal of Experimental Biology, 210,* 3559-3567.

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.

Peissig, J.J., Young, M.E., Wassterman, E.A., & Biederman, I. (2000). The pigeon's perception of depth-rotated shapes. In: Fagot, J. (Ed.), *Picture perception in animals*. Psychology Press Ltd, East Sussex, UK, pp . 37-70.

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

Plowright, C.M.S., Evans, S., Chew Leung, J., & Collin, C.A. (2011). The preference for symmetry in flower-naïve and not-so-naïve bumblebees. *Learning and Motivation, 42*, 76-83.

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.

Raguso, R.A. (2004). Flowers as sensory billboards: progress towards an integrated understanding of floral advertisement. *Current Opinion in Plant Biology*, *7,* 434-440.

Raine, N.E. & Chittka, L. (2005). Comparison of flower constancy and foraging performance in three bumblebee species (*Hymenoptera: Apidae: Bombus*). *Entomologia Generalis, 28*(2), 081.

Raine, N.E. & Chittka, L. (2007). Flower constancy and memory dynamics in bumblebees (*Hymenoptera: Apidae: Bombus*). *Entomologia Generalis, 29*(2/4), 179.

Reinhard, J., Srinivasan, M.V., Guez, D. & Zhang, S.W. (2004). Floral scents induce recall of navigational and visual memories in honeybees. *Journal of Experimental Biology, 207,* 4371-4381.

Reinhard, J., Mandyam, V. & Srinivasan, S.Z. (2006). Complex memories in honeybees: can there be more than two? *Journal of Comparative Physiology, 192,* 409-416.

Rescorla, R.A. & A.R. Wagner (1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In: Black, A.H. & Prokasy, W.F. (Eds.) *Classical Conditioning II: Current Research and Theory*. Appleton-Century-Crofts, New York, pp 64-99.

Riffell, J.A. (2011). The nueroecology of a pollinator's buffet: Olfactory preferences and learning in insect pollinators. *Integrative and Comparative Biology, 51*, 781-193.

Rodríguez, I., Gumbert, A., Hempel de Ibbarra, 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. (1998). How do bees learn and 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,* 1-7.

Sherry, D.F., & Strang, C.G. (2014). Contrasting styles in cognition and behaviour in bumblebees and honeybees. *Behavioural Processes, doi:10.1016.*

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

Spetch, M.L. & Friedman, A. (2006). Pigeons see correspondence between objects and their pictures. *Psychological Science, 17*(11), 966-972.

Srinivasan, M.V. (2010). Honey bees as a model for vision, perception, and cognition. *Annual Review of Entomology, 55*, 267-284.

Srinivasan, M.V., & Lehrer, M. (1984). Temporal acuity of honeybee vision: behavioural studies using moving stimuli. *Journal of Comparative Physiology, 155,* 297-312.

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

Stout, J.S. & Goulson, D. (2001). The use of conspecific and interspecific scent marks by foraging bumblebees and honeybees. *Animal Behavior, 62*,183-189.

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.

van Hest, A., & Steckler, T. (1996). Effects of procedural parameters on response accuracy: lessons from delayed (non-)matching procedures in animals. *Cognitive Brain Research, 3*, 193-203.

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

Weinberg, D. & Plowright, C.M.S. (2006). Pollen collection by bumblebees (*Bombus impatiens*): the effects of resource manipulation, foraging experience and colony size. *Journal of Apicultural Research and Bee World, 45*, 22-27.

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

Woteki, C. (2013). The road to pollinator health. *Science*, *341*, 695

Wu, W., Moreno, A.M., Tangen, J.M., & Reinhard, J. (2013). Honeybees can discriminate between Monet and Picasso paintings. *Journal of Comparative Physiology*, DOI 10.1007/s00359-012-0767-5.

Ydenberg, R. (1998). Behavioral decisions about foraging and predator avoidance. In: Dukas, R. (Ed.), *Cognitive ecology: The evolutionary ecology of information processing and decision making*. The University of Chicago Press, Chicago, USA, pp343-378.

Zhang, S., Bock, F., Si, A., Tautz, J. & Srinivasan, M.V. (2005). Visual working memory in decision making by honeybees. *PNAS, 102*(14), 5250-5255.

Zhang, S.W., Lehrer, M., Srinivasan, M.V. (1999). Honeybee memory: Navigation by associative grouping and recall of visual stimuli. *Neurobiology of Learning and Memory, 72,* 180-201.

Zhang, S.W. & Srinivasan, M.V. (1994). Prior experience enhances pattern discrimination in insect vision. *Nature, 368*, 330-332.

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

Zhang, S., Schwarz, S., Pahl, M., Zhu, H. & Tautz, J. (2006). Honeybee memory: a honeybee knows what to do and when. *Journal of Experimental Biology*, *209*, 4420-4428.

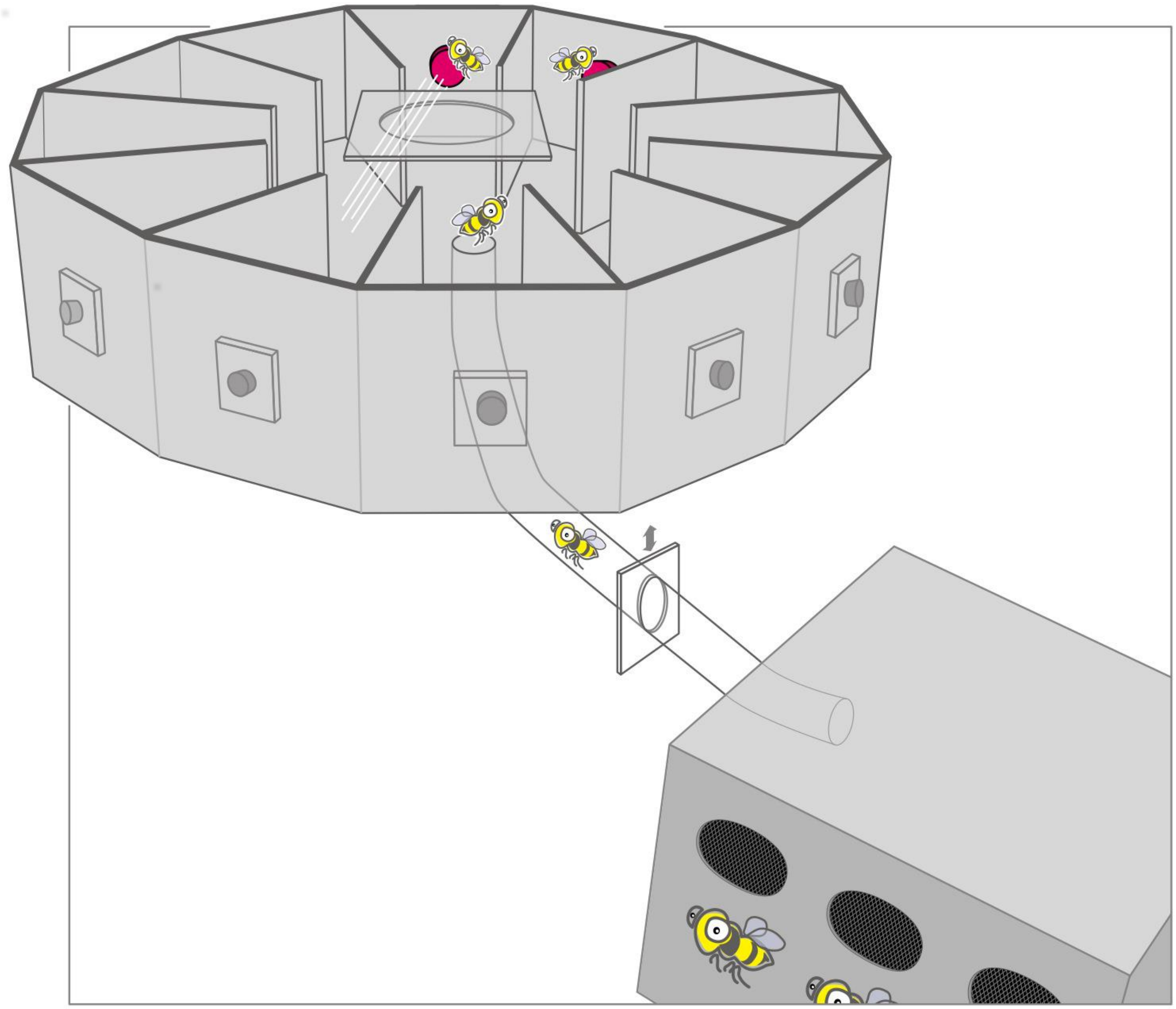


Fig. 1: Radial maze and connected hive box. Stimuli (photographs or objects) were placed at the ends of the corridors in the positions marked with a red circle.

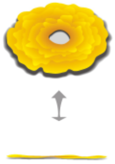
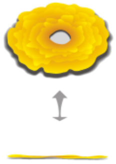
Experiment 1: Confusion	**Object+/Picture- (O+/P-)**		**Picture+/Object- (P+/O-)**	
Training	 Object A+	 Photo A-	 Object A-	 Photo A+
Testing	Unrewarded object A & unrewarded photo A			
Experiment 2: Independence	**Object A+/Object B- (OA+/OB-)**		**Object B+/Object A- (OB+/OA-)**	
Training	 Object A+	 Object B-	 Object A-	 Object B+
Testing	Unrewarded photo A & photo B			
Experiment 3: Equivalence	**Object A+/Object B- (OA+/OB-)**		**Object B+/Object A- (OB+/OA-)**	
Training	 Object A+	 Object B-	 Object A-	 Object B+
Testing	Unrewarded pictures: Black and white silhouettes & drawn images of objects A & B			
	 Silhouette A	 Silhouette B	 Drawn A	 Drawn B

Fig. 2: Experimental design for experiments 1, 2 and 3.

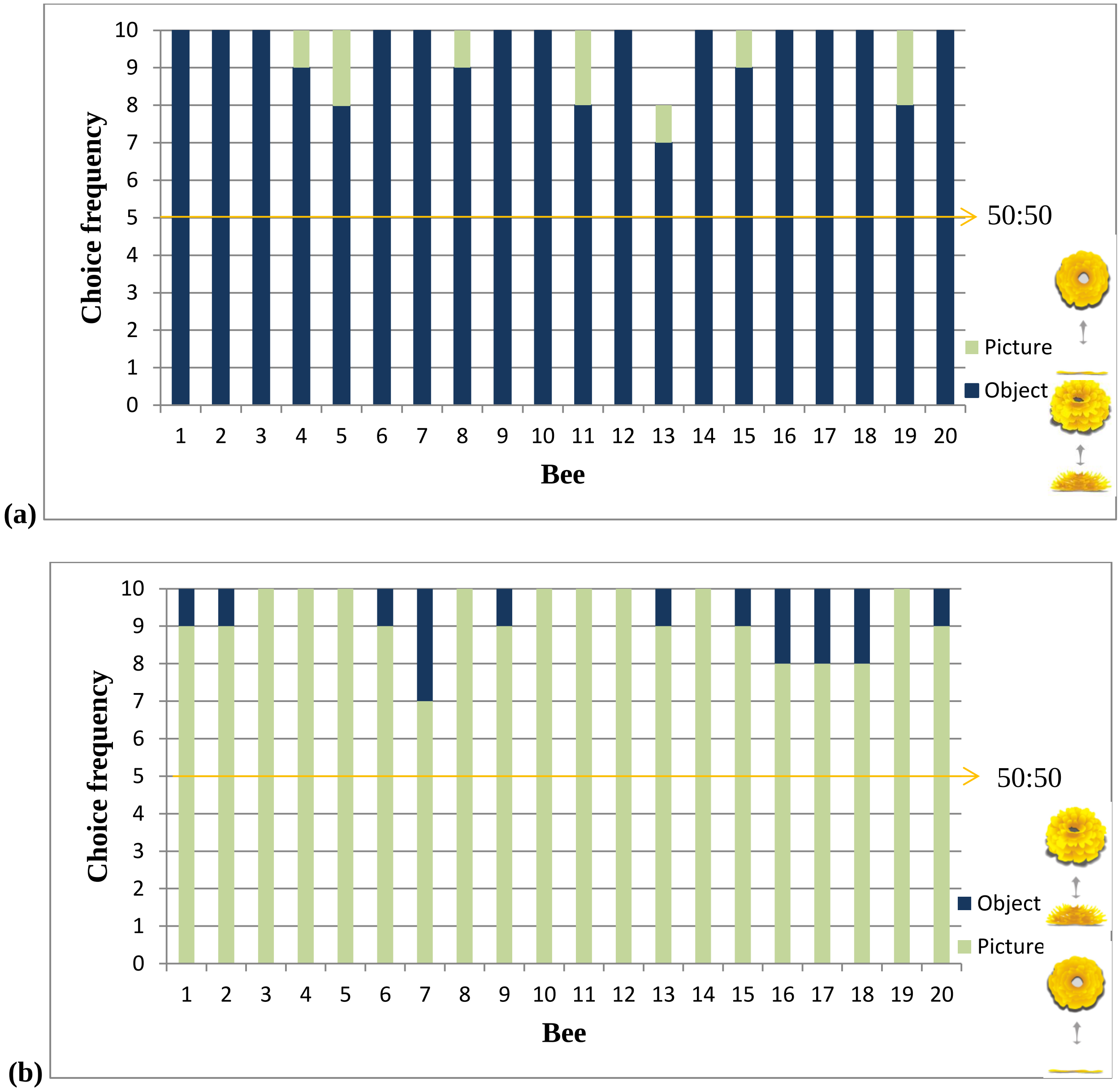


Fig. 3 Choice frequencies in experiment1 of unrewarding objects *versus* unrewarding pictures by individual bees compared to a chance value of 50:50. Two groups were given different discrimination training: (a) Group O+/P- (b) Group P+/O-.

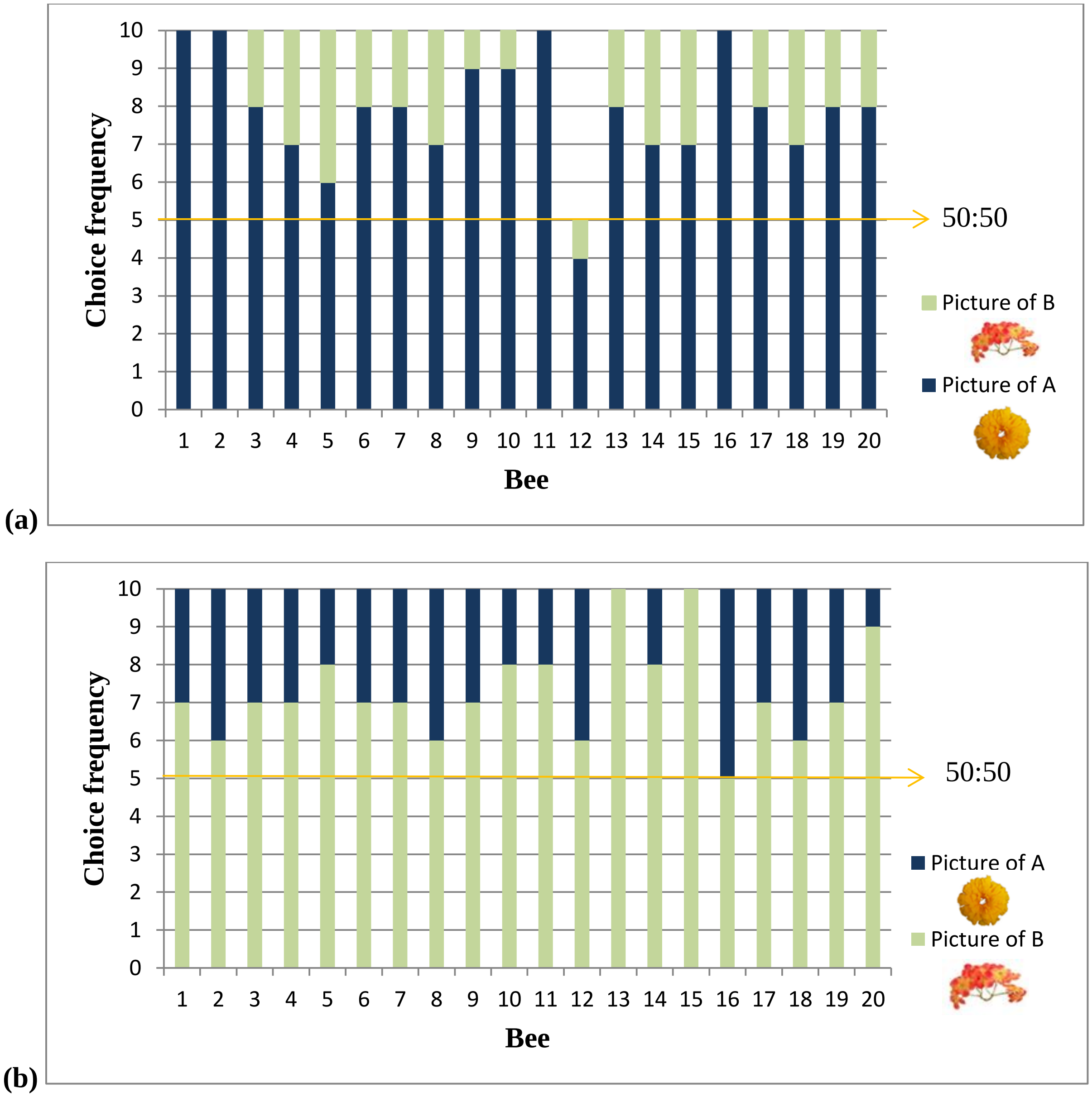
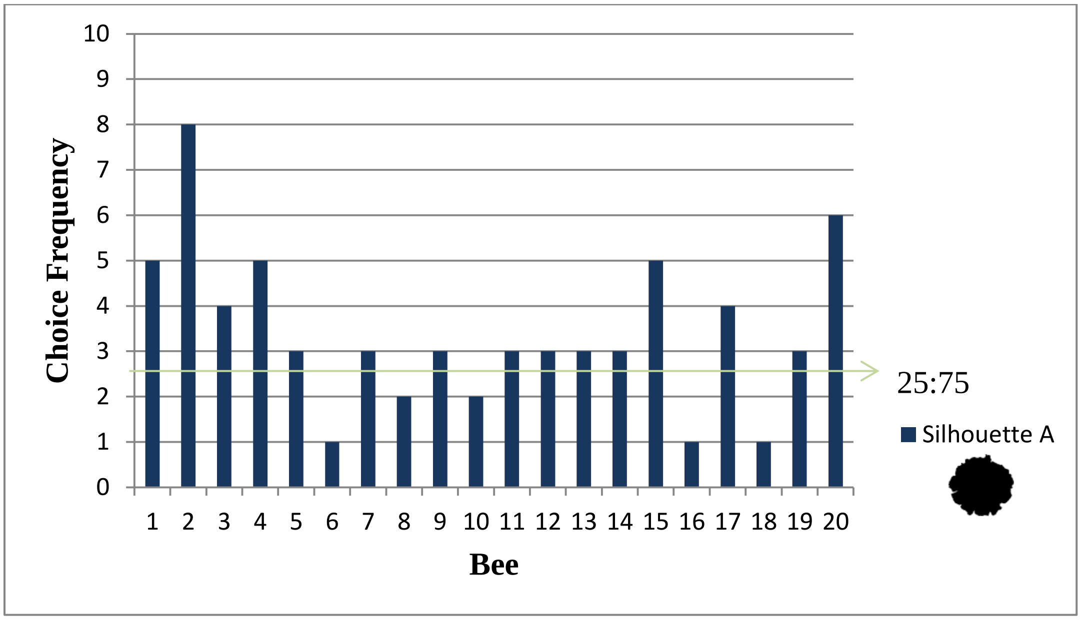
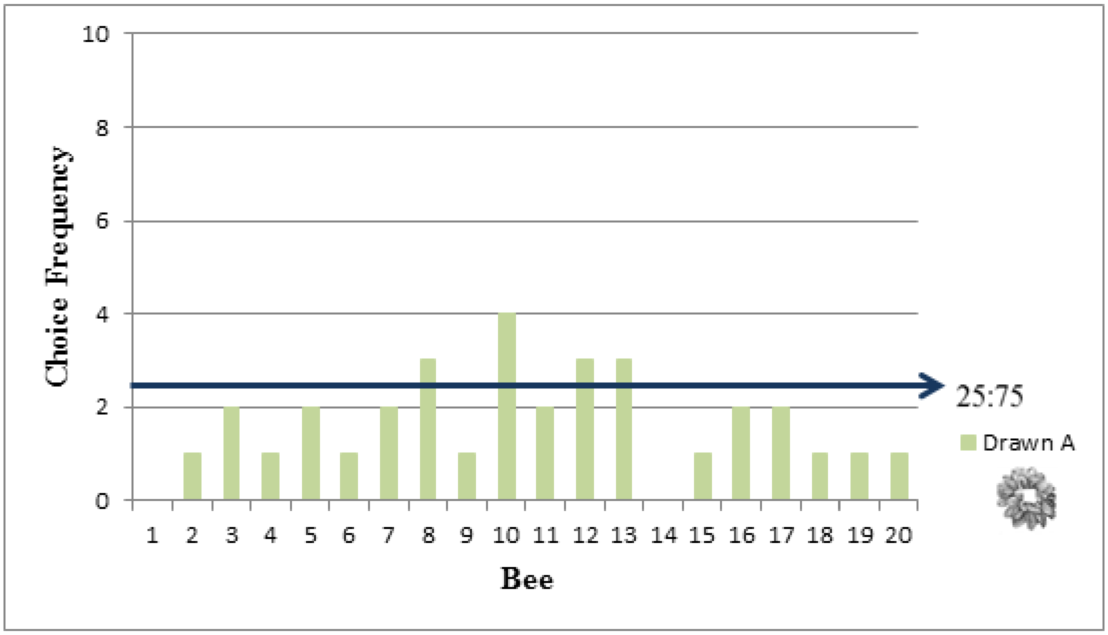


Fig. 4 Choice frequencies in experiment 2 of unrewarding pictures by individual bees compared to a chance value of 50:50. Two groups were given training on different object discriminations**:** (a) Group OA+/OB- (b) Group OB+/OA- .


Choice Frequency
10
9
8
7
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Bee
25:75
Silhouette A

(a)


Choice Frequency
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Bee
25:75
Drawn A

(b)

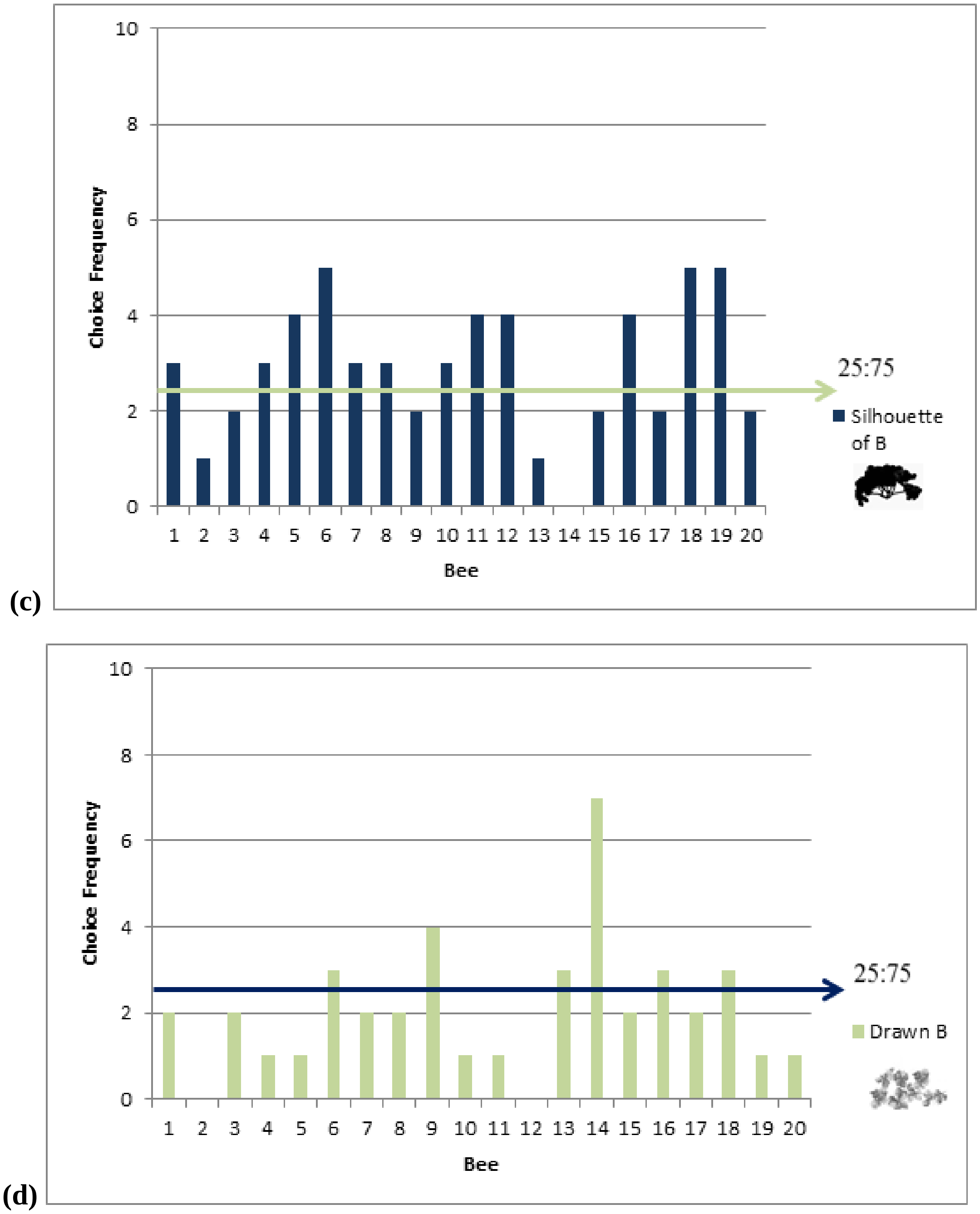
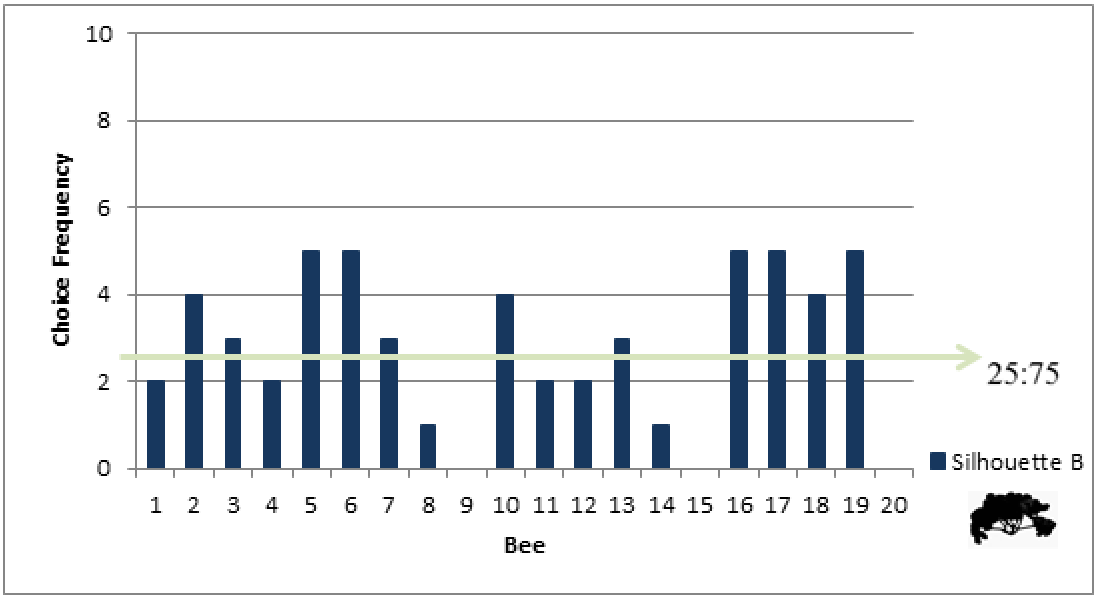


Fig. 5 Choice frequencies in experiment 3 of unrewarding altered pictures by individual bees in one group (OA+/OB-) compared to a chance value of 25:75 (a) a significant preference for the silhouette of A (b) a significant avoidance for the drawn image of A (c) no significant difference from chance for the silhouette of B (d) no significant difference from chance for the drawn image of B.


10
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2
0
Choice Frequency
1
2
3
4
5
6
7
8
9
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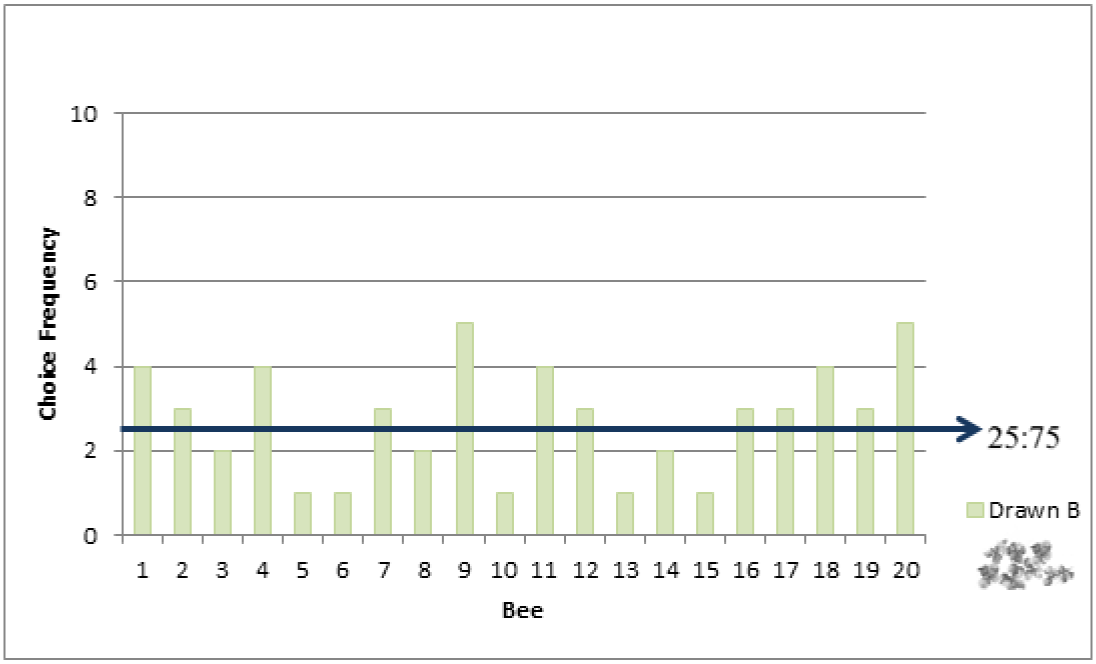
11
12
13
14
15
16
17
18
19
20
Bee
25:75
Silhouette B

a)


10
8
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Choice Frequency
1
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3
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5
6
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15
16
17
18
19
20
Bee
25:75
Drawn B

b)

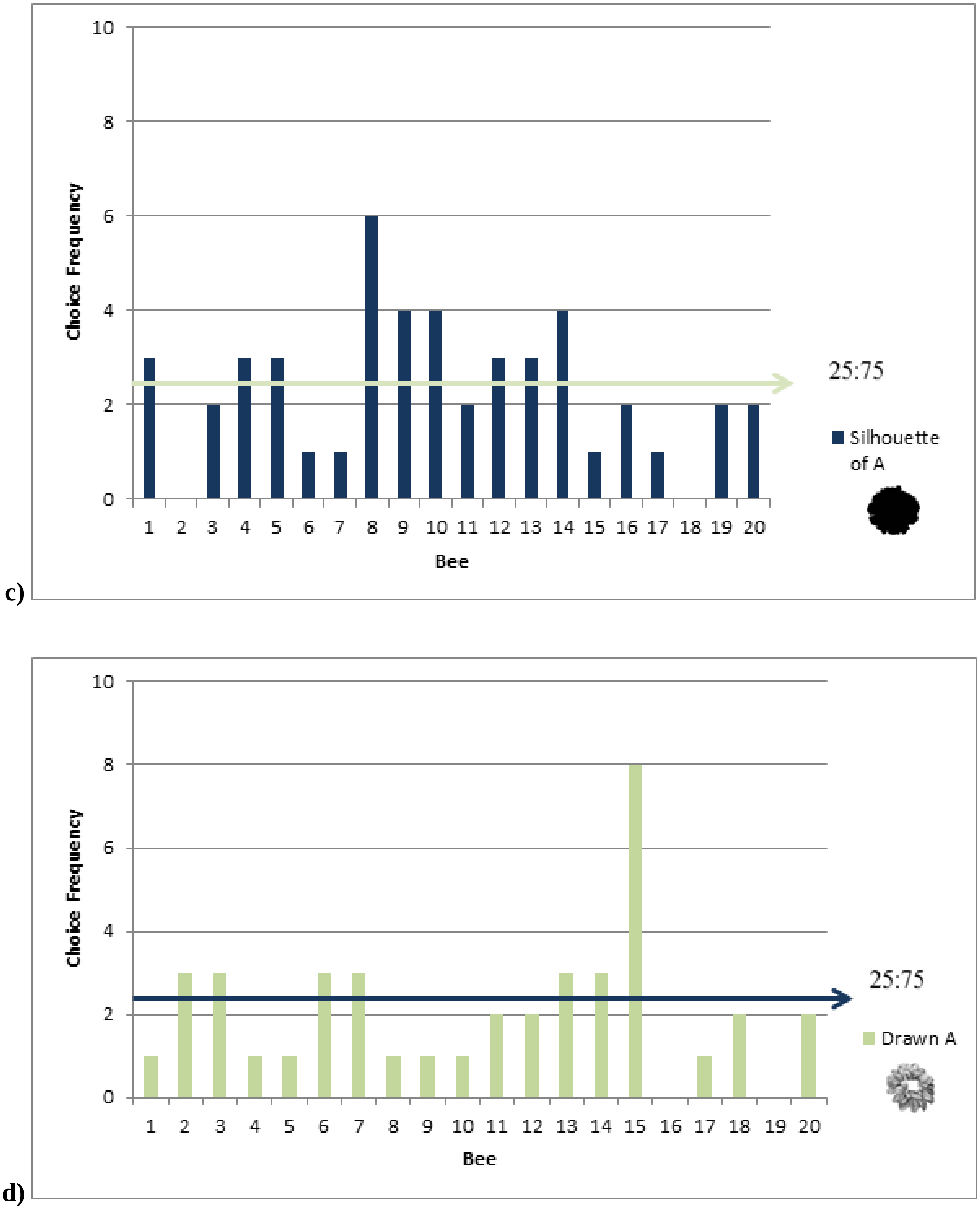

Fig. 6 Choice frequencies in experiment 3 of unrewarding altered pictures by individual bees in one group (OB+/OA-), compared to a chance value of 25:75**.** No significant preferences were exhibited.

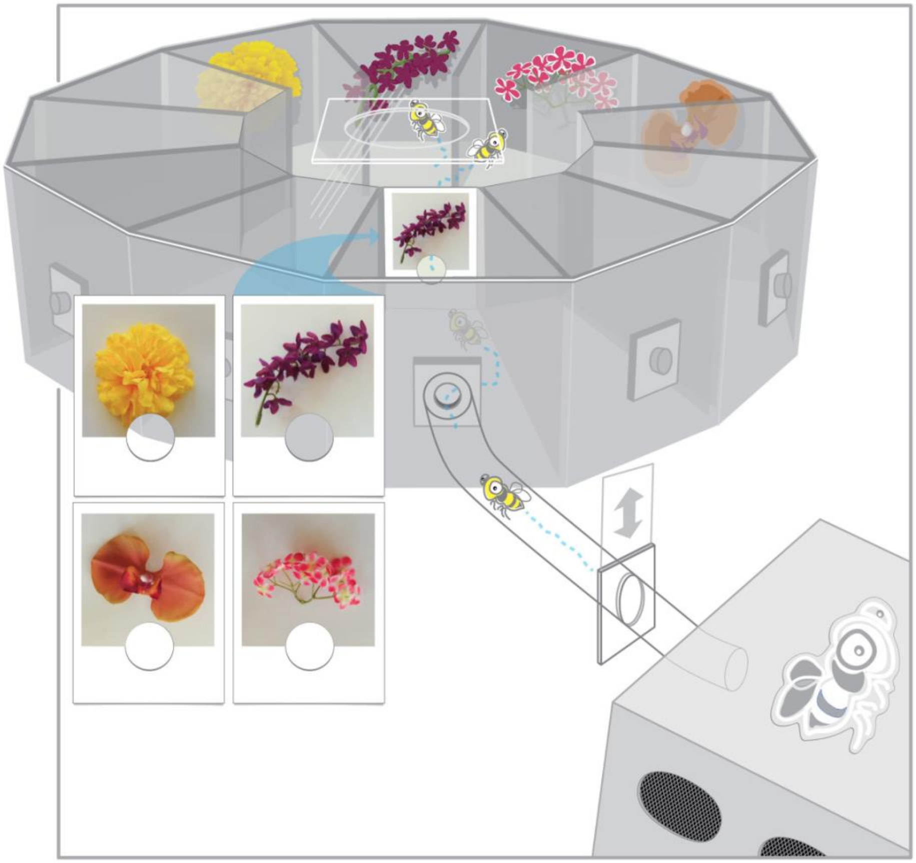

Fig. 7 Radial arm maze with connected hive box used in in experiment 4. A photograph cue was placed on a gated entry way to the centre of the maze, requiring bees to fly through or near the image before entering the radial maze. Four of the corridors were open with artificial flowers placed on the end wall of each surrounding a feeder trough. The picture cue viewed upon entering the maze corresponded to a matching object in a stable location within one of the maze corridors rewarded with sugar solution. For the three ‘flowers’ that did not match the photograph cue, the feeder troughs were empty.

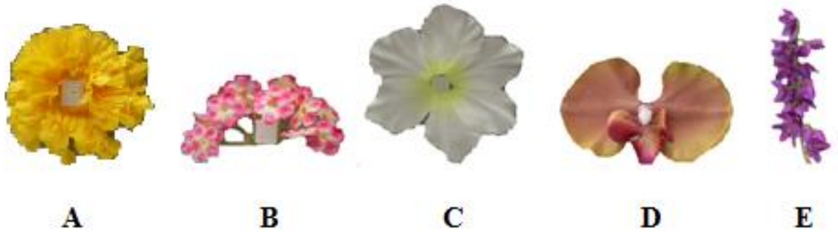

Fig. 8 Four of the five artificial flower stimuli were used for each colony (stimuli A,B,D & E for colony 1 and A,B,C & D for colonies 2 and 3) in experiment 4. An unaltered photograph of each was used as a cue to signal reward at the corresponding object.

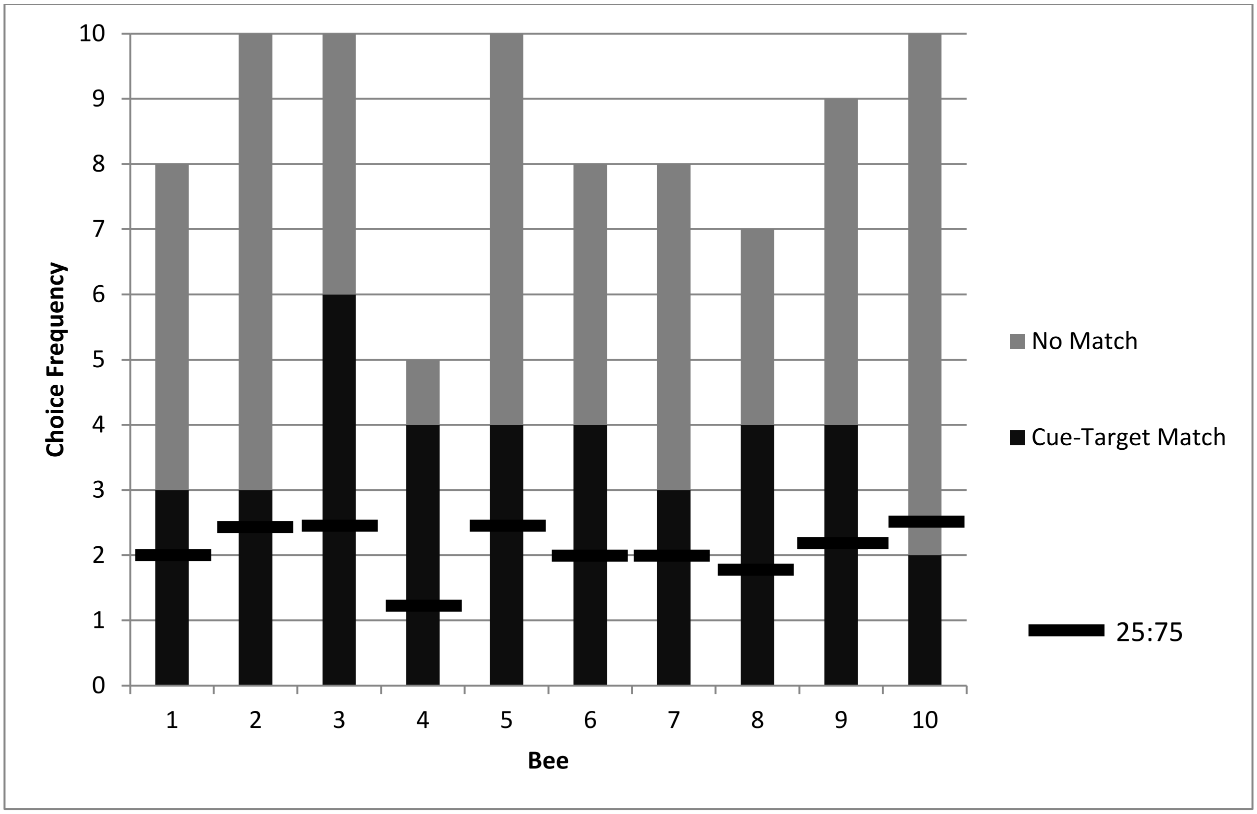

Fig. 9 Choice frequencies of rewarded testing for 10 bees in colony 1 in experiment 4 showed a significant preference for object stimuli corresponding to a picture cue when compared to a chance value of 25:75. No significant individual differences were found, but the group choice proportion was significantly higher than chance.

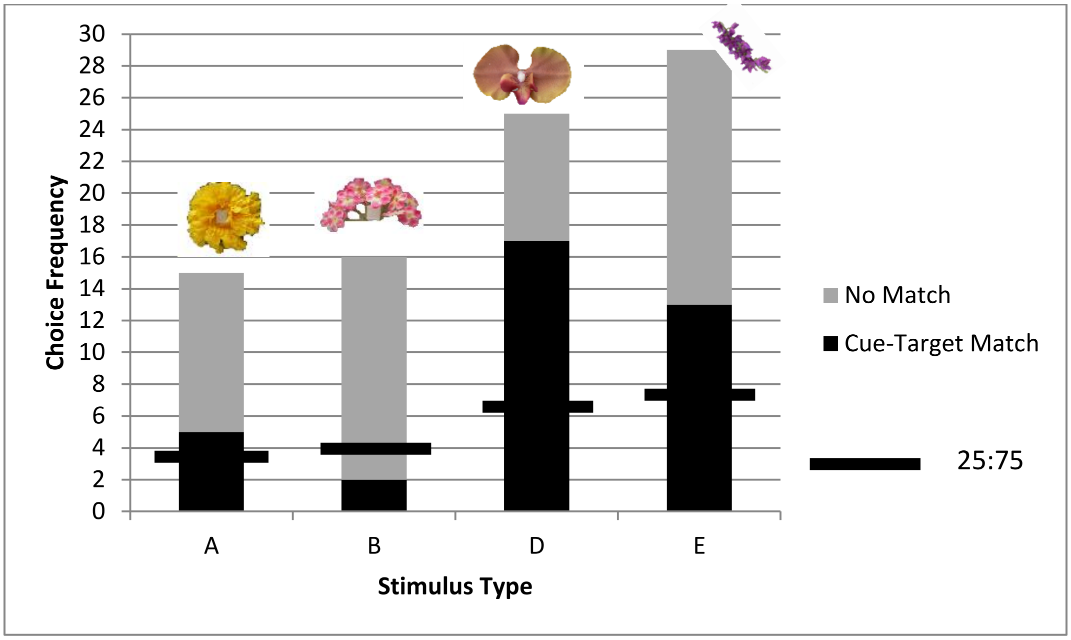

Fig. 10 Despite overall significant matching for objects corresponding to picture cues in rewarded testing of colony 1 in experiment 4, one stimulus, ‘B’, was found to be matched at significantly lower levels than the other 3 stimuli (A, D and E).

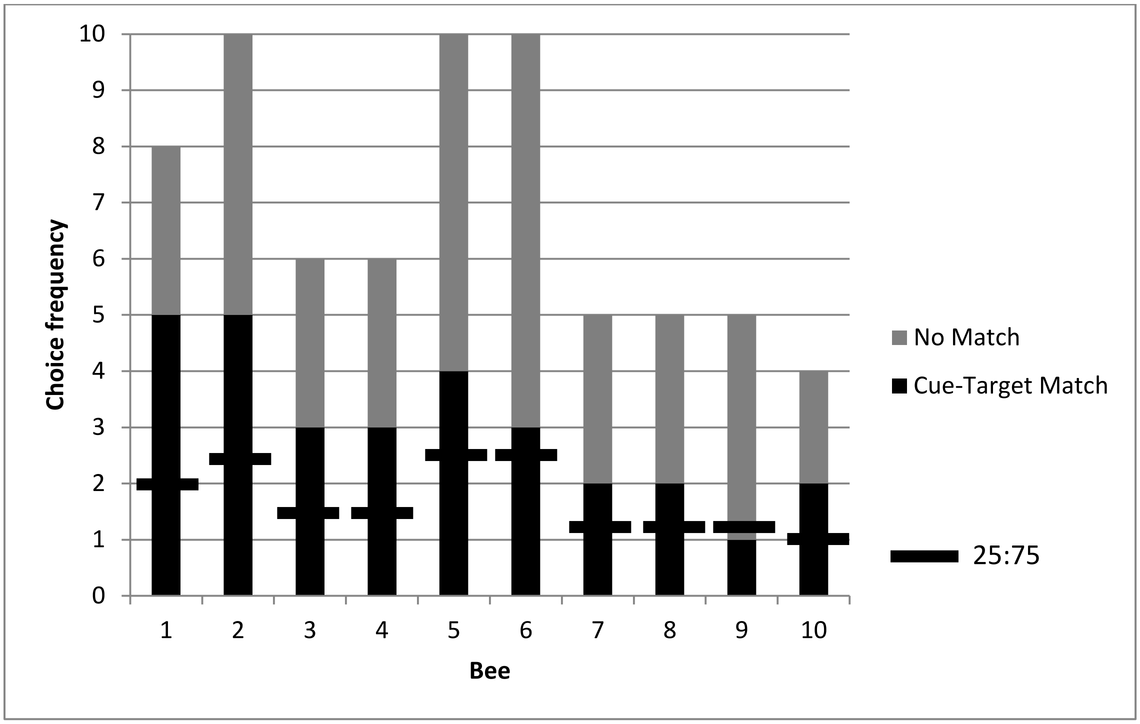

Fig. 11 Choice frequencies of rewarded testing for 10 bees in colonies 2 and 3 in experiment 4 showed a significant preference for object stimuli corresponding to a picture cue when compared to a chance value of 25:75. No significant individual differences were found, but the group choice proportion was significantly higher than chance.

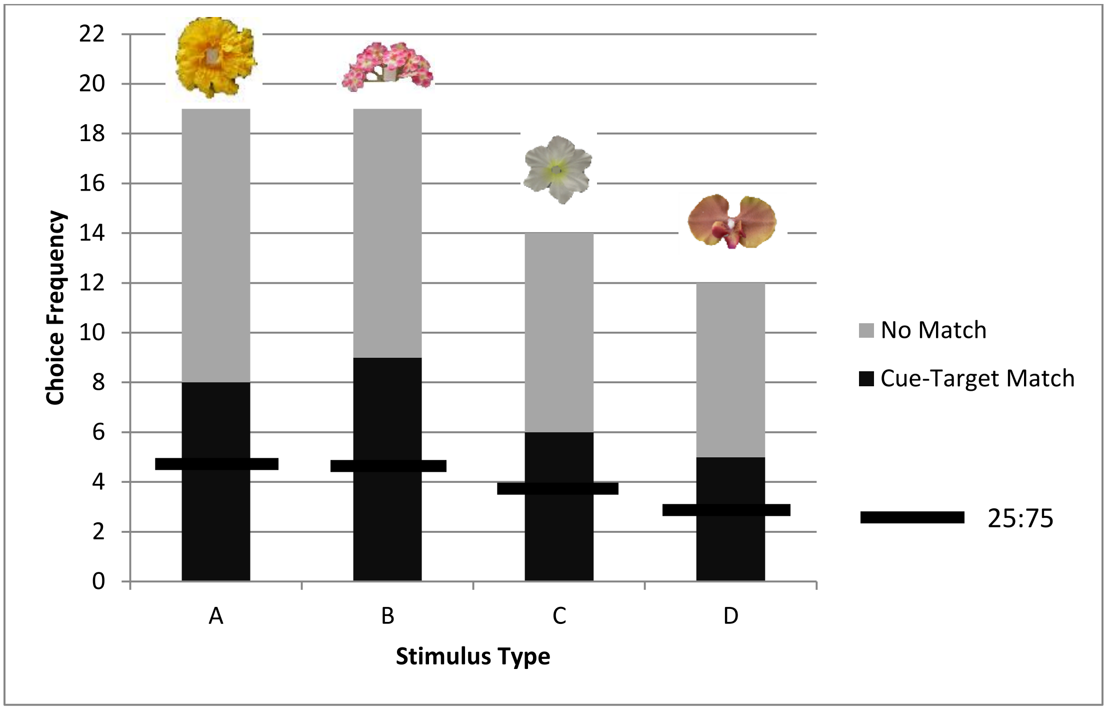

Fig. 12 Combined choice frequencies in rewarded testing for colonies 2 and 3 in experiment 4 for each by stimulus type (A, B, C & D) showed significant preference for the object corresponding to a picture cue across all stimuli types with no significant differences between A, B, C or D.

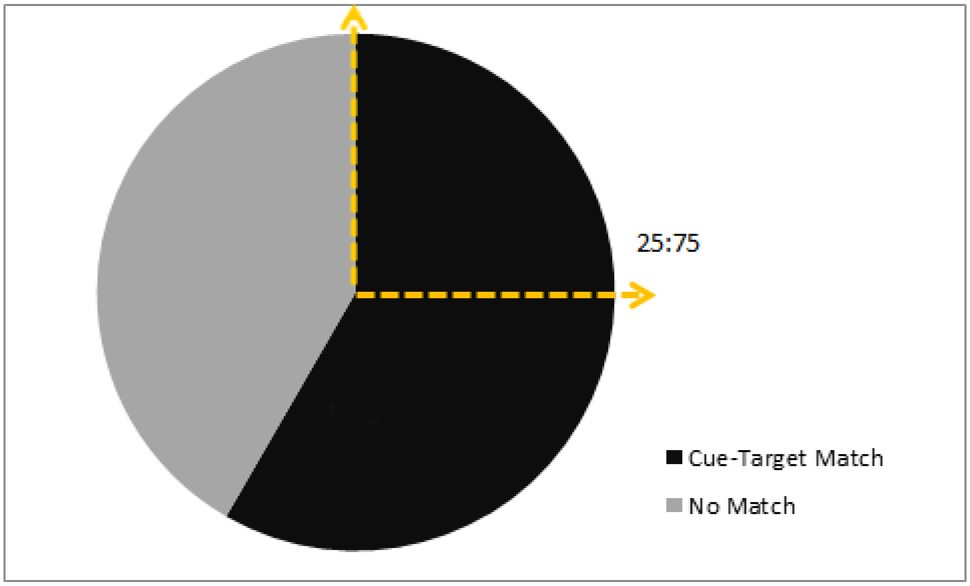

Fig. 13 Choice frequencies of unrewarded testing for colonies 2 and 3 in experiment 4 showed a significant preference for object stimuli corresponding to the picture cue over non-matching objects; matching occurred 58% of the time with a chance proportion of 25%.