

Behavioural studies and computational models exploring visual properties that lead to the first floral contact by bumblebees

by

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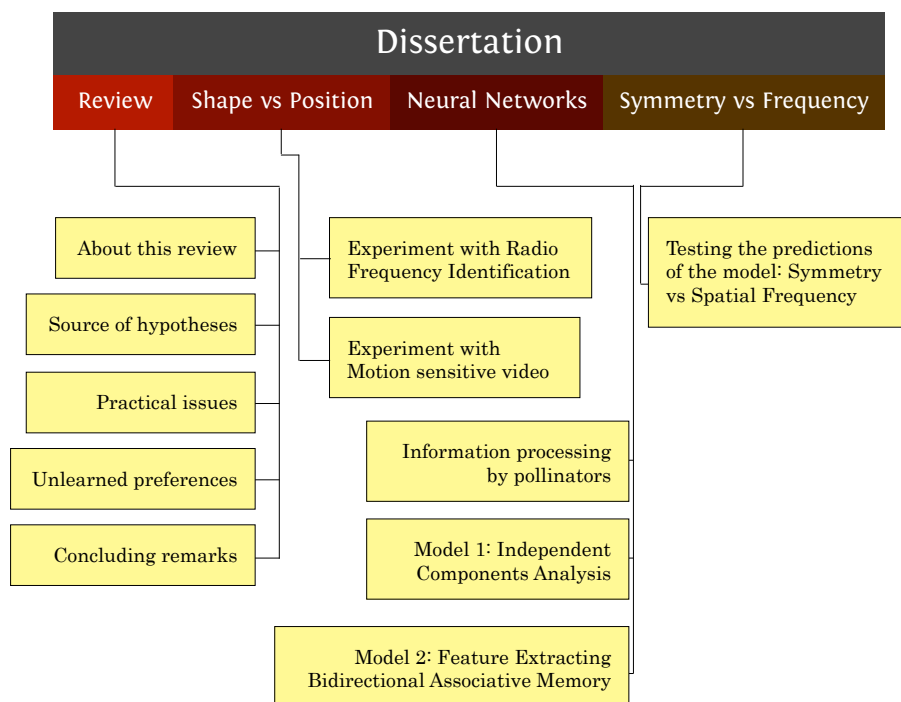


Figure 1: Thesis Layout

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Preface

At the heart of this thesis is a curiosity about the way bumblebees first find flowers. The aim is to explore how cognitive processes work together within the confines of perceptual constraints to enable bumblebees to discover the first rewarding flower. Perception in this manuscript refers to the category of cognitive processes that deal specifically with the detection and initial transformation of environmental information. The method of exploration includes both behavioural experiments and mathematical models simulating biologically plausible neural processes. The schema of this thesis is outlined in Figure 2 (Coombs, 1983).

In abstract terms, the first component of this dissertation begins by making observations of a small segment of the real world, unlearned visual preferences. We form generalizations about unlearned visual preferences, which then, become the basis for the second component. As part of the second component, we abstract the empirical observations and develop a computational model that describes our observations using a precise mathematical system. The exploration will be based on a set of axioms, which refer to a series of unproven assertions, including basic assumptions such as laws of logic, and contradiction, and postulates that underlie mathematical structures. The third component attempts to verify the conclusions of the computational model by testing a novel prediction using empirical observations.

The first component concerns itself with the empirical exploration of choice behaviour

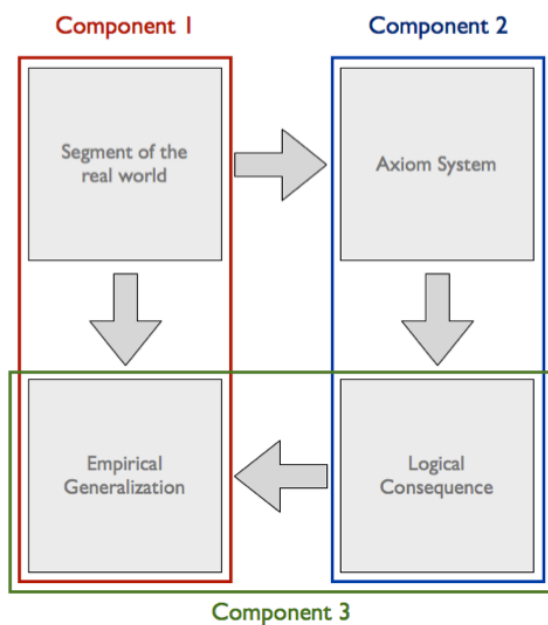


Figure 2: The relationship between the empirical studies and mathematical models in this thesis.

in bees. More specifically, young flower-naïve bumblebees choice behaviour at visual patterns will be explored. The second component concerns itself with the construction of a computational model. Through the implementation of a biologically plausible Independent Components Analysis (ICA), we ask what information processing neural functions account for unlearned visual choice behaviour. The third component of this dissertation aims to bridge the mathematical model with the empirical observations. A novel prediction from the computational model will be sought and tested using behavioural methods. Before elaborating on the three components, the next chapter reviews the literature on unlearned preferences by bumblebees and honeybees. The reader may find it unusual that the review refers to each of the three components of this dissertation as published literature. The reason for this peculiarity is that this literature review has been submitted for publication as a review paper.

Chapter 1

Getting to the start line:

How unlearned visual preferences guide
bumblebees (*Bombus spp.*) and
honeybees (*Apis spp.*) toward their
first floral contact

Inexperienced bees discover flowers in less than an hour of leaving their nest, without any prior floral experience. What characteristics of flowers give away to bees that there may be rewards there? Is it colour, size, a special configuration of patterns, or some other visual properties? Bumblebees may have a familiarity with floral odours as they are fed by the queen or conspecifics in their larval form (Dornhaus and Chittka, 1999; Tully et al., 1994), but there is no assurance those flowers are even in bloom when it is the workers' turn to forage. So the first cohort leaves their nest equipped with a familiarity

of floral odours, which may or may not be present, and their ability to see some aspects of the environment. The decisions workers make during this sensitive period is the result of specific neural wiring that gives rise to cognitive biases, which we can observe as unlearned choice behaviour.

Studying unlearned visual choice behaviour is useful to understand cognitive biases or inherent features of information processing. By unlearned, we refer to behaviours prior to learning from specific experience with floral rewards. Indeed, not all prior experience is excluded: bees have experience inside the nest and in larval form. The word “unlearned” also activates meanings shared with “innateness” and “instincts”, but these concepts are problematic because they oversimplify the relationship between genetic information and environmental signals (Mameli and Bateson, 2011; Bateson and Mameli, 2007) and because the words have taken on dozens of meanings (Oyama, 2000; Scholz, 2002). By “unlearned”, we mean the behaviour is not acquired through specific experience with flowers; or another related word is “pre-functional”, that is, the behaviour prior to functional experience with rewarding flowers (Hogan, 1994). Behavioural trunks (ie, unmodified behavioural patterns) exposed to the floral stimuli that the exploratory behaviours were designed to encounter, give rise to finely tuned, learned, goal directed behaviours. Though the question of how bees first discover flowers dates back to at least Manning (1956b), it has only recently become an active area of investigation. Much of the work in bee cognition explores associative learning: behaviour that has been reinforced by floral resources. Here, we review the literature on the behaviour that lead to the first-time discovery of food – in other words, the visual properties that bring bees to the start-line of their foraging careers.

Social bees are a suitable model for studying these cognitive functions due to their foraging life-style and sophisticated visual system (Srinivasan, 2010; Real, 1991). The

two genera of commonly studied bees include honeybees (*A. mellifera*) and bumblebees (typically *B. impatiens* by North-East American researchers and *B. terrestris* by European researchers). These species are often studied in the field, but field studies are not typically useful in this context because it is methodologically intractable to observe inexperienced bees in their natural environment.

Unlearned cognitive abilities of bees are the building-block of sociality and social behaviour. Initial information processing characteristics of the visual system will guide each worker toward an environment in which the discovery of floral rewards and a lifetime of foraging is a distinct possibility. For example, a discussion about floral characteristics would be very narrow if bees' cognitive abilities were not considered. Preference for symmetry is a case in point: one approach is to argue that bees prefer symmetry because symmetry is a sign of health in flowers, but the other is to argue that flowers signal redundancy of symmetrical patterns offers a computational incentive for visual processing, and bees find symmetry "easy on the eyes". While the two hypotheses have very different origins, generated from an ecological theory and cognitive theory, respectively, both hypotheses make a prediction about the co-evolutionary relationship between flowers and pollinators. Each hypothesis implies a different evolutionary pressure: the ecological approach implies flowers lead bees to most rewarding flowers *vs* the cognitive approach implies the bees lead the characteristics of flower displays.

While these questions are addressed with social bees, fundamental concepts may generalize to other insects or animals. We know that learning mechanisms such as classical conditioning are fairly general across different organisms. Unlearned behaviour, and the manner in which visual information is processed could also be similarly general. One indication for this is that the computational models described later in this manuscript are not unique to social bees' information processing characteristics. In fact many of

these models were developed to simulate human cognitive abilities, but capture aspects of floral choice behaviour by social bees.

Bumblebees and honeybees emerge with specific information processing characteristics that help them navigate a complex environment rich with visual and chemosensory signals. Untrained bees inexperienced with flowers (ie, “flower-naïve” Giurfa et al., 1995) rely largely on their compound eyes and antennae to receive, process and integrate environmental signals. While chemosensory information may well play a part in discovering the first flower (e.g., foraging without vision Chittka et al., 1999), it is not part of this review. Olfaction, when compared with visual or even auditory signals, provides less precise information about the exact whereabouts of flowers because odour propagation is subject to the turbulent flow of its medium (ie, most commonly air, but other atmospheric compositions have been tried, (see Nardone et al., 2012)), making detection intermittent and unpredictable (Laurent, 1999). For a review of honeybee olfaction, see Riffell (2011). How the bee sees the world is also not a primary focus here, although a brief discussion of basic visual characteristics is necessary to understand the boundaries of visual abilities (for a review of bee visual recognition, see Horridge, 2009). Studies involving learning are also not a focus except in cases where an inference to unlearned behaviour is made (for a recent review on visual learning, see Avarguès-Weber et al., 2011a).

Tinbergen (1963) suggested four complementary approaches to the analysis of behaviour: functional (ie, adaptive explanations), phylogenic (evolutionary explanations), causal (mechanistic, proximate explanations), and ontogenic (developmental explanations). This review approaches the subject from a mechanistic perspective with functional explanations informing mechanistic answers only to the extent that they are needed. A recent review of behavioural and cognitive mechanisms of foraging can already be found

in the literature (Dukas and Ratcliffe, 2009).

This review is divided into three main parts. In Part I, we will discuss the source of hypotheses and some of the theoretical perspectives that include cognitive, ecological, neuroscientific and computational modeling. In Part II, we will discuss practical issues such as the observation of choice behaviour, the design of testing environments, and technological advances in tracking behaviour. These issues are key to understanding that (a) most research of unlearned behaviour resort to less than ideal methods due to the difficulty of studying this subject, (b) recent advances in miniaturization and tracking technology allow overcoming most of these difficulties, and (c) non-linear computational modelling techniques are becoming more and more useful in explaining the behaviour of inexperienced bees and generating hypotheses that are not obvious from behavioural studies. Part III of this review will discuss findings from studies that explored unlearned visually guided behaviours. Data are available for choice behaviour at various visual properties such as colour, symmetry, spatial frequency, patterns such as radial and concentric shapes, different background colours and patterns, spatial frequency, and social cues. We conclude with potential areas of exploration, and suggestions for different theoretical perspectives to inform one another.

1.1 Source of Hypotheses

At one end of the spectrum, the source of hypotheses is a “variable-box”, which is shorthand for an a-theoretical systematic manipulation of potential target variables. To some extent, the study of unlearned preferences by bees suffers in this condition as visual properties are examined one-by-one in a long series of experiments. There are, however, tacit assumptions because variables and objects are not randomly chosen (eg, tissue papers or car tail-lights have never been used). Theoretical approaches in different scientific

branches have also been constructed to explain unlearned behaviour but with slightly different focusses in different scientific branches. Some of the branches that study unlearned behaviours include cognition, neuroscience, ecology and computational science. Cognitive scientists are typically occupied with questions relating to how the brain uses information in the context of perception, learning, memory, decision making and attention (eg, Dukas, 2004). On the other hand neuroscientific approaches are concerned with mapping out and understanding the structure and behaviour of neuronal populations associated with an organism's unlearned behaviours (ie, the physical underpinnings of behaviour). Ecological approaches also generate theoretical predictions from a perspective that deals with the interaction between organisms and their environment. Here, focus is not on the organism but on the consequences of the organism's behaviour on system variables, such as pollination, population or biomass. Finally, computational models are theories that have been written in the precise and non-ambiguous language of mathematics. These "ultimate" theories can be tuned with a high degree of precision, and generate testable and novel hypotheses.

Consider the example of unlearned preference for symmetry: cognitive scientists would explain a finding that bees land on symmetric flowers by suggesting a bias in information processing that leads bees to approach this visual property. Neuroscientists would explain this finding by pointing to a particular neural structure in the brain that shows a specific activation pattern when viewing symmetric patterns. Ecologists would explain symmetry as flowers signalling good health and more rewards (Møller, 1995). Finally computational scientists would implement an algorithm that captures the behavioural results and adjust the model's parameters to match those of the experimental conditions.

While each approach has its advantages and limitations, the aim is arguably the

construction of sound computational models that describe the phenomenon accurately, stay consistent with other theoretical approaches, and generate several novel and testable hypotheses. However, due to technological and methodological constraints, the kinds of hypotheses we can test, and therefore, the precision and versatility of theories we can construct, are often seriously limited. In the next section we explore these limitations in the context of unlearned preferences and suggest paths to overcome them.

1.2 Practical Issues

Visual preferences in social insects are typically studied by releasing bees into a test environment all at once, or one-by-one, and observing their choices at test stimuli. Test environments typically vary from 1 - 4 m^3 flight-cages, and mazes vary from two-arms (ie, Y-maze) to 12-arms (ie, radial-arm maze). Field research is difficult to implement because not only foraging experience of bumblebees is usually unknown, but it is difficult to identify the exact species under observation (Møller and Sorci, 1998). New technological advancements make studies of exploratory behaviour by pollinators in greenhouses a distinct possibility.

1.2.1 Measurement Techniques

Due to the difficulty of observing spontaneous unrewarded behaviour, many studies resorted to inferring unlearned preferences from different learning manipulations such as rewarded training (ie, pre-training) prior to testing choice behaviour.

Facets of learning A rewarded experimental strategy can be used to infer unlearned preferences indirectly by considering (1) an inability to learn visual properties, or (2) learn visual properties but with lower accuracy or at a slower rate. For example, some

studies found that bees associated reward with an ultraviolet (UV) reflecting-purple faster compared to other colours. Here, bees are said to have a biological “preparedness” for UV wavelengths (Lavery, 1994; Menzel, 1985). The opposite of learning rate comparisons is to study the rate of decline in preferences: how long the presumably unlearned preference for blue persists before association with yellow, a rewarded but not initially preferred colour takes over (Ings et al., 2009). For example, *B. terrestris audax* persist with the unlearned preference for blue longer than *B. terrestris dalmatinus* in the face of rewarded yellow.

Pre-trained behaviour An extensively used method is to pre-train bees in the testing environment on stimuli that appear neutral to humans, such as checkerboard patterns, grey discs or transparent feeders. Researchers use pre-training on “neutral” patterns because they are effective at conditioning bees to enter the testing environment and are thought not influence subsequent choices during an unrewarded testing session. For example, Lehrer et al. (1995b) studied choice preferences to different black and white patterns by pre-training *A. mellifera* workers to randomized checkerboard patterns. The extent to which workers’ choices deviated from the checkerboard pre-training pattern towards any of the test patterns indicated the strength of an unlearned preference towards the test patterns. Using this method, Lehrer et al. (1995b) tested four dozen patterns in groups of four. Gumbert (2000) suggested that as long as the colours of the pre-training discs are so different from the tested colours that bees cannot generalize their experience, naïve preferences are possible to measure. This finding may be true for studying colour, but it may not be true for studying other visual properties. For example, pre-training bees even to a grey disc still conveys a particular symmetry and spatial frequency configuration.

Untrained behaviour Studying untrained behaviour means that subjects have not been rewarded in the testing environment prior to measuring choice behaviour. During testing, spontaneous choice behaviour is observed. Studying spontaneous choice behaviour at visual elements is common with many insects such as crickets (Matsumoto and Mizunami, 2000), beetles (Domingue et al., 2011) or butterflies (Weiss, 1997), but has not been as popular in bees. There are a handful of studies that compared the behaviour of *B. impatiens* workers at various stimuli with and without pre-training (Plowright et al., 2011; Séguin and Plowright, 2008). The results in both of these studies showed that choice proportions significantly changed as a direct result of pre-training.

1.2.2 Methodological issues

Measurement criteria Choice behaviour can be measured in multiple ways, differing in level of “strictness”. Choice can refer to approach flight or hovering in front of an artificial flower (eg, Lunau, 1992), walking into the arm of a testing maze such as a radial maze or a Y-maze (eg, Séguin and Plowright, 2008), antennal contact with a test pattern (eg, Pohl et al., 2008), landing on a test pattern (eg, Milet-Pinheiro et al., 2012; Leonard and Papaj, 2011), or even floral exploration (eg, walking into an artificial flower) (eg, Orbán and Plowright, 2013). These behaviours are typically lumped together and discussed as “bee preference” even though not all choice behaviours are equivalent. For example, Orbán and Plowright (2013) found an interaction between landing and floral exploration: bees were more likely to explore a flower if its patterns were positioned near the center of an artificial flower, but bees were more likely to land on a flower if it displayed a radial pattern. Similarly, Lunau (1992, 1990) found that bumblebees readily approached unrewarding star-shaped artificial flowers with two dots at the center, but were far less likely to land on them when compared with star-shaped flowers without

central-dots. In sum, depending on the choice criterion, bees may or may not show a preference for a particular pattern.

Measurement criteria may also be conceptualized as a speed *vs* accuracy trade-off. Landing and explorations indicate a longer examination time of the stimulus thereby trading off accuracy over speed. On the other hand hovering and antennation require much less time, thereby valuing speed over accuracy. The consequence of conceptualizing criteria in this manner is that hovering and antennation may introduce more noise in the observations from the researcher's point of view.

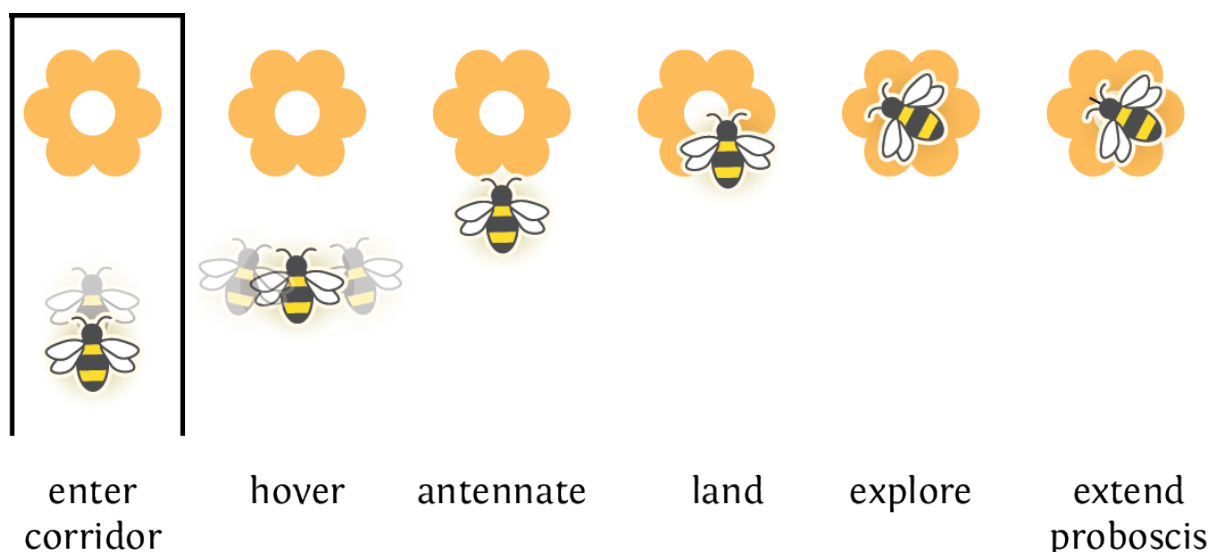


Figure 1.1: Preference as defined by choice behaviour has typically meant landing. However, there are a series of behaviours before and after landing that are also associated with preference or aversion.

Kinds of choices There are multiple issues relating to choice paradigms. First, bees may be observed in a forced (ie, similar to the 2 alternative forced choice method “2AFC”) or a free choice environment. Second, the evaluation of choices may be successive or simultaneous. Third, independence of choices may be an issue if it is not possible to tell whether the same bee or different bees contributed to a set of choices.

There are key differences between *forced vs free choice* experimental designs. When studying forced choice, as in Y-mazes or 12-arm radial mazes, the relative preference of patterns is being measured. With this design it is not clear if any of the patterns contribute to floral choice *per se*; we can only estimate that one pattern appears more preferable in relation to another. In fact it is not even clear whether preference or aversion is measured. It is possible that the most chosen pattern in a forced-choice environment is the least repellent pattern. If a bee is free not to make a choice, as in many free-flying paradigms, then absolute preferences can be measured. The estimation of absolute preferences may be calculated by dividing the number of bees that entered the flight cage with the number of bees that landed on a pattern. Experimental configurations that use free-flying bees but present multiple patterns use a hybrid method.

There is also the question of whether bees make *successive or simultaneous choices*. Successive choices refer to the idea that stimuli are presented without temporal overlap: bees must “take-it-or-leave-it”. Simultaneous choice refers to multiple stimuli displayed concurrently such as in a radial-arm maze. As the current state of research stands, it is impossible to tell how bees make choices when multiple patterns are displayed at the same time. This obstacle will be present as long as experimenters cannot tell to what bees’ attend at any given moment. When stimuli are presented simultaneously, the delay between two choices of different patterns can be a matter of a few seconds, but in other cases minutes, hours or even days. In a free-flying setup, bees that return to a different pattern over 24 hours later may make an independent choice provided the stimulus does not offer rewards, but this distinction becomes problematic when the choices are made within a few seconds or minutes. If habituation is any indication of correlated choices, bees dishabituate from an unrewarded pattern after 24 hours (Plowright et al., 2006), but anything less than this interval may indicate a correlated choice.

Finally *independence of choices* is an issue when bees are not individually identified and a large number of choices is reported. When more than a few dozen choices are recorded, it is important to know whether it is 50 bees making one choice each, or one bee making 50 choices. Individually identifying bees allows distinguishing between two sources of variations: between and within individuals. This can be a real problem because worker activity patterns typically roughly match an exponential function, where a few very active workers contribute to a large proportion of foraging.

Observation techniques While human observation of bee behaviour has been most common over the past century, new technologies are offering more experimental control, more naturalism, and larger scale studies.

Human observations typically progress by waiting for a worker to emerge from the nest and enter the testing area (eg, Plowright et al., 2011; Forrest and Thomson, 2009). At this point the researcher records the tag number of the worker, and lifts the gate to allow the bee into the test area where behavioural observations are made by taking notes on the bee's choices. There are at least two difficulties with this method: (1) observations are recorded for a single individual at a time, making it impractical to study the behaviour of a full colony that can have as many as 500 workers (or 5000 in the case of honeybees). Studying a large proportion of workers in a colony is necessary to minimize sample bias towards the "researcher friendly" or less aggressive workers, and most active workers that may very well be systematically different from less active or aggressive individuals. (2) The recording tool (ie, human attention) performs poorly when observing scarcely occurring events, also known as vigilance decrement (Warm et al., 2009). Waiting for bees to emerge, and then waiting for them to make a choice is a textbook example of keeping sustained attention while observing infrequent signals.

Video recordings are taking a hold in insect research (eg, Orbán and Plowright, 2013;

Sibbald and Plowright, 2012; Lihoreau et al., 2012; Leonard and Papaj, 2011). Motion-sensitive camcorders record a specified length clip only when a specific pattern of movement is detected in the viewfinder. Motion is characterized by changes between two adjacent frames, and the proportion of change within a frame. Motion detection is a critical feature because flower-naïve bees are not frequently visiting unrewarded stimuli. Some workers only visit a stimulus once during their life-cycle, while others may return a few times, and a small number of workers will visit stimuli throughout an experiment. Continuously recording a video for the whole duration of testing would be very difficult to analyze. The result would be potentially hundreds of hours of video for relatively few and short behaviours. Alternatively, it is also impractical to record a limited duration of video each day because the relevant behaviours could be missed. Motion-detection camcorders generate 5-15 s video clips of the relevant behaviour that can be quickly analyzed.

Finally, radio-frequency identification (RFID) offers new ways of automating research by electronically detecting bee behaviour at particular points in the testing environment (Nachev et al., 2012; Silcox et al., 2011; Decourtye et al., 2011; Ohashi et al., 2010; Stelzer and Chittka, 2010; Carbunar et al., 2009; Sumner et al., 2007; Streit et al., 2003). In an RFID enabled study, every worker can be tracked by gluing an identification tag on the thorax of the bee, using the same protocol as Opalith Plättchen tagging (ie, coloured number). Tags can be detected by RFID readers, which can be placed in multiple locations: colony entrances, checkpoints in a maze, or even camouflage RFID readers in artificial-flowers (Orbán and Plowright, 2013). One challenge with today's RFID systems is that tags must come to a distance of about 3 mm from the reader for reliable detection. Currently, transponders only from Microsensys (mic3-TAG 32k transponders) and Hitachi-Maxell (Coil-on-Chip transponders) have been successfully

used in bee experiments. Hitachi also has a promising transponder, the μ -chip, which can offer detection from a range of up to 30 cm, but requires a 52 mm external antenna. Such a long antenna makes it prohibitive for bee research at this time. A tag with a reading distance of about 5 - 6 cm and the ability to detect multiple transponders simultaneously would greatly increase the versatility of stimuli designs, testing space designs and therefore the hypotheses that could be tested.

1.3 Unlearned Preferences by Bees

This section is organized by the visual properties that have been examined, more or less in order of intensity of research. Some visual properties such as colour and shape have received much attention while others are scarcely researched, or are newly emerging as an interest for scientists. The primary focus is on studies that examined unrewarded and untrained behaviour.

1.3.1 Colour

Unlearned colour preference is the most intensively studied floral visual property that includes the investigation of different frequencies of the electromagnetic spectrum, colour saturation, and contrast between patterns and backgrounds. Neurophysiological experiments show that bumblebees and honeybees have peak electromagnetic sensitivities at approximately 350 nm, 450 nm and 550 nm, which correspond to UV, green and blue (Skorupski et al., 2007). There are no receptors with peak sensitivity near red, which corresponds with behavioural findings relating to poor learning of red even in bumblebees that are known to visit red flowers (Lunau et al., 2011; Martínez-Harms et al., 2010; Chittka, 1997). However, colour vision functions only at relatively short distances: up

to 10 cm for a grating with a spatial period of 2 cm (Chittka and Raine, 2006; Macuda et al., 2001; Lehrer et al., 1988). Bees use green-contrast (ie, grayscale vision) to up to an additional 40 cm but beyond this point, the shapes of objects become indistinguishable (Dyer et al., 2008). Exploring any unlearned colour preferences should take into account these parameters in terms of the kinds of colours that are tested, and the configuration of the testing space should meet distance requirements.

The role of ultraviolet The colour of nectar guides was studied on four spectral frequencies of 41 flower species: 360 nm (UV), 450 nm (blue), 520 nm (green) and 680 nm (red) (Penny, 1983). Flowers displayed nectar guides with better colour contrast on the insect visible spectrum (360 nm and 450 nm) when compared with the human visible spectrum (520 nm and 680 nm). The colour contrast effect was weaker when only UV was considered, suggesting that UV does not have a disproportionate contribution to preference: bees will respond to yellow, and violet as well as to UV. This finding is consistent with a study that showed flower colours without UV reflectance are more common, and another study that highlights the absence of pure UV flowers (Chittka et al., 1994). These findings correspond with a more recent investigation of UV signals (Kevan et al., 2001).

Floral colours Giurfa et al. (1995) tested approximately 1200 *A. mellifera* paint-marked workers' preferences in a flight-cage in which bees were pre-trained on brushed aluminum, grey cardboard or hardboard patterns. Workers' choices were tested on UV-blue, green, blue and blue-green colour discs, presented simultaneously with geraniol scent. The results indicate a complex picture: test pattern preferences depended on the pre-training stimulus, and initial preferences not only shifted over time, but in some cases disappeared. One of Giurfa et al.'s interpretations is that initial preferences are towards

green, and deviations towards UV and blue indicate learning during pre-training.

In a test of floral reflectance spectra, Heuschen et al. (2005) found that the inner portion of flowers show less diversity of colours compared with the corolla portion. Further, the inner portions tend to resemble the yellow colour of pollen. Subsequently, untrained *B. terrestris* workers were tested at unrewarding star-shaped artificial flower stimuli. Workers clearly preferred bi-colour over single colour stimuli, and stimuli with violet corolla and relatively large ($d = .6mm$), spectrally pure, UV-absorbing yellow inner portions. This preference for yellow has been shown even in male *B. impatiens* workers (Church et al., 2001).

Spectral purity Lunau (1990) hypothesized that unlearned preferences are driven by an attraction towards the yellow colour of pollen, in its most spectrally pure form. A spectrally pure colour is one that contains no other colours (ie, reflect a single wavelength). To test the spectral purity hypothesis, choice behaviour, as defined by hovering and landing, was measured in untrained *B. terrestris* and *B. lucorum* at star-shaped artificial flowers with two central dots as floral-guides, and a green background. Ten corolla and seven floral-guide colours were tested, generating 70 combinations. Workers were found to show more hovering behaviour in front of spectrally pure corollas, but not in front of spectrally pure floral-guides. Contrast between the floral guides and corolla contributed to more landings. This suggests that the importance of a spectrally pure yellow may be highly specific to particular parts of a flower, and relevant only during specific portion of choice behaviour.

In a subsequent study, Lunau et al. (1996) reanalyzed the data from Lunau (1990) and performed two additional experiments that showed bees' sensitivity not only to contrast between corolla and background but the directionality of this colour difference. Bees preferred corolla with maximal colour difference from the background, and chose floral

guides of highest spectral purity.

In a discrimination test of floral guide colours, *B. terrestris* workers failed to associate conspicuous yellow floral marks with non-reward (Pohl et al., 2008), but were able to learn this task with a brown mark. The results are interpreted to mean that yellow, the common colour of pollen is so important that this innate preference is not easily eliminated. The structure of this study is akin to another study comparing two sub-species of bumblebees at a colony level. Ings et al. (2009) found that the unlearned preference for blue persists longer in *B. terrestris audax* than in *B. terrestris dalmatinus*.

1.3.2 Pattern types, their positioning and size

Visual acuity of the bee eye is severely limited due to design constraints of the ommatidia (Land and Fernald, 1992). In order to minimize the effect of diffraction, the ommatidial array's curvature is adapted to detect images at higher resolution on the frontal region of the eye (where they are most likely to encounter floral patterns), and at lower resolution on the lateral region of the eye. The eye also under-samples the environment slightly, which results in an image that would be analogous to seeing through a piece of gauze. The shape of floral patterns need to anticipate the approach angle and distance of bees, and guide them to the pollen.

Bumblebees and honeybees are known to choose non-randomly between different types of patterns (ie, shapes). While not working strictly with flower-naïve honeybees, the study described in Section 2.1 by Lehrer et al. (1995b) tested choice behaviour at 64 distinct shapes. *A. mellifera* workers pre-trained on randomized checkerboard patterns, preferred radial properties to others, such as stripes and concentric properties. When comparing multiple radiating shapes, ones with proportionally higher area of black patterns (ie, radial sectors) were preferred over bars configured in a radial configuration.

This study thoroughly tested shape preferences, though pre-training on ostensibly neutral patterns creates a slight doubt in the findings as to whether the preferences were those of truly flower-naïve bees. The results indicate that a relatively low spatial frequency radial pattern are most preferred. In terms of frequency, the researchers suggested that it is the optimal contrast to the background that low spatial frequency patterns typically offer rather than the spatial frequency *per se*. In terms of patterns, radial features were preferred, which mimics many flower’s sunburst patterns more so than other tested patterns.

Preferences towards radial features are indeed not a new finding. Often called *honey-guides* or *floral-guides*, their role in guiding bees the location of rewards has been discussed as early as the 19th century by Sprengel (as cited in Manning, 1956a). Even completely inexperienced *B. impatiens* foragers have shown preference for radial over concentric patterns (Plowright et al., 2006; Simonds and Plowright, 2004).

The study we described earlier, which manipulated the presence or absence of two central dots on discs (Section 3.1) was originally studied in the context of colour, but may also be interpreted in the context of shape preferences (Lunau, 1992, 1990). It is possible that workers preferred the presence of the central dots rather than the yellow markings as Lunau suggested, though Pohl et al. (2008) suggests that the colour and contrast of markings is also relevant. Lunau et al. (2006) indicated that bees behave differently by antennating the central markings if such markings are present, and antennating the margin of the artificial flowers if the central markings are absent.

Pattern positioning of radial or concentric patterns was studied to test whether bees show preference for the shape of a pattern or its placement at the center of an artificial flower (Orbán and Plowright, 2013). Free-flying flower-naïve *B. impatiens* workers were exposed to blue discs with yellow patterns that displayed either radial (ie, “sunburst”) or

concentric (ie, “bull’s eye”) patterns. The yellow patterns either appeared only near the center or the periphery of a blue 3-dimensional conical shaped artificial flower. Choice was defined to be landing on the artificial flower or exploring the inside of a three dimensional RFID equipped artificial flower. The results have two key features. First, positioning predicts floral exploration: bees are more likely to explore a centrally positioned pattern even if the pattern is concentric. Second, pattern-type predicts landing: bees are more likely to land on a pattern that displays a radial shape, even if it is positioned only on the periphery of the flower (see Figures 1.2 and 1.3).

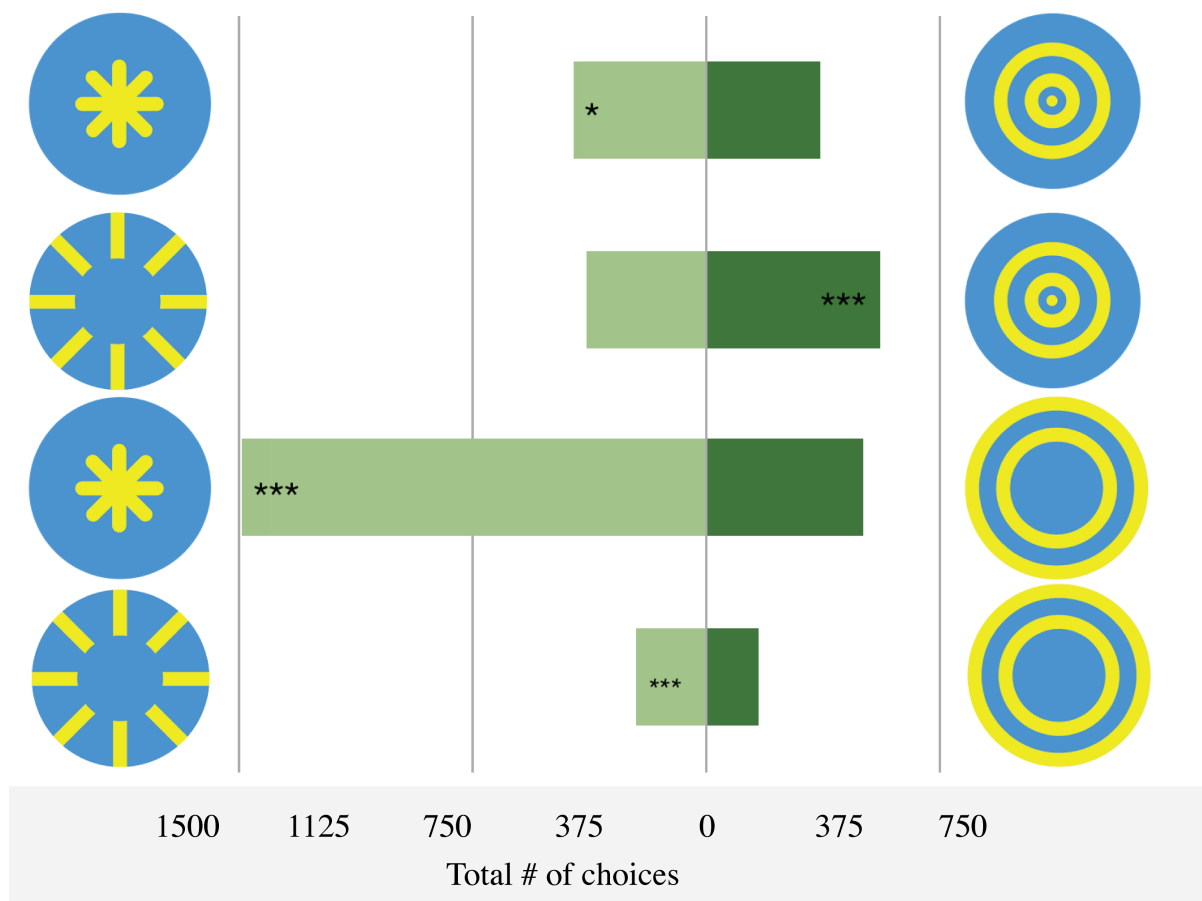


Figure 1.2: In an RFID experiment, bees explored the inside of conical RFID-enabled flowers when patterns were centrally located

In terms of floral appearance, Dafni and Kevan (1996) found that the external con-

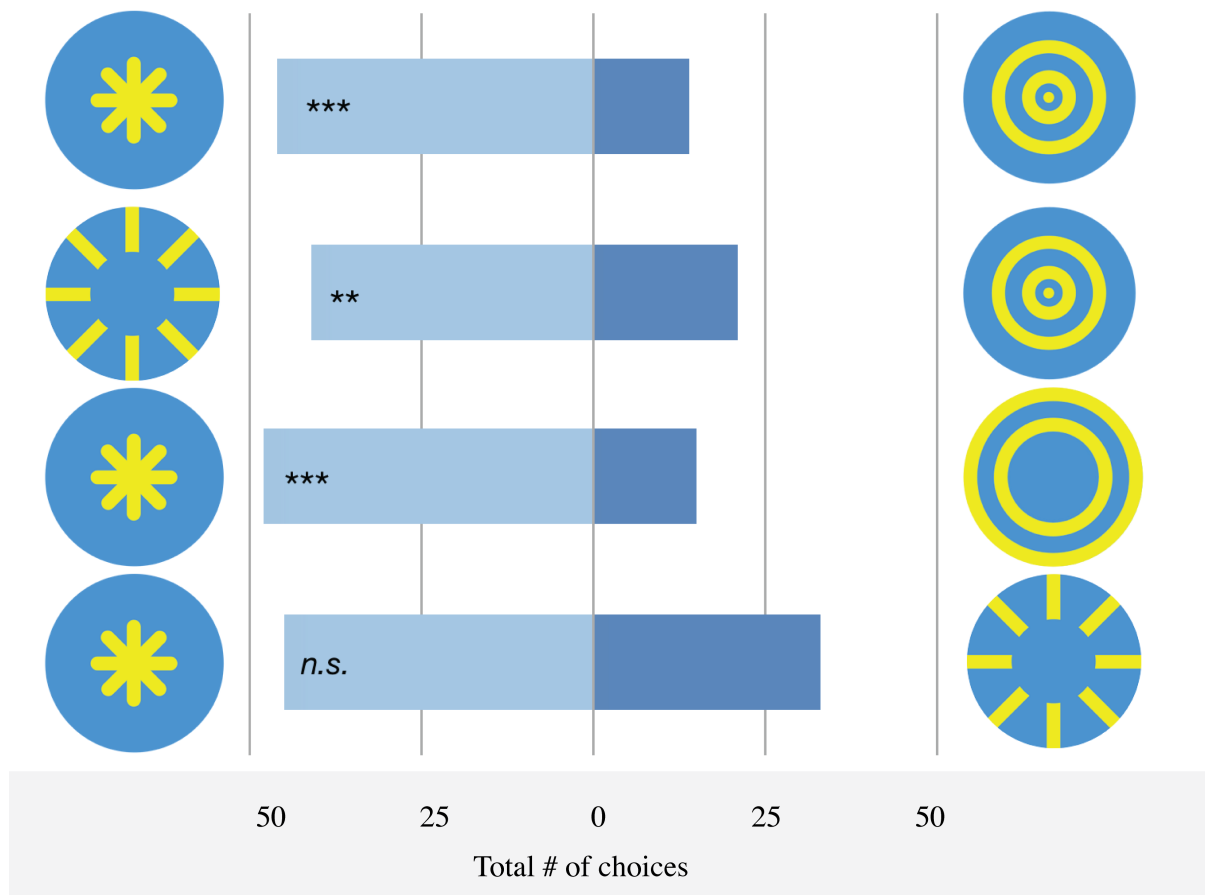


Figure 1.3: In a motion-sensitive video recording, bees landed on conical artificial flowers when patterns displayed radial features

tours of floral patterns showed significant correspondence with inner venation patterns on its petals. For example, if the external contour of a flower was circular, its internal contour was also similarly shaped. The significance of this finding may lie with the way bees scan floral patterns: following the contours of flowers would help bees discover a source of reward.

There is indirect evidence for increased fitness of pollinators and flowers where flowers display a radial feature. Compared to artificial flowers that did not display a radial element, *B. impatiens* workers not only discovered rewards quicker but were faster to find and extract that reward (Leonard and Papaj, 2011). This seems to be a general



Figure 1.4: *B. impatiens* worker tagged with RFID chip

cognitive feature observed also in other species of insect pollinators such as in *Prosoeca* *sp. nov.* (Hansen et al., 2012).

The question of pattern size also emerges from time to time, but unlearned size preferences have been elusive (Hudon and Plowright, 2011; Blarer et al., 2002; Lehrer et al., 1988). Perhaps size preferences develop only through experience with flowers. Alternatively, the usual explanations for the failure to reject the null hypothesis may be invoked to explain failures to detect unlearned floral size preferences (eg, ecologically irrelevant manipulations, small sample sizes, or not sufficiently strong experimental manipulations).

1.3.3 Symmetry and spatial frequency

Empirical studies Findings relating to unlearned symmetry preferences have been mixed. While radially or bilaterally symmetrical flowers are very common in nature, it is not clear whether bees have an unlearned preference towards such visual properties

or bees learn to prefer symmetrical displays because they are more rewarding (Møller and Sorci, 1998). The former hypothesis is consistent with the idea that inherent characteristics of the bees' visual system drove the emergence of symmetrical flower displays whereas the latter hypothesis is more consistent with the idea that symmetry signals floral health, which is reminiscent of hypotheses suggesting human facial symmetry as indicators of health (eg, Rhodes et al., 2001).

Rodríguez et al. (2004) suggest that symmetry preference is unlearned. Free-flying *B. terrestris* workers were tested on unrewarded symmetric and asymmetric random black and white patterns, but pre-trained on plain black and plain white disks. This finding has quickly permeated the literature (eg, Goulson, 2010; Kalisz et al., 2006; Biesmeijer et al., 2005; Benard et al., 2006), but Giurfa et al. (1999) point out that in order to test unlearned symmetry preferences, animals must not have had uncontrolled visual experiences. This not only includes functional experience with real flowers, but also with reward with a disc. Case in point is the study by Plowright et al. (2011) where spontaneous symmetry preferences were not observed in completely flower-naïve *B. impatiens* workers. However, when pre-trained on plain black discs and plain white discs as in Rodríguez et al. (2004), workers showed a significant preference towards bilateral symmetry in a subsequent unrewarded test. The plain discs were radially symmetrical, which may have conditioned workers to symmetrical patterns.

In a conditioning experiment of *A. mellifera* workers, Giurfa et al. (1996) found that bees did not have a spontaneous preference for symmetry, but appear to have a predisposition to learn symmetry faster compared with asymmetrical patterns. In a rewarded experiment, bees were capable of extracting symmetrical features in as few as seven trials with at least 60% accuracy. Bees not only learnt symmetric features, but generalized them to novel stimuli. This finding corresponds with the results of West

and Lavery (1998), who also found an absence of preferences for symmetry, but found that *B. impatiens* workers handled symmetrical flowers 20% faster than asymmetrical flowers. Computational models that implemented feed-forward artificial neural networks to describe this behaviour also imply a visual by-product for symmetry that is a result of learning dynamics (Enquist and Johnstone, 1997; Enquist and Arak, 1994, 1993).

Computational models A feed-forward genetic network was used to test the symmetry preference as a by-product hypothesis (Johnstone, 1994). Two networks were trained to recognize bilaterally symmetrical “tails” (represented by 3 black pixels) of varying levels of fluctuating asymmetry: one network exposed to a perfectly bilateral pattern, whereas the other network only exposed to various imperfect variations. The trained networks did not differ in their preferences for bilateral patterns. The network that was not exposed to symmetric patterns generalized to symmetric patterns during training on forms of imperfect variations.

In a similar model, Enquist and Arak (1993) suggested that animals learn to prefer symmetry as a result of generalizing knowledge of signals to their various forms (ie, translations, rotations, transformations). A feed-forward network illustrated this idea by training the model using 6 x 6 pixel input signals to discriminate between three stimuli: “birds” (represented by a bird-like cluster of black pixels) with short tails and wings and “birds” with long tails and wings, and random pixels. The activation function was defined as the weighted sum of the hidden layers reaching a threshold of 0.5. Results show that the network activated with longer tailed and longer winged patterns, but not tail-less or random patterns. Further iterations progressively increased preference for exaggerated features. This finding indicates that a preference for symmetric floral patterns may be a by-product of repeated rewarding encounters with flowers, but not a unlearned preference that would be observed in flower-naïve pollinators.

There are other types of computational models that propose an alternative hypothesis (Orbán and Chartier, 2013). Models based on Independent Component Analysis (ICA) and Feature-Extracting Bidirectional Associative Memory (FEBAM) suggest an incentive to prefer symmetrical patterns only when the computational cost of processing high spatial frequency patterns is high relative to a pollinator’s brain available computational resources. As the spatial frequency increases, and therefore the amount of information contained in a pattern increases, the informational redundancy inherent to symmetrical displays becomes more relevant. Information redundancies may offer a bees’ visual system a “computational incentive”. Orbán and Chartier (2013) implemented two unsupervised neural networks that indicate symmetry preferences to be unlearned. Two unsupervised neural networks, a FEBAM and an ICA were used to decompose images of test stimuli (identical to stimuli used in behavioural studies) into their underlying components, and then reconstruct the original test image from these feature components. The quality of image reconstruction, measured in Peak-Signal-to-Noise-Ratio (PSNR), is an indicator of information processing cost. A poorly reconstructed image implies a “computationally expensive” pattern, and a well reconstructed image implies “computationally affordable” pattern. The assumption of these models is that bees prefer stimuli that have lower costs of information processing. This assumption is consistent with evolutionary hypotheses relating to energetic and temporal pressures on the design of brain functioning (Laughlin et al., 1998).

The key differences between the two breeds of models is that Johnstone (1994) and Enquist and Arak (1993) suggest symmetry preferences to be a by-product of learning dynamics. Feed-forward networks work on the principle that the correct solution is repeatedly experienced by the network. The behavioural implication of these models is that symmetry preference arises with functional and rewarded experience with floral

patterns. On the other hand, the models of Orbán and Chartier (2013) suggests that symmetry and other pattern preferences are a by-product of information-processing by the bees' visual system. These distinctions lead to testable behavioural hypotheses: if the feed-forward networks captured behaviour correctly, preference for symmetry should not be observed in flower-naïve bees. However, if the unsupervised neural networks captured reality more accurately, unlearned symmetry preferences should be observed when the spatial frequency of a pattern is high. Symmetry in this case offers a meaningful computational efficiency in cognitive processing.

Briefly, there are several distinct hypotheses that may give rise to symmetric preferences. First, flowers drive the preference for symmetry by offering more reward, and thereby teaching pollinators that symmetry is good. Second, pollinators drive the preference for symmetry due to a by-product in learning mechanisms. As bees experience more rewarding flowers, their memory of these flowers leads them back to more and more symmetric variations. Finally third, pollinators drive the preference for symmetry due to a computational constraint in visual information processing. Flowers that offer the most computational savings for pollinators while standing out from the foliage background gain the visits of inexperienced flower-naïve pollinators.

1.3.4 Background Effects

In addition to the properties of the floral stimuli themselves, the backgrounds on which those stimuli are displayed have also been examined in a couple of experiments. For example, Lunau et al. (1996) explored the question of whether bees evaluate a floral property in relation to its background colour. More specifically, by reanalyzing behavioural data from an earlier experiment (Lunau, 1990), two hypotheses were set to compete: *B. terrestris* workers unlearned preferences reflect the spectrally purest colours, or workers

unlearned preferences reflected the maximal colour difference between the stimulus and its background. In three experiments, Lunau et al. (1996) concluded that workers did not choose stimuli with the most colour difference from their background. Therefore, the spectral-purity hypothesis was supported. However, both spectral purity and colour contrast may play a role in preference because a later study lends support to the relevance of colour contrast (Spaethe et al., 2001).

1.3.5 Social cues

Up to now we have been considering aspects of flowers that elicit initial choice. Recent research has addressed the question of whether the presence of foragers on flowers functions the same way as floral properties: perhaps an individual on a flower attracts other bees toward it. From a mechanistic point of view, local enhancement or stimulus enhancement, whereby one individual attracts another to a particular location or stimulus, are commonplace in animal behaviour (Shettleworth, 2009). From a functional point of view, however, such a possibility is only one of other plausible scenarios. Perhaps in nature floral characteristics are such strong predictors of reward that additional social cues carry little additional informational value. Another possibility is that while the presence of a forager signals that a flower has indeed been discovered, that flower is empty or on its way to being depleted. In other words, other foragers may act as informers or as competitors (Baude et al., 2011). In view of these considerations, it might be expected that the predictive value of foragers on flowers might depend on local environmental conditions and might only be learned from experience. Recent reports, however, have shown that a preference for “occupied” flowers is apparent as soon as bees first begin to search for food.

The interest in giving bees that leave their colony for the first time a choice between

occupied and unoccupied flowers lies in understanding their behaviour in nature. The experimental control of studying first time choices of floral stimuli that differ along one dimension can only be achieved, however, in the laboratory. Inexperienced bumblebees given a choice between two rewarding artificial flowers, one of which was occupied by a dead pinned bee and the other not, first landed on the occupied flower more frequently than chance (Kawaguchi et al., 2006). Similarly, inexperienced bumblebees given a choice among 12 unrewarding artificial flowers, four of which were occupied by a dead pinned bee and eight of which were not, first landed on an occupied flower more frequently than chance (Leadbeater and Chittka, 2009). More recently, Plowright et al. (2013) tested bees leaving their colony for the first time in a radial arm maze with occupied and unoccupied unrewarding floral stimuli mounted on the wall of each corridor. A preference for occupied stimuli was found, but only under a restricted set of conditions: when the occupied flowers were rare compared to unoccupied flowers and, in addition, the ratio of the size of the occupier relative to the size of the flower was comparatively large. Otherwise, choice proportions did not differ from chance.

There is evidence that flowers adapted their visual appearance to exploit the salience provided by the presence of other foragers. For example, a South-African daisy species, (*Gorteria diffusa*) displays dark spots that resemble other foragers on the central portion of the petals (Whitney et al., 2011; Thomas et al., 2009).

Currently, research in this area is in its infancy. Little is known about how the presence of other foragers is perceived by bees making floral choices. They may be possibly perceived as parts of the flowers such as nectar guides (Baude et al., 2008), or they may be perceived as other inanimate objects such as a coin or a plastic disk (Dawson and Chittka, 2012). More importantly, little is known about the ecological relevance of laboratory demonstrations of an unlearned preference for occupied flowers. Beyond floral

size and occupier frequency, the important variables remain to be identified in efforts to bridge the gap between the lab and the field.

1.4 Concluding Remarks

1.4.1 Summary of Findings

Bees discover all kinds of flowers that have few similarities. Lilacs, thistles, blueberry flowers, sunflowers and tomato flowers do not share colour, contrast, symmetry, spatial frequency or size properties. At the very least, we know bees explore their environment non-randomly. Non-random exploration tells us something about the way they process environmental input guides floral discovery. The visual parameters that lead to floral discovery have been studied by decomposing flowers into their constituent parts, and observing bees' choice behaviour. Several visual properties have been successfully studied. First, multiple chromatic parameters appear to draw flower-naïve bees: UV-reflective blue, and spectrally pure yellow with a background consisting of less pure colours. We also know that UV in itself is not preferred over other bee-visible colours. Second, patterns and their positioning matter: radiating, “sunburst” shapes are preferred over most other shapes. This result is shown in many test environments and using multiple methods, making it one of the reference properties on which scientist build. However, the role of pattern positioning is also becoming clear. A centrally positioned pattern is more likely to elicit floral exploration behaviour even if the shape itself is less ideal. Daisies that display centrally positioned forager-like patterns are consistent with this idea. Floral size has not been shown to make a difference to flower-naïve bees, but it does not necessarily indicate the absence of an effect. Third, symmetry is a topic of much interest but few answers: some empirical studies suggest an unlearned preference,

some empirical studies and computational models suggest a learned preference, and some computational models suggest an unlearned preference under specific conditions. Only a behavioural experiment setting all of these hypotheses in competition with one another can decisively answer this dilemma. Fourth, spatial frequency is also a visual property to which inexperienced bees respond. Lower spatial frequencies are typically preferred over higher spatial frequencies, although this behavioural finding has not been operationally concretized. While physiological capacities have been well defined, unlearned preferences have not been studied with the same precision. Fifth, the role of social cues has been found to make a difference: artificial flowers to which a dead bee was pinned is visited more often than artificial flowers without one, but an abundance of social cues appears to diminish this effect.

1.4.2 Future Directions

The cognitive architecture needs to be sufficiently flexible to allow some learning to adapt to the many varieties of flower displays, but computational processes need to place reasonable boundaries that narrow search options, thereby conforming to temporal and energetic pressures on survival.

Computational models are now starting to reach sufficient complexity and performance to investigate the dynamics of information processing by small brains. The activation pattern and interaction of thousands of neural cells is not easily explored through behavioural, imaging or theoretical models. Even if the whole brain's activity could be recorded with a technique such as calcium imaging (Ahrens and Keller, 2013), activation patterns still need to be interpreted. Computational models, like those of Enquist and Arak (1993) and Orbán and Chartier (2013) will be necessary to understand the “Rosetta Stone” of the brain. However, the results of models are not always easily evalu-

ated behaviourally. One method is to design behavioural experiments and computational models together in such a way that results could be directly compared. For example, a computational model that uses visual patterns that are identical to those used in behavioural experiments can be key to evaluating a model's performance. Models also need an outcome measure that is analogous to behavioural choice data. For example, strength of choice can be measured through a discriminant function, correlation coefficients, or the quality of image reconstruction. But what about behavioural data and computational model results that already exist, but were not developed together in this way? The solution may lie in a new analytical technique used in neuroscience.

The analytical evaluation of results from multiple kinds of data is possible through a new technique called Representational Similarity Analysis (RSA) (Kriegeskorte et al., 2008). The information within visual properties can be characterized by computing the correlation coefficient between each stimulus for a given kind of observation (eg, choice behaviour, model prediction, neural activation, physiological measurement, etc). This technique abstracts the functional properties of results by constructing a series of Representational Dissimilarity Matrices (RDM) for each type of observation. An RDM is a square symmetric matrix where each value is a measure of dissimilarity (ie, 1-correlation) associated with two stimuli. This analytical technique could help integrate the quantitative analysis of unlearned floral behaviours by social bees, and more broadly, cognitive information processing.

Theoretical perspectives also need to be integrated. Cognitive, neuroscientific, ecological, genetic and other perspectives can contribute to novel hypotheses and predictions, but only within a sound evolutionary framework. For example, functional hypotheses can be evaluated through the generation of a nomological network of hypothetically interrelated findings (Schmitt and Pilcher, 2004). A nomological network is a system of laws

that may be related in terms of observations and theories (Cronbach and Meehl, 1955). For example, consider a proposed adaptive hypothesis: the visual system of bumblebees and honeybees is adapted to aid in the discovery of floral rewards. For example, the ecological finding that violet inflorescences attract bees is consistent with the neuroscientific finding that bees are capable of seeing violet, which in turn is consistent with the cognitive behavioural finding that bees tend to land on violet discs.

The focus of research has been largely on the exploration of single properties on a single behaviour (ie, landing). The observation of all behaviours associated with preference should be studied, rather than restricting investigation to one behaviour such as landing. For example, while many landing behaviours are preceded by hovering, a hovering that terminates without landing may indicate the opposite of a preference. Another perspective is that one cue may be necessary to draw bees to hover in front of a flower and a different cue to elicit landing. For this reason, a constellation of properties need to be studied together that lead to floral exploration rather than any one property. It may be only through the activation of a chain of reactions that preference emerges. Visual properties may not show relevance if they do not appear in conjunction with other properties that contribute to earlier stages of naïve preference behaviour. For example, shape, a particular pattern type may be key to attract bees to detect the flower from a distance, but a particular colour combination (eg, blue and yellow), which is only visible from a nearer distance, may be key to elicit antennation or landing behaviour.

Recent technological advancements in recording and miniaturization enable the study of truly untrained flower naïve bumblebees and honeybees, and advancements in automation allow more experimental control and large scale studies that stay sensitive to individual differences. These technological advancements, in combination with analytical and mathematical modeling, and a shift to a unifying functional perspective will propel

ahead research in unlearned bee behaviours. Whether or not such forager-like patterns attract flower-naïve bees has not yet been tested.

Chapter 2

The effect of flower-like and non-flower-like visual properties on choice of unrewarding patterns by bumblebees

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2.1 Introduction

Sensitivity to radial patterns is suggested to be an adaptation that helps bumblebees both to discover flowers and to reduce floral handling time (Leonard and Papaj, 2011; Medel et al., 2003; Waser and Price, 1985; Penny, 1983). Radial patterns are composed of distinctly coloured lines extending from the periphery of a flower, converging to the centre where nectar and pollen are usually found, thus often called *nectar guides*. This study investigates the visual properties that draw bumblebees to their first flower, with

the objective of testing the boundaries of the nectar guide hypothesis. Specifically, we test the relative influence of floral pattern type and pattern position on choice behaviour by bumblebees that have had no prior rewarded experience in the testing environment (ie, “flower-naïve” bees, see Lunau et al., 2006; Giurfa et al., 1995). Here, a stricter interpretation of flower-naïve is used to mean a bumblebee has never received any reward in the testing environment, and workers did not have any experience with flowers or foraging in the flight room prior to the experiment. Indeed the bees in the present study left their colonies for the first time in our experiments.

Approach and landing on artificial flowers by flower-naïve bumblebees is more likely when two small dots are located at the centre of the floral stimulus when compared with no dots at all (Lunau, 1990). The presence of two small dots at the centre of a colour disc elicit approaches directly towards these visual properties, but a colour disc without central dots elicit approaches only to the periphery of the colour disc (Lunau et al., 2006). Moreover, the colour of the visual cue is crucial; bumblebees make antennal contact with artificial flowers where the floral guide’s colour is distinct from the corolla colour, and the corolla colour is distinct from the background colour (Lunau et al., 1996). These results on visual property positioning underscore the importance of the presence *vs* absence of particular visual cues and their positioning in attracting flower-naïve bumblebees to stimuli that are likely to be flowers, and guiding them to the potential source of food on those flowers.

The type of visual pattern has also been explored using honeybees. A study of visual patterns compared preferences for three categories of cues, each with four variations of spatial frequencies and orientations (Lehrer et al., 1995a). The unrewarded testing patterns included black bars on a white background arranged in radiating, concentric and random formations, horizontal and vertical gratings, and plain white and grey discs

(ie, 50% black). Bees showed a clear preference towards radial patterns over other non-random patterns. Because bees had been trained on rewarding checkerboards, however, they were not, strictly speaking, flower-naïve (Plowright et al., 2011). Nonetheless, preference for radial patterns over concentric patterns by bumblebees in a 12-arm radial maze has been confirmed even without prior rewarded experience (Séguin and Plowright, 2008). Leonard and Papaj (2011) found that pre-trained bumblebees not only prefer flowers with radial patterns over the absence of patterns, but handling time to extract a reward is also shorter, promoting higher pollinator fitness. The potential function of radial patterns may well be to guide bees to the source of pollen (Pohl and Lunau, 2007; Dafni and Kevan, 1996; von Frisch, 1914). What remains unclear, however, is whether it is the radial elements per se that are salient to bumblebees, or it is the relatively high spatial frequency gradient (the “busyness”) that typically occurs at the centre of radial patterns that is salient to bees. If so, radial patterns lacking the central elements may fail to elicit approach or landing and conversely, concentric patterns with added central elements may draw bumblebees to them.

Two distinct methodologies were implemented in the present study to observe unlearned choice behaviour: using radio-frequency identification (RFID) and motion-sensitive video recordings. Both are automated systems, as recommended by Döring and Chittka (2011). RFID systems have been successfully implemented in studies using bumblebees and other eusocial insects (Molet et al., 2008b; Sumner et al., 2007; Streit et al., 2003), and most recently in a study involving the detection of rewarded bumblebee visits to feeders (Ohashi et al., 2010). This latest implementation consists of an automatic sugar-solution dispensing mechanism that allows finely tuned nectar secretion adjustments without refilling for up to 7 h. The RFID system implemented here is distinct from Ohashi et al.’s in two crucial elements. The visits are not rewarded, and the stimuli dis-

play visual cues that are manipulated. The second methodology recorded artificial floral landings using a motion-sensitive video camcorder that allowed the continuous observation of freely exploring workers in a flight room. Video recording is a common approach to observing insect behaviour, and motion sensitive recording allows the efficient recording of relatively rare events (Lihoreau et al., 2012).

The research question in Experiment 1 relates to how different visual parameters interact when displayed together. We seek to explore the relative importance of pattern positioning on a flower in relation to pattern type using a 2 x 2 design in which the radial and concentric pattern types are placed centrally or peripherally.

The questions of the second experiment are threefold. First, is flower-entry as measured by RFID, and landing as measured by video recordings equivalent choice criteria (see Figure 2.5 a). Second, what is the effect of central *vs* peripheral positioning? It is not known whether or not workers would choose a central pattern if a combination consisting of two radial patterns in different positions were presented (see Figure 2.5 b). Third, what is the relative importance of pattern position *vs* pattern type? In other words, will bumblebees land on patterns of the preferred pattern type, or the preferred pattern location? Bees could prefer central radial to a peripheral concentric pattern, but it could be due to the pattern type or its central positioning. In this experiment, we pit the two variables against each other (a strategy borrowed from Brodbeck and Shettleworth, 1995)(see Figure 2.5 c,d). Finally, is the RFID recording technique equivalent to video recordings of landing behaviour? Three of the four combinations are equivalent across the two experiments allowing for a direct methodological comparison.

A confound between radial and concentric patterns, or no patterns is the presence or absence of a visual property positioned at the centre of the flower. An inherent characteristic of the radial pattern is the meeting of bars at the centre of the floral

stimulus. However, this is not the case with concentric patterns, or many previously tested visual properties (eg, checkerboard, parallel bars, random bars). Perhaps there is something special about lines crossing at the centre to which bees are responding. In the second study, a concentric pattern with a central dot will be used to eliminate this confound.

2.2 Methods

2.2.1 Experiment 1: Radio-Frequency Identification

A total of 375 workers were used from a colony of commercially initiated bumblebees (*Bombus impatiens* Cresson; Biobest Biological Systems, Leamington, Canada) with ten initial workers and a queen. The colony was housed in a bipartite wooden box (30 x 15 x 15 cm) and received ad libitum sugar solution (1:2 water: sugar by volume) and pollen prior to the experiment. The experiment did not include any training sessions prior to testing, and bumblebee workers were not rewarded at any of the artificial flowers. Bumblebees never received a reward outside of the colony.

Apparatus Testing was performed inside a wire-mesh flight cage (1.6 x 1.6 x 1.6 m) with a white wooden frame, and a green wooden floor. Entry to the flight cage was through a 1.5 cm radius opening, which was connected to the nest-box via glass-covered 5 cm wide and 20 cm long wooden tunnel. Daylight was supplemented with ten compact fluorescent light bulbs lit the flight cage with electronic ballasts running at 60Hz lamp frequency, and with bulbs performing at 3500 K colour temperature.

Three radio-frequency identification (RFID) readers were used (2k6 head; Microsensys GmbH, Erfurt, Germany): one placed at the entry point to the flight cage and two in

pecially designed artificial flowers (see Figure 2.1, and see videos of flight-room and examples of behaviour at www.beelab.ca/Resources.html). RFID tags (mic3-TAG 64 bit RO, Microsensys GmbH) were glued to the thorax of workers. The funnel shaped RFID-enabled artificial flowers guided workers to 5 mm distance from the reader where tag identifications are reliably recorded. This distance was optimal in that bumblebees of multiple sizes could fit into the flower and the chance of non-readings were minimized. Workers walking on the outer edges of the pattern or flying near it could not trigger the reader.

Artificial flowers were made of oven-baked clay, shaped into a 3-dimensional funnel shape, with a cylinder on its posterior end (Sculpey, sculpey.com; Staedtler, staedtler.com). The cylinder had a rectangular opening on its superior portion to allow for the placement of the RFID reader (see Figure 2.1). The body of the artificial flower was blue (RGB: 8,43,108) with yellow (RGB: 200,180,42) patterns on the inside of the funnel portion. “RGB” is a common colour-space that is defined by the amount of red, green and blue in the composition of a mixture colour. The choice of colours is a matter of practicality: to emphasize the presence objects in a room with white walls. A new set of artificial flowers was used for each experimental stage to avoid any visual and olfactory marks to carry over between testing sessions.

The visual configuration of artificial clay flowers consisted of a blue background, and yellow lines. The configuration of a visual pattern refers to the different orientation of yellow-lines used to display on the artificial flower (see Figure 2.1). Two kinds of patterns were used: radial and concentric (i.e., multiple circles of varying radii) patterns, and two presentation areas were defined: patterns appeared either on an inner portion of the funnel that extended from the centre to half-radius of the stimulus, or the outer portion of the funnel that extended from half-radius to the radius of the stimulus. The yellow

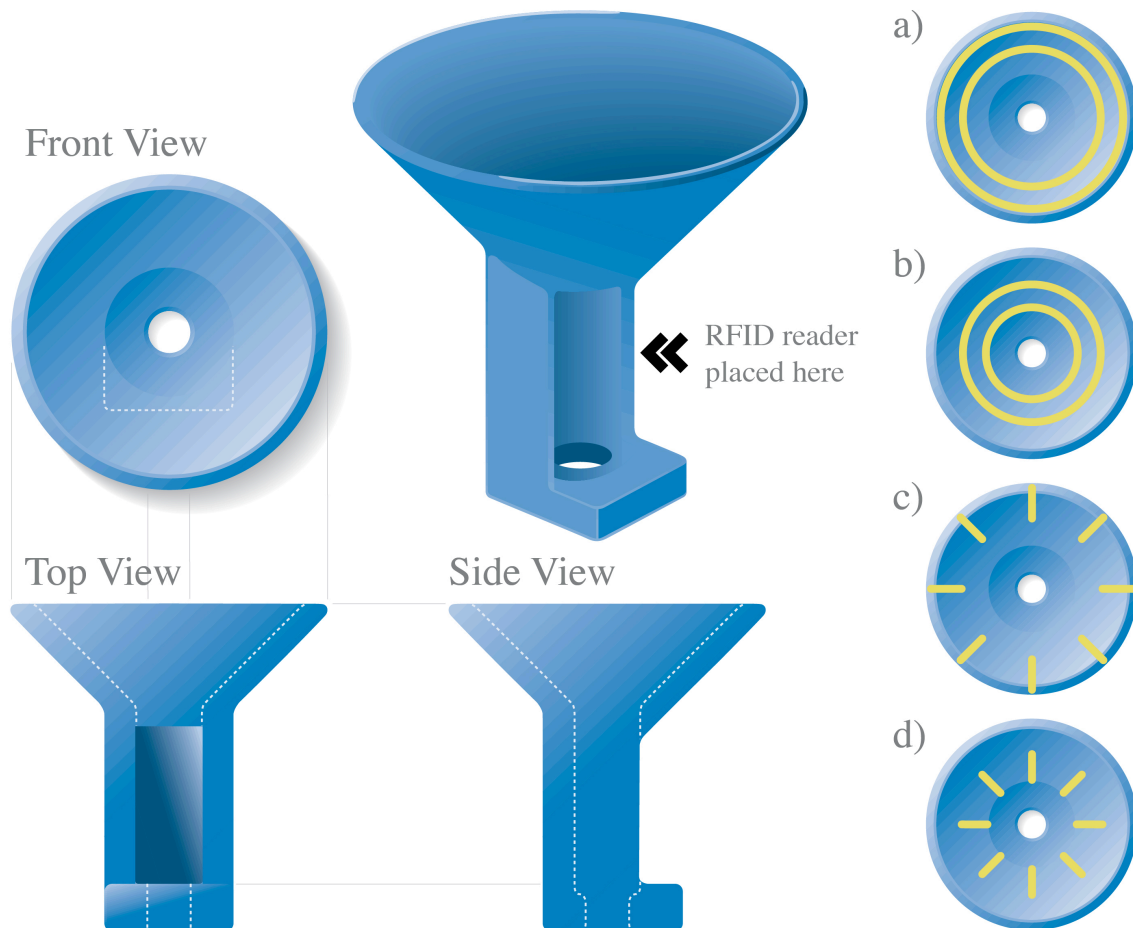


Figure 2.1: Schematic diagram of the RFID-enabled artificial flower used in Experiment 1. The RFID reader rested on top of the open cylinder through the centre of the flower. Stimuli patterns and positions: a. peripheral concentric, b. central concentric, c. peripheral radial and d. central radial.

lines on both concentric patterns and radial patterns were 4 mm in thickness. Similar 4 mm radius dots were recognized by bumblebees in another free-flight experiment (Lunau et al., 2009). The areas covered by the yellow markings were made as similar as possible by using two concentric lines, and eight radial spokes.

Procedure Workers were tagged throughout the life of the colony. Each worker was tagged within one to three days of emergence. Tagged workers were placed back in the colony and were free to move in and out of the nest-box throughout the experiment. Pairs of artificial flowers were displayed inside the flight cage. All workers in the colony had access to the flight cage freely between 9:00 and 20:00. Each stimulus combination was displayed until at least 25 different workers visited a flower. All stimulus combinations were displayed over a 44 d period, presented during four sessions in which the pairs of stimuli were: Central-Radial vs Central Concentric (Session I: 11 d); Peripheral-Radial vs Central-Concentric (Session II: 11 d); Central-Radial vs Peripheral-Concentric (Session III: 10 d); Peripheral-Radial vs Peripheral Concentric (Session IV: 11 d) (see Figure 2.1 a-d) for exemplars). The placement of each artificial flower with respect to the other (left vs right) in the four pairs was counterbalanced. In other words, if a particular pattern appeared on the left side one day, it would appear on the right side on the next day. Artificial flowers were located 1.5 m above the floor of the flight cage attached to the left side edge, and the two artificial flowers were 1 m apart.

The RFID reader recorded tag detections every 1 ms, which resulted in multiple data points for a single visit, where a single visit is defined as one entry into the artificial flower. One of three types of RFID events were used (1: date of tagging, 2: detection at flight cage entry, and 3: detection at flowers). The raw data, including the date of workers' natural death, are available on request. The number of data points for a single visit was directly proportional to the time the worker spent under the RFID reader. RFID

data were downloaded from the readers periodically and further processed in a database application (MySQL; Oracle Corp., Redwood Shores, United States of America).

2.2.2 Experiment 2: Motion-Sensitive Video Recordings

A total of 136 bumblebee workers were used from five colonies. Colonies with 10 - 50 initial workers and a queen were donated by Koppert Biological Systems, Inc. (Ann Arbor, U.S.A.). They were housed and fed as in Experiment 1. Each worker received a colour number-tag (Opalithplättchen) to allow visual identification. Workers were free to move between the nest-box and the testing environment throughout testing, 24 hours a day. As in Experiment 1, there was no training session and workers were not rewarded at any of the artificial flowers. Bumblebees never received a reward in the testing environment.

Apparatus The nest-box was connected to the flight room (389 x 455 cm) with a 10 cm long 2.5 cm diameter wire-mesh tube. Twelve fluorescent daylight bulbs (Sylvania model FO32/841/XP/SS/EC03) with electronic ballasts (40 Khz Sylvania Quicktronic T8 InstantStart) provided lighting. Temperature was maintained at 24°C, and humidity levels varied between 20% and 40%.

Two high-resolution Internet Protocol (IP) camcorders were used to record worker behaviour near the conical portion of the artificial flowers (Vivotek, Model: IP8161; Chung-Ho, Taiwan). The camcorders were configured to record at 1600 x 1200 pixels, 15 frames per second in H.264 video format in colour, and without audio.

Stimuli Flower patterns made of paper were shaped into a 3-dimensional cone. The visual patterns were printed using a colour laser printer with a blue background (RGB: 57, 127, 208) and yellow lines (RGB: 243, 251, 9) formed visual patterns. The cone's physical

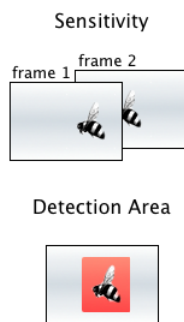


Figure 2.2: Schematic diagram of how sensitivity and detection area is measured by the motion sensitive camcorders.

properties consisted of 5 cm diameter, and 2.5 cm vertex. The width of the yellow lines was 0.5 cm. The central patterns extended 2.5 cm from the centre, including a visual mark at the centre, and the peripheral patterns extended 2.5 cm from the periphery of the flower. Four types of patterns, relevant to the study's predictions were used in four sets of choice combinations. The first combination consisted of a radial and a concentric pattern, both of which were located centrally; the second combination consisted of two radial patterns, one of which was centrally located and the other peripherally located; the third and fourth combinations each included one radial and one concentric pattern type: one pattern was centrally and the other peripherally located. The pattern combinations were placed on a stand 1.3 m high from the ground and 1.7 m away from the nest entrance.

Procedure The camcorders were configured to begin recording a 10 s long video clip only when motion was detected at the artificial flowers. Sensitivity threshold was set to 85%, and detection area was set to 5%. Sensitivity threshold refers to the motion detecting algorithm's criterion that measures the number of pixels that changed between two adjacent frames, and the detection area refers to the percentage of changed pixels of the total motion detection trigger area (see Figure 2.2).

All workers in a colony were tagged upon arrival. Visual stimuli were placed on a wooden stand in the flight room 30 cm apart from each other. Each pattern was centred on a photo (5 x 5 cm) of foliage background (Forrest and Thomson, 2009). The foliage background was introduced in this experiment in an attempt to enhance pattern visibility. An eight-day period allowed the presentation of all possible floral combinations with left and right floral positions counterbalanced.

The video clips were analyzed frame-by-frame using Finder's Quick Look, a feature of Mac OS X 10.7.4 (Apple Inc, Cupertino, U.S.A.). Visits from untagged workers, and workers with tags that could not be identified due to very quick movement, poor orientation or poor camcorder focus were discarded. In these cases, it was impossible to determine whether the visit was from a single bee, or multiple bees, which would have led to incorrect calculation of degrees of freedom and therefore, choice heterogeneity. Landings on stimuli occupied by another worker were also discarded because the displayed pattern was altered by the presence of the other worker.

2.2.3 Design and Statistics

Both RFID and video data were analyzed with replicated goodness of fit tests for categorical data (Sokal and Rohlf, 2012). Replicated G-tests are designed to analyze repeated measurements from distinct bumblebees, thereby avoiding pseudoreplication. G_h values are a test for heterogeneity of choices (ie, individual differences), and G_p values test for the deviation between a group choice proportion (ie, pooled data) and a theoretical value of chance (50:50). The G value is compared to the χ^2 distribution, but the test is not a χ^2 test. Four planned tests were performed that might justify the use of Bonferroni-correction, but due to increased criticism of this statistical technique in many fields (Lieberman and Cunningham, 2009; García, 2004; Nakagawa, 2004; Perneger, 1998), we

Table 2.1: Results of the replicated Goodness of fit test pooled values, their degrees of freedom and probabilities, and heterogeneity values, their degrees of freedom and probabilities in Experiment 1, as shown in Figure 2.3.

Conditions	Pooled			Heterogeneity		
	G_p	df	p	G_h	df	p
Central Radial <i>vs</i> Central Concentric	3.96	1	.047	197.55	41	.000
Peripheral Radial <i>vs</i> Central Concentric	33.77	1	.000	210.61	42	.000
Central Radial <i>vs</i> Peripheral Concentric	508.31	1	.000	345.78	30	.000
Peripheral Radial <i>vs</i> Peripheral Concentric	7.42	1	.006	84.06	24	.000

report precise p -values in place of Bonferroni-correction.

2.3 Results

2.3.1 Experiment 1: RFID Data

All 375 workers in the colony were tagged with an RFID transponder and 318 (84.8% of colony total) of these workers entered the flight-cage at some point during the study. A total of 197 (52.5% of colony total) visited at least one of four artificial flower stimuli.

Four replicated goodness of fit tests were performed on all choices from a bees naïve session to compare choice deviations to a theoretical value of chance (Sokal and Rohlf, 2012). A bee’s naïve session refers to the first testing condition in which the bee “participated”. The G-tests reveal a preference for central positioning (see Table 2.1) and radial pattern type. Figure 2.3 (b) shows that pattern preference is reversed when the concentric pattern is positioned centrally and the radial pattern is positioned peripherally. However, if positioning is held constant as in Figure 2.3 (a) and (d), pattern preference is towards the radial pattern. Figure 2.3 shows that the relative proportions of first choices for each pattern for each combination were comparable to the proportions shown for all choices. Heterogeneity of choices was statistically significant indicating much noise in

Table 2.2: Results of the Sinusoidal Regression analysis to quantify the relationship between the number of pattern visits over time and choice proportions.

	df model	df error	R^2_{sin}	R^2_{linear}
Peripheral Radial <i>vs</i> Central Concentric	3	9	98.8%	28.2%
Peripheral Radial <i>vs</i> Peripheral Concentric	3	7	97.7%	21.8%
Central Radial <i>vs</i> Central Concentric	3	9	98.1%	36.7%
Central Radial <i>vs</i> Peripheral Concentric	3	9	98.6%	47.6%

preferences. Preference for the opposite patterns were noted especially in workers that made relatively few choices (< 10 choices per worker) compared to most workers (mean choices ranged from 20 - 60 per worker, depending on condition).

Subsequent choice proportions were analyzed for shifts in preference. Preference refers to the proportion of bee landings to one radial pattern *vs* all landings. If this proportion deviates significantly from chance level of 0.5, we call it preference. Figure 2.4 shows bumblebee worker choices for each condition. Tag recordings were filtered to retain one choice per one-minute interval to minimize the possibility of treating a long visit as a series of repeat visits. Average choices were calculated for each 5 visits until a total of 60 visits. Figure 2.4 shows a cyclical shifting of preferences. At first, initial choice proportions become amplified, and then weaken, in some cases reverse for a short period. The stronger the observed preference for a radial pattern, the less likely it is that a reversal is observed during the fluctuation (ie, proportion to dip below 0.5). A sinusoidal model was fitted to the data using least squares estimation as the error term. The model explains a significant amount of the variance for all four conditions (see Table 2.2). The results of the model indicate that in the absence of reward, choice preferences shift cyclically. While choice proportions fluctuate in all four conditions, depending on the relative attractiveness of a given stimulus, in case of a strongly preferred stimulus such choices never reverse (eg, Figure 2.4 b,d), but in case of a weakly preferred stimulus, choice preferences reverse during periods of maxima or minima (eg, Figure 2.4 a,c).

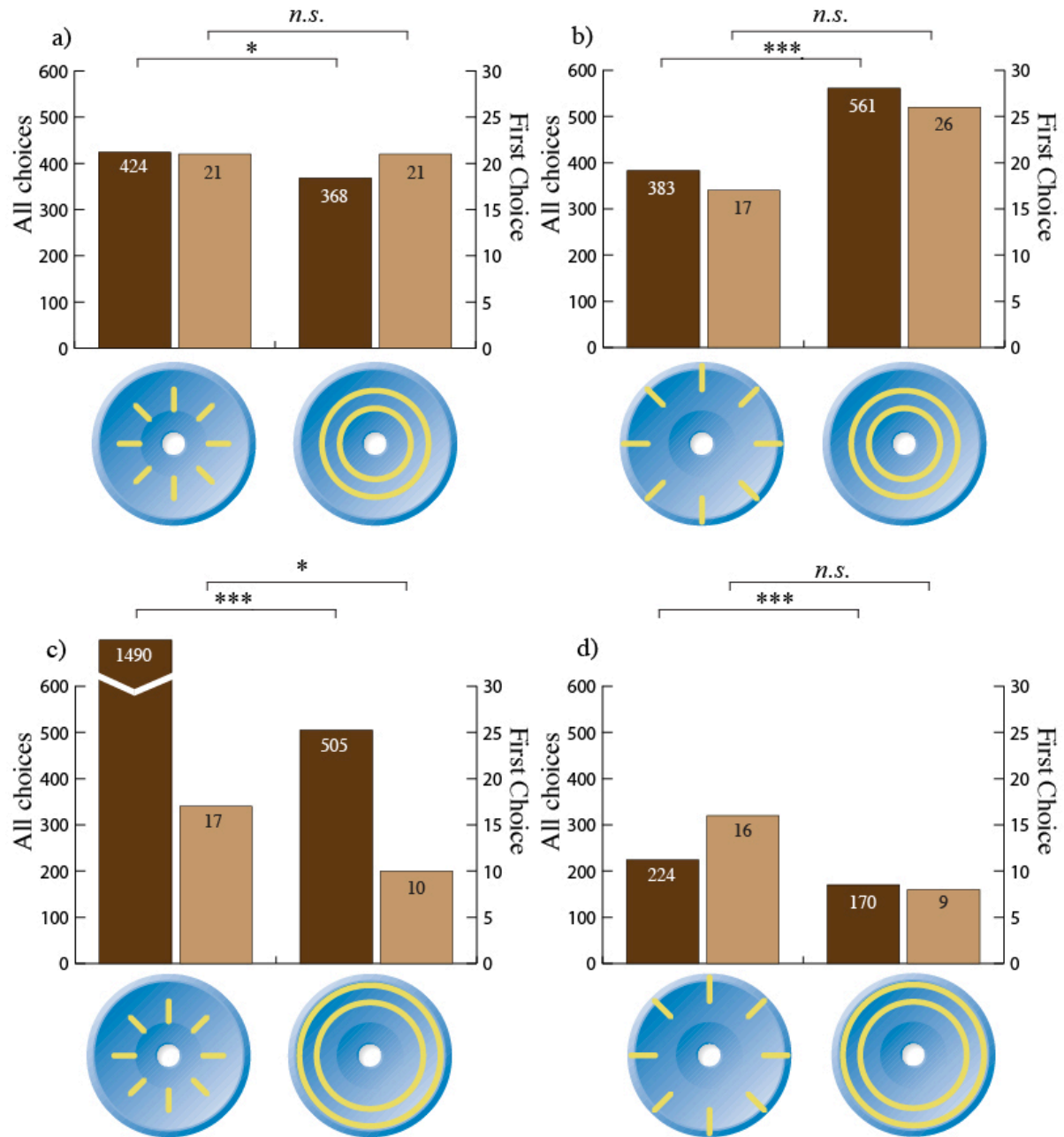


Figure 2.3: Choice frequencies at the four different flower combinations in Experiment 1. The dark brown bar graphs show all choices from the bee's naïve session (left-side y-axis), and the light brown choices indicate the first choice of each worker (right-side y-axis). Asterisks indicate a choice proportion that is significantly different from chance. Note. * $p < .05$, ** $p < .01$, *** $p < .001$

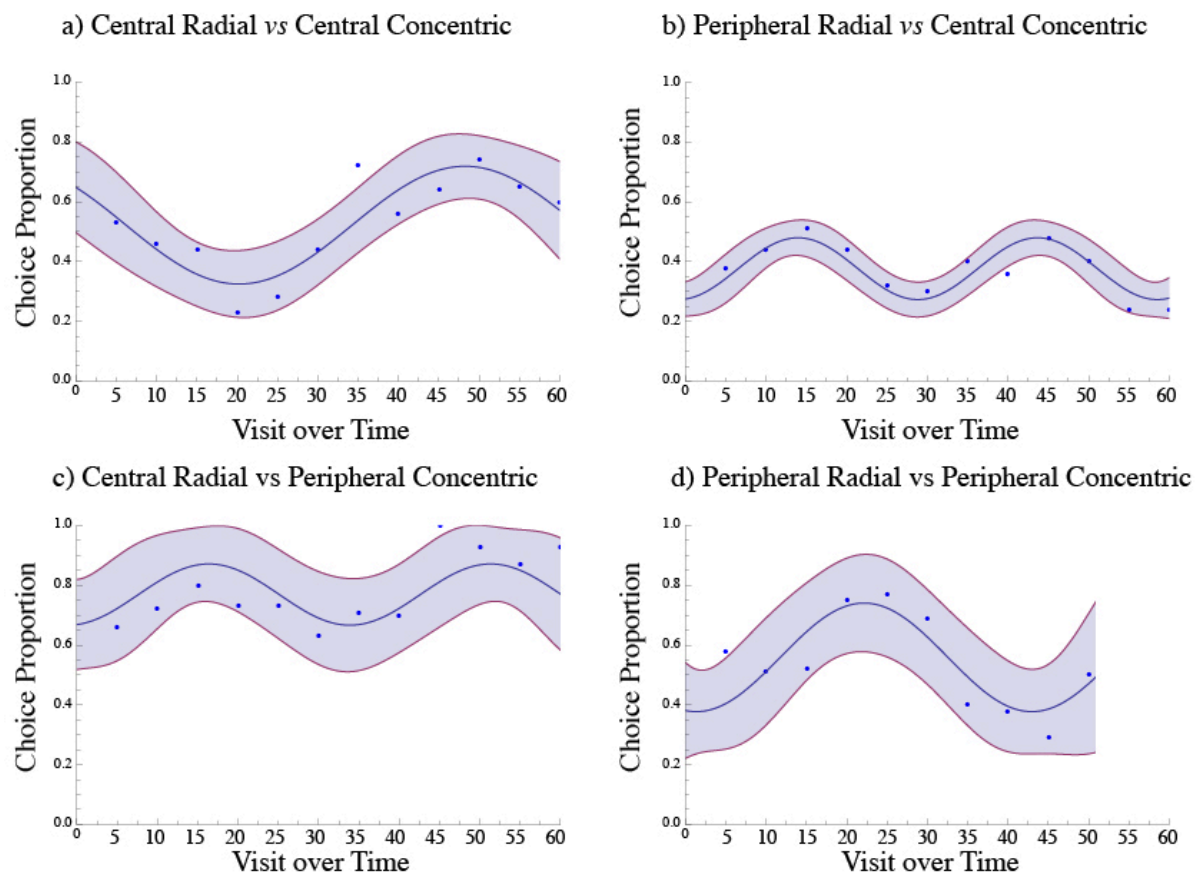


Figure 2.4: The data points indicate proportions of choices towards the radial pattern in Experiment 1. Each data point represents the average of five choices. The blue line shows the sinusoidal regression model prediction, and the purple lines show the 95% confidence interval.

Table 2.3: Total number of choices recorded at the artificial flowers in Experiment 2 for each colony, and the number of workers making these choices.

	Session 1		Session 2		Session 3	
	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	
No. of Workers	45	7	2	8	23	
No. of Choices	151	25	2	20	65	

2.3.2 Experiment 2: Video Data

A total of 264 choices were recorded across the four conditions over three testing sessions. Table 2.3 shows the number of workers and choices contributed from each colony. Four replicated goodness of fit tests found three group proportions that deviated significantly from chance, and one non-significant overall proportion (see Table 2.4 and Figure 2.5). Statistical significance is defined as group choice proportions that deviated significantly from a chance value of 50:50. (1) Pattern is important: the central-radial and central concentric pattern resulted in significant preference towards the central-radial pattern (see Table 2.4). (2) Position of the radial pattern is not so important: the presentation of the central-radial and peripheral radial combination showed no significant difference from chance. (3) The central-radial and peripheral-concentric combination resulted in a strong preference towards the central-radial pattern. The central-concentric and peripheral-radial combination elicited significant preference towards the peripheral-radial pattern. Pattern trumped location. Individual differences were non-significant in all four combinations as indicated by non-significant heterogeneity tests (see Table 2.4).

Our observation that bees tended to follow the yellow lines of the visual stimuli led us to explore the behavioural characteristics of landing in terms of the duration of stay on the floral stimulus, and the bees movement on the stimulus. An analysis of initial landing position during the third session shows that 92% of all landings were on the yellow lines. Workers that landed on the peripheral-concentric pattern stayed for an average

Table 2.4: Results of the replicated Goodness of fit test pooled values, their degrees of freedom and probabilities, and heterogeneity values, their degrees of freedom and probabilities in Experiment 2, as shown in Figure 2.5.

Conditions	Pooled			Heterogeneity		
	G_p	df	p	G_h	df	p
Central Radial <i>vs</i> Central Concentric	17.98	1	.000	40.72	29	.073
Central Radial <i>vs</i> Peripheral Radial	1.85	1	.173	53.63	39	.060
Peripheral Radial <i>vs</i> Central Concentric	6.57	1	.010	26.31	27	.500
Central Radial <i>vs</i> Peripheral Concentric	18.18	1	.000	41.92	37	.266

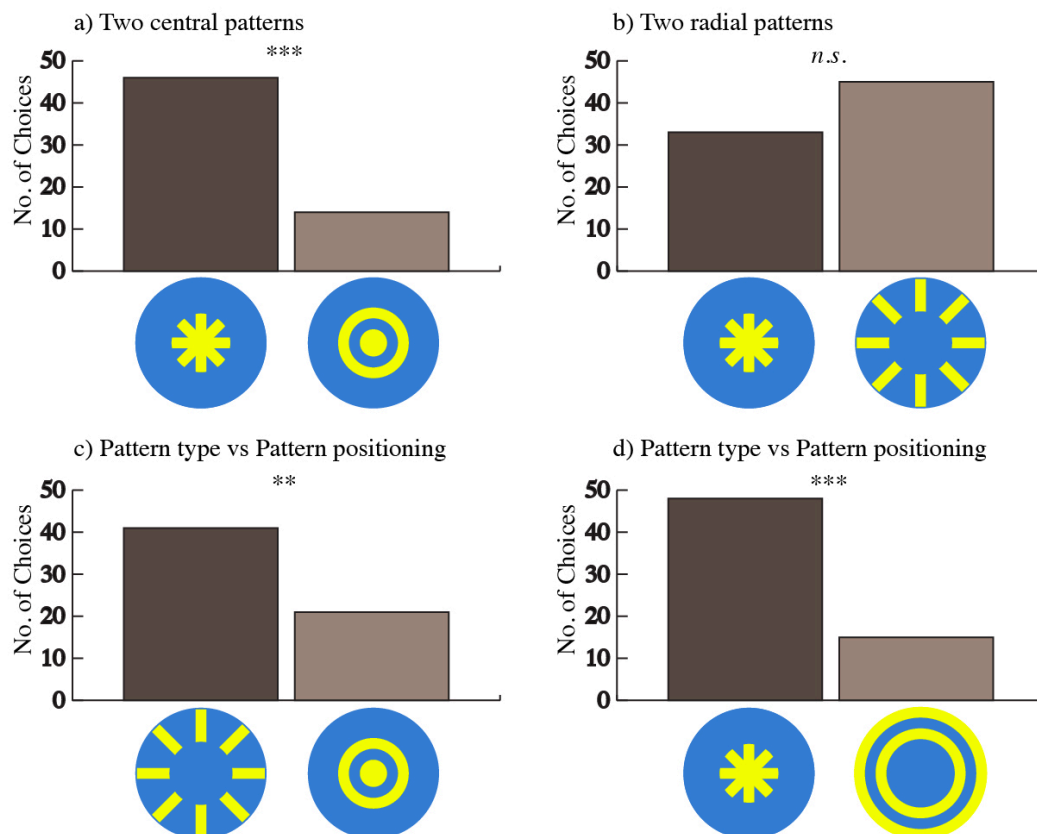


Figure 2.5: Choice frequencies at the four different flower combinations in Experiment 2. Values indicate the number of choices of the displayed pattern. Asterisks indicate a choice proportion that is significantly different from chance. Note. ** indicates $p < 0.01$, *** indicates $p < 0.001$

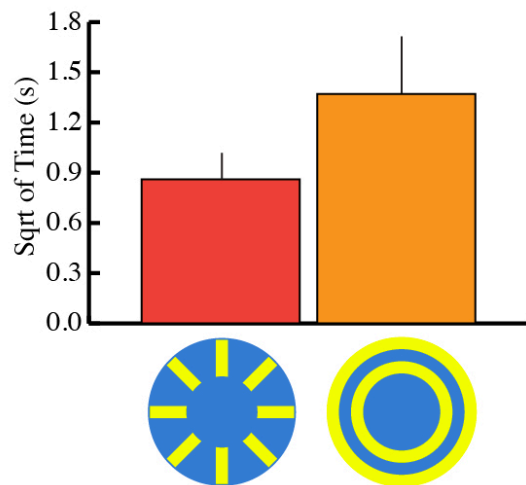


Figure 2.6: Landing duration analysis. Error bars show M.S.E. A *t*-test indicates significant difference in landing duration between the peripheral concentric and peripheral radial patterns in Experiment 2. Time values were square-root transformed to meet *t*-test assumptions of normality. Note. * indicates $p < .05$.

of 2.2 s (M.S.E. = 0.20) compared with 0.9 s (M.S.E. = 0.13) on the peripheral-radial pattern ($t(21) = 2.31$, $p = .031$; square-root transformed to meet normality assumptions; see Figure 2.6). The path of movement from each individual worker was recorded and visualized (see Figure 2.7). The paths on the peripheral-radial pattern appear to show more movement near the centre of the stimulus, and the peripheral-concentric pattern shows more circular paths around the periphery of the stimulus.

2.4 Discussion

2.4.1 Pattern Type and Position Effects

Results of Experiment 1 indicate that the central positioning of patterns is most indicative of choice preference: workers explored a centrally positioned pattern more often, regardless of whether a radial or concentric pattern was displayed. Further, the first

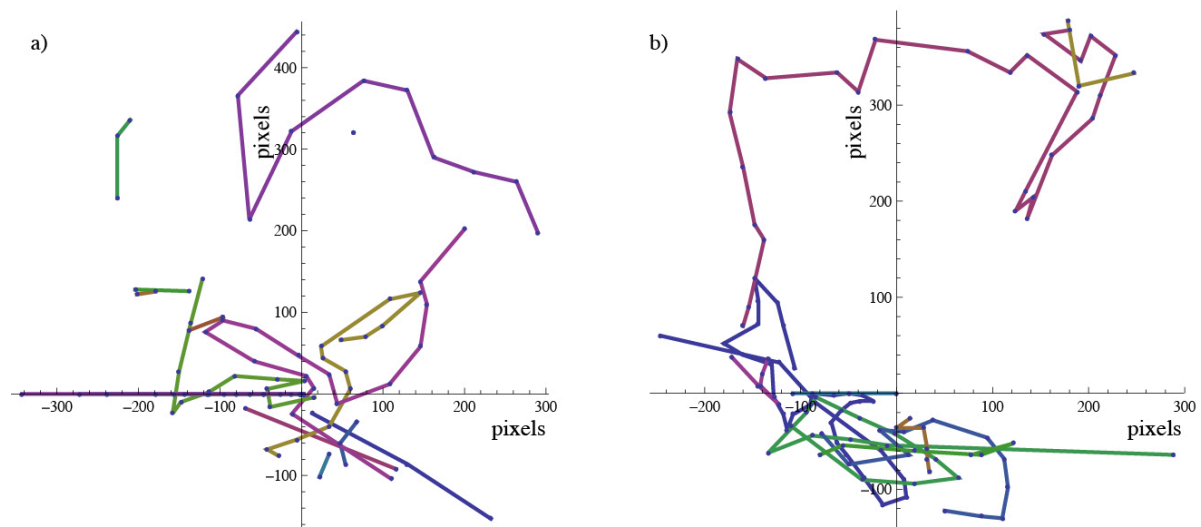


Figure 2.7: Individual workers' motion path on the floral stimulus in Experiment 2; $(0,0)$ coordinates represent the centre of the flower; different colour paths represent different workers. (a) Peripheral Radial; (b) Peripheral Concentric. Paths are slightly skewed due to camcorder view angle of stimuli: positive y -axis values are closer to the centre, and negative y -axis values are further away from the centre than they appear. Number of crossings through the centre are not affected.

choices of workers did not substantially differ from all of their unrewarded choices. This is a surprising finding given how well the preference for radial patterns has been shown previously.

On the other hand, results of Experiment 2 show the relative importance of pattern type is greater than pattern position. When pitted against concentric patterns, radial patterns are always preferred, and when a radial pattern on the centre is pitted against a radial pattern on the periphery, choice proportions are not different from chance, indicating that the influence of location on the flower is smaller. Further, nearly all landing behaviours are observed on the pattern, further underscoring the importance of pattern over location. The preference for radial pattern holds even though the central concentric pattern included a small dot marking the centre.

Experiment 2 also shows that a visual cue can also be used to guide bees to the

periphery of the flower, as is the case for peripheral-radial preference over a central-concentric pattern in Experiment 2. Lunau's suggestion (Lunau, 2006; Lunau et al., 2006) that visual cues guide bees to the centre of the flower is extended by showing that bees will land on parts of a flower other than the centre, if that is where the spectrally pure yellow markings, resembling the colour of pollen, are found. As Lunau's results suggest, most flowers display such markings at the centre of the flower, but centrality is not the only dimension bees evaluate when approaching a flower. Our results are consistent with the pattern preference findings of Lehrer et al. (1995a) and Plowright et al. (2006) and extend them by showing that preference for radial patterns persists even when another dimension of visual cues, pattern position is introduced.

There are key differences between the results of Experiment 1 and Experiment 2. The peripheral radial *vs* central concentric pair appears in both experiments, but we recorded preference for the central concentric type in Experiment 1 and a preference for the peripheral radial type in Experiment 2. As RFID technology in insect research gains significant traction (Robinson et al., 2012; Silcox et al., 2011; Streit et al., 2003), methodological comparisons become increasingly important to investigate. We interpret these differences between RFID recordings and motion-sensitive video recordings to be due to measuring choice behaviour differently, though we can not rule out the possibility that methodological differences between the experiments such as pattern positioning, the background for the patterns, size of the flight cage or lighting conditions might have somehow produced the observed differences. In Experiment 1, there may have been landings that were not followed by flower-entry, which is consistent with landing-path patterns (Figure 2.7). Future studies could benefit from viewing distinct choice behaviours in the context of releasing mechanism of behavioural reactions (Heuschen et al., 2005; Lunau, 1992): different floral cues have a role at different stages of preference

behaviour (ie, approach, antennal reaction, landing, flower-entry). Applying the releasing mechanism hypothesis in this context, radial cues that elicit landing behaviour do not necessarily elicit floral-exploration behaviour. In the future, an RFID system that is time-locked to video recordings would allow exploring landing and floral exploration simultaneously.

2.4.2 Cyclical Pattern Preferences

It is the first time choice proportions at unrewarded flower-like stimuli were recorded at high-temporal resolution. These recordings show a remarkable alternating amplification and attenuation of choice proportions. This finding is consistent with Plowright et al's observation of habituation of pattern preferences in the absence of reward (Simonds and Plowright, 2004). Because this is a serendipitous finding, because complete cycles were not obtained in all conditions, and because time was measured in number of visits, we can draw no further conclusions regarding the temporal dynamics of attention.

Additionally, many bumblebees did not persist with visiting the artificial flowers in Session IV beyond 60 choices. More importantly, in all four conditions the number of bumblebee workers that persisted in visiting the flowers drastically decreased: most workers visited the flowers less than 20 times. However, the few workers that persisted, contributed a disproportionate number of choices, showing great individual variability from bumblebee to bumblebee in terms of activity levels.

It does not appear that social or chemosensory learning could account for these results. In terms of social learning, most of the research has been conducted in the context of rewarded experience (eg, Leadbeater and Chittka, 2007), which does not apply here. It is unclear what if any social learning would take place in the face of unrewarded experience. In terms of chemosensory learning, there is little empirical evidence or theo-

retical justification for bumblebees to leave olfactory cues on unrewarded objects. There are studies that indicate early olfactory learning inside the nest but such odours (ie, sugar-water and pollen) were not present in the testing environment (eg, Molet et al., 2008a).

2.4.3 Methodological Considerations

RFID technology enables studying hundreds of individual workers with ease and high precision, but the characteristics of the recorded behaviour varies. The observed choice behaviour can be described as flower-entry, in spite of the absence of any floral odour or food source, which is a very strict criterion of preference compared to criteria used in other studies, such as approach (Church and Plowright, 2005), entry into a maze-arm (Plowright et al., 2006), antennal reaction (Lunau et al., 2006), or landing on a pattern (Chittka and Walker, 2006; Chittka et al., 2003). In order to compare the validity of choice behaviour definitions, and to validate the new RFID method, we observed landing video recordings in Experiment 2.

A useful element of testing in a flight-cage or flight-room versus a more constrained environment such as a Y-maze or a radial maze is that bumblebees can freely optimize their distance from artificial flower, thereby ensuring that the test object's characteristics are visible to the subjects. Distance from the artificial flower is important in terms of colour vision and pattern resolvability (eg, Wertlen et al., 2008; Land and Fernald, 1992), and the ability to hover in front of the objects reveals additional information about the patterns' shape and precise distance, which is impossible for bumblebees to do in constrained testing environments (Land, 1999).

Researchers using bees often study unlearned choice behaviours with pre-training sessions, which offer reward at a stimulus considered as neutral (e.g. gray, transparent or

checkerboard patterned disk). This pre-training makes the approximation of spontaneous choice behaviour easier and quicker (eg, Ings et al., 2009; Raine and Chittka, 2007; Rodríguez et al., 2004; Møller and Sorci, 1998; Giurfa et al., 1995). The alternative methodology of studying spontaneous choice behaviour without pre-training is difficult to implement because bees that were not trained to feed at artificial stimuli do not readily approach and land on them. However, a number of studies showed that even stimuli that appear neutral will influence choice behaviour in unexpected ways (Plowright et al., 2011; Simonds and Plowright, 2004; Gumbert, 2000), and so bees that have been rewarded for visiting visual patterns can not be considered to remain flower-naïve. Spontaneous choice behaviour has been generally used for exploring choice behaviour of flies, beetles, crickets (Silcox et al., 2011; Desurmont and Weston, 2010; Zhang et al., 2007), which are not easily trained. This study departs from the traditional methods of studying choice behaviour in Hymenoptera using conditioning paradigms. This modification makes the conclusions about naïve choices stronger, but also makes it more difficult to compare the results to studies using learning paradigms because very different processes take place in the absence of reward. For example in one study where brain activity for odour representations was studied using in-vivo calcium imaging, brain activity increased for rewarded odours, but decreased for unrewarded odours (Faber et al., 1999).

2.4.4 Visual Cognition and Floral Signals

Future research in this area might profitably address questions on the visual cognition underlying the behaviour of flower-naïve bumblebees in much the same way that questions on the visual cognition of experienced foragers have been addressed in honeybees and other social insects, such as configural processing (Avarguès-Weber et al., 2011a, 2010), feature detection (Horridge, 2009), local feature assembly (Stach and Giurfa,

2005; Stach et al., 2004) and concept learning (Avarguès-Weber et al., 2011b; Giurfa et al., 2001; Brown and Sayde, 2013), and stimulus generalization and categorization (Dyer and Griffiths, 2012; Benard et al., 2006; Stach and Giurfa, 2005). Much of this work shows that choice behaviour changes with experience, but our work here concentrates on the effects of unrewarded experience rather than rewarded experience. Positive or negative outcomes of decisions are signposts that guide learning and behaviour. The potential for learning by social insects from a series unrewarded experiences remains an understudied line of inquiry.

Another caveat of the findings is that while bumblebees perceive differences between the stimuli as evidenced by the non-random choice proportions, we do not claim to know how patterns were perceived here. Moreover, we do not know whether the flowers were compared by the bumblebees, and choice of one flower over the other was made. Alternatively, the bumblebees may have made approach and landing decisions for flowers on an individual basis as they encountered each flower independently. Again, further research into cognitive mechanisms underlying choice may elucidate this question.

The broader question is whether unlearned preference behaviour for radial patterns is (a) an adaptation specific to pollinating insects such as bumblebees, (b) pollinating animals that include non-insects, such as hummingbirds, (c) or perhaps the preference relates to a by-product of the information processing property of the nervous system found in other, even non-pollinating animals. The findings of this study along with the findings of Leonard and Papaj (2011), are consistent with the idea that radial pattern preference is an adaptation that increases workers' fitness. It is useful to keep in mind that in the plant-pollinator co-evolutionary process, plants developed signals that their pollinators would take better notice of, so the second and third hypotheses remain distinct possibilities.

Chapter 3

How do bees first find flowers?

Unsupervised neural networks to
quantify the computational cost of
information processing

3.1 Information Processing By Pollinators

Cognitive systems were shaped by selective pressures to minimize costs related to temporal delay and energy requirements of information processing (eg, Buss, 2005; Laughlin et al., 1998). These broad pressures have shifted organisms to use as little and as relevant information as possible to make fast and optimal choices in an uncertain environment (Gigerenzer and Gaissmaier, 2011; Hasenstaub et al., 2010; Dukas, 2002; Dall et al., 2005). However, even strong selective pressures cannot push organisms to function beyond the boundaries of the physical laws that govern information processing: also known as per-

formance envelopes (Levin, 2011). The purpose of the models here is to test the amount of information required to process visual stimuli that is independent of the energetic requirements to maintain the information processor unit (i.e., brain tissue).

The energetic requirements of information processing have been explored in various insects (Laughlin, 2011; Niven et al., 2007; Laughlin et al., 1998). Measured in bits, which refer to the amount of information required to reduce uncertainty, blowflies (*C. vicina*) have experimentally been shown to process information at a rate of 1000 bits per second, while fruit flies (*D. melanogaster*) process information only at a rate of about 200 bits per second. A bit refers to the number of ATP molecules required to transmit a bit of information. Information and energy consumption rate is proportionately increasing with increasing amounts of available light. In other words, the more information available, the more it costs to process it. However, there is a minimum amount of energy required to process information: approximately 20% of a photoreceptor's maximum consumption (Niven et al., 2007). This cost seems to be incurred us to keep the information processing "equipment" functional.

What are the computational costs inherent to the physical properties of visual signals? In principle, this computational cost is the minimum amount of energy required for processing, assuming a perfect information processing architecture, without any maintenance costs. But is it possible to measure it? Computational models attempting to solve the visual problem of unlearned floral choice behaviour by bees may help answer the question.

Neural network models by Enquist and Arak (1994) and Johnstone (1994) suggested that symmetry preference is a by-product of the brain's learning dynamics, which are shaped by pressures to minimize information processing. Symmetry has the property that a large proportion of information (ie, approximately half in the case of bilateral

symmetry) can be discarded without any information loss.

Enquist and Arak (1993) suggested that animals learn to prefer symmetry as a result of generalizing knowledge of signals to their various forms (ie, translations, rotations, transformations). A feed-forward network using a genetic algorithm illustrated this idea by training 6 x 6 pixel input signals to discriminate between stimuli resembling birds with short from long tail lengths and from random pixels. The activation function was defined as the weighted sum of the hidden layers reaching a threshold of 0.5. Results show that the network activated with longer tailed and longer winged patterns, but not tail-less or random patterns. Further mutation of the network produced a progressively increasing preference for exaggerated features. Symmetry may be viewed as a form of exaggeration of non-symmetrical variants.

Another feed-forward network was also used to test the symmetry preference as a by-product hypothesis in another way (Johnstone, 1994). Here, two networks were trained to recognize bilaterally symmetric tails of varying levels of fluctuating asymmetry: one network exposed to a perfectly bilateral pattern, whereas the other network exposed to various imperfect variations. The trained networks did not differ in their preferences for bilateral patterns, indicating that the network that was not exposed to symmetric patterns generalized to symmetric patterns during training on various forms of imperfect variations.

Both of these neural networks assume that symmetry preference emerges as a by-product of learning. However, the behavioural literature on symmetry preferences has been mixed: some studies showed symmetry preference as an unlearned capacity, but others showed symmetry as a learned ability. If unlearned symmetry preferences are indeed possible, an alternative explanation is needed.

3.2 Bees as a Model of Information Processing

Bees are an ideal model to study symmetry preferences, and information processing in general, due to their simpler but very capable brain (Menzel, 2012). Many behavioural studies have focussed on studying the kinds of visual characteristics of a flower that enable bees to identify them as a potential food source. Preferences for symmetry, along with properties such as shape, colour and background-foreground characteristics have been studied extensively. Studies typically deconstruct flowers into their constituents and choice behaviour is measured at these flower-like visual properties by pitting two or more artificial flower patterns against one another (Brodbeck and Shettleworth, 1995).

Unlearned preferences for visual properties can only be studied by truly flower naïve bees, meaning that they have not received any rewards in a testing environment. Unlearned preferences have been observed for colours (Simonds and Plowright, 2004), shapes (Lehrer et al., 1995b), symmetry (Plowright et al., 2011), foliage background complexity (Forrest and Thomson, 2009), and pattern positioning (Orbán and Plowright, 2013).

For example, yellow and blue colours, nectar guides (i.e. radial, sunburst pattern), and floral symmetry are often suggested to be an adaptation by flowers to attract bees. How could a bee discover these flowers prior to any experience with such properties, as would be the requirement by the models of Enquist and Arak (1993) and Johnstone (1994). There is another possibility: preference for symmetry is an inherent characteristic of the visual system, rather than an artefact of learning.

The goal of this study is to test the hypothesis that inherent properties of visual signals are processed differently: some visual signals are computationally cheaper to process than others. Further, we hypothesize that the initial unrewarded preferences by insect pollinators are related to the computational cost of processing floral signals. The class of models that are ideal for evaluating this hypothesis are ones that are able

to extract features from a high dimensional space and reduce it to a small (and more manageable) feature space. In other words, the compressibility of a signal is an indication of how easy it is to process by the visual system. There are several models that meet this criteria, and the ones chosen in this study are two unsupervised neural network models.

Two unsupervised neural networks will be tested using visual stimuli that are identical to ones used in three behavioural experiments: (a) pattern shape and positioning experiment (Orbán and Plowright, 2013), (b) foliage background (Forrest and Thomson, 2009) and (c) symmetry and spatial frequency (Plowright et al., 2011).

3.3 Model Descriptions

The choice of models was greatly narrowed by the purpose of the simulations. Our goal is to simulate how a brain region in an insect uses electrochemical visual signals from the ommatidia to extract useful information. The model is not concerned with the way physical properties of electromagnetic waves are translated into electrochemical signals, nor is it concerned with more advanced cognitive operations such as decision making, learning or memory related computations. There are numerous unsupervised non-linear neural networks that capture various aspects of low level visual processing with good results (Baek et al., 2002). Our choice fell on point-models that attempt to capture key aspects of how visual information may be treated by brain structures in an insect. We test two algorithms here, Independent Component Analysis (ICA), and Feature-Extracting Bidirectional Associative Memory (FEBAM) that detect local components in images of natural scenes with high performance. Both neural networks accomplish perceptual feature extraction in an unsupervised fashion to keep useful information and discard noise.

3.3.1 The ICA Model

ICA can be performed on natural images by processing the observed signal in a statistical generative model, the components of which yield a representation of the original data (Luo and Boutell, 2005). The process is applied to centred (Eldar and Oppenheim, 2003) principal components of overlapping grayscale image patches, extracted from the source image. Figure 3.1 shows the architecture of the network.

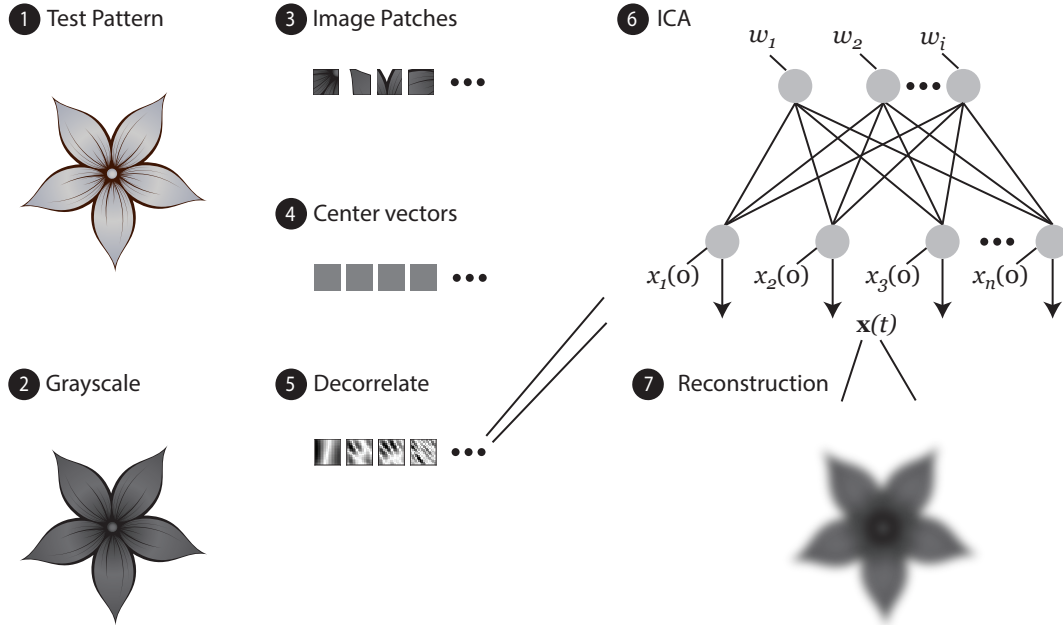


Figure 3.1: Network 1 Architecture: ICA

ICA input mixture signals were grayscale 3 x 3 overlapping image patches generated from a 100 x 100 pixel byte level image. After centering the image patch vectors, Principal Components Analysis (PCA) was performed on each image to decorrelate the signal. The principal components were fed into the deflation fixed-point variant ICA. This method uses *tanh* function to minimize mutual information. Independent components were generated using the fastICA algorithm, implemented in Mathematica (Langlois et al., 2010).

Using the inverse of the estimation matrix $\mathbf{W}$, images used in behavioural studies

were reconstructed. Here $\mathbf{x}$ refers to the test pattern or floral test images, and $\mathbf{y}$ is the matrix of statistically independent component vectors.

$$\mathbf{y} = \mathbf{W}\mathbf{x} \quad (3.1)$$

Image $\mathbf{x}'$ was reconstructed using:

$$\mathbf{x}' = \mathbf{W}^T \mathbf{y} \quad (3.2)$$

The model's parameters have been adjusted to reflect the physiological capacities of bees. The images were converted to grayscale using green-channel data. This configuration mimics how bees might see a pattern with spatial resolution of 0.30 - 0.35 *cyclesdegree*⁻¹ from a distance of 30 cm. Next the images were cut into overlapping image-patches, producing 9000 fragments of each pattern. Finally, the original image was reconstructed using the feature vectors produced by the ICA process. The quality of the reconstructed image was compared with the original image using Peak-Signal-to-Noise-Ratio (PSNR).

3.3.2 The FEBAM Model

FEBAM is a modified version of Bidirectional Associative Memory (BAM) (Chartier et al., 2007) where one set of connections were removed. This modification makes the network act as an unsupervised associative memory. Figure 3.2 shows the architecture of this model:

The $\mathbf{W}$ weights send information to the output layer, and the $\mathbf{V}$ weights send information back to the x-layer in a top-down bottom-up manner. Activation is expressed by the following relations:

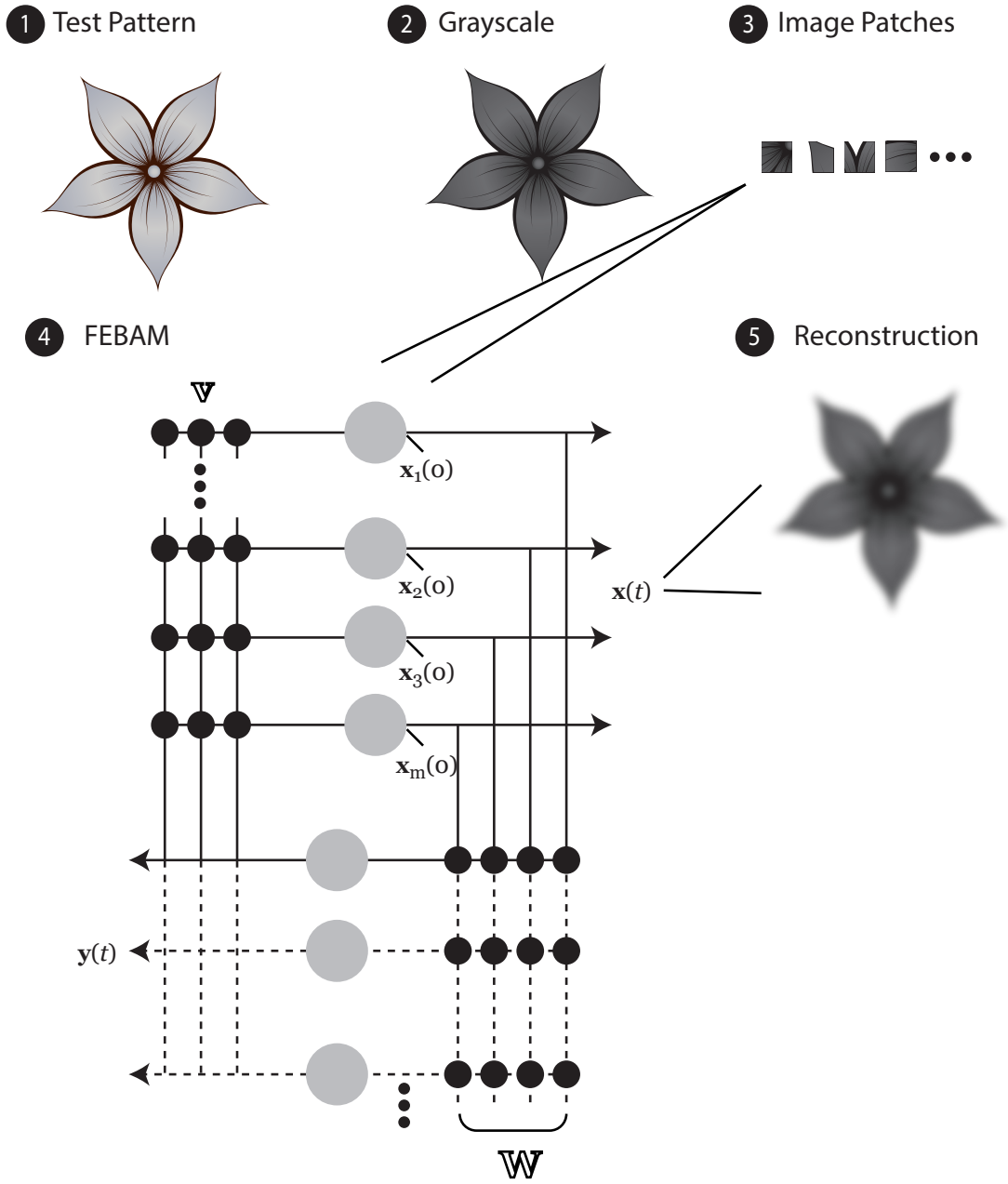


Figure 3.2: Network 2 Architecture: FEBAM

$$\mathbf{y}(t+1) = g((\delta+1)\mathbf{W}\mathbf{x}(t) - \delta(\mathbf{W}\mathbf{x})^3(t)) \quad (3.3)$$

$$\mathbf{x}(t+1) = g((\delta+1)\mathbf{V}\mathbf{y}(t) - \delta(\mathbf{V}\mathbf{y})^3(t)) \quad (3.4)$$

where $\mathbf{W}$ and $\mathbf{V}$ are weight matrices, $\mathbf{y}$ refers to the distributed filters across the units, $\mathbf{x}$ is the original image input, and the reconstructed image is $\mathbf{y}$. δ is a general output parameter that determines the type of attractor the network will exhibit (fixed-point, cyclic, chaotic). This value was held constant in this implementation at 0.1 to produce fixed-points. The output of the piecewise function g behaves like a sigmoid-type function, but without the asymptotic property:

$$g(z) = \begin{cases} +1, & \text{If } z > 1 \\ -1, & \text{If } z < -1 \\ z, & \text{Else} \end{cases} \quad (3.5)$$

The images were also pre-processed in the same manner as in ICA. Images were reconstructed using the $\mathbf{W}$ and $\mathbf{V}$ matrices and compared to the original image using PSNR.

3.3.3 Outcome Measures

Image correlations were calculated on the raw overlapping image patches to ensure the results were not due to inherent image characteristics (see Table 3.1). The quality of the reconstructed image was compared to the original image using two measures: Peak-Signal-to-Noise-Ratio (Huynh-Thu and Ghanbari, 2008) and Mean Squared Error (MSE). The rationale for choosing this measure is that reconstruction quality of images may indicate the cognitive cost to process the test patterns. All parameters kept constant across simulations, better quality of reconstruction means that a fixed number of filters (i.e., 3 filters for both ICA and FEBAM) captured more relevant information to reconstruct

	Rad-In	Rad-Out	Conc-In	Conc-Out
Rad-In	1.	.443	.574	.157
Rad-Out		1.	.483	.453
Conc-In			1.	.477
Conc-Out				1.

Table 3.1: Table showing raw correlations between artificial flower test pattern images

the image. Therefore, we suggest that the inherent characteristics of test patterns are computationally more affordable to process and thereby should be more preferred by pollinators, provided prior reward has not been associated with any other visual property.

3.4 Simulations

Three categories of images were used. First, test patterns manipulating pattern positioning and pattern type, which mimic ones used in a behavioural experiment (Orbán and Plowright, 2013). Second, images of background and artificial floral stimuli, which mimic ones used in Forrest and Thomson (2009). Third, test patterns manipulating symmetry and spatial frequency that were generated through the same algorithm that generated patterns for a behavioural experiment (Plowright et al., 2011). All simulations used Monte Carlo sampling method and performed 200 times.

3.4.1 The *Nectar Guide* hypothesis: Radial vs concentric patterns

Many behavioural studies document an unlearned preference for radial patterns (ie, sunburst pattern) over concentric patterns (ie, bull’s eye pattern) and over other shapes not typically found in nature (Orbán and Plowright, 2013; Leonard and Papaj, 2011; Plowright et al., 2006; Simonds and Plowright, 2004; Lehrer et al., 1995b). For exam-

ple Orbán and Plowright (2013) displayed radial and concentric patterns to free-flying flower-naïve *B. impatiens* workers where the appearance of the pattern was manipulated to be visible only at the centre or only at the periphery of the artificial flowers. Flower naïve means that the bees left the nest for the first time and have never received any reward in the testing environment. The results showed that regardless of positioning, workers preferred to land on patterns with radial shapes (See Figure 3.3). Other studies have also shown similar effects for radial patterns (Leonard and Papaj, 2011; Plowright et al., 2006; Simonds and Plowright, 2004; Lehrer et al., 1995b). The rationale originates in the 19th century when Sprengel suggested that “honey guides” help bees discover the source of rewards (as cited in Manning, 1956a).

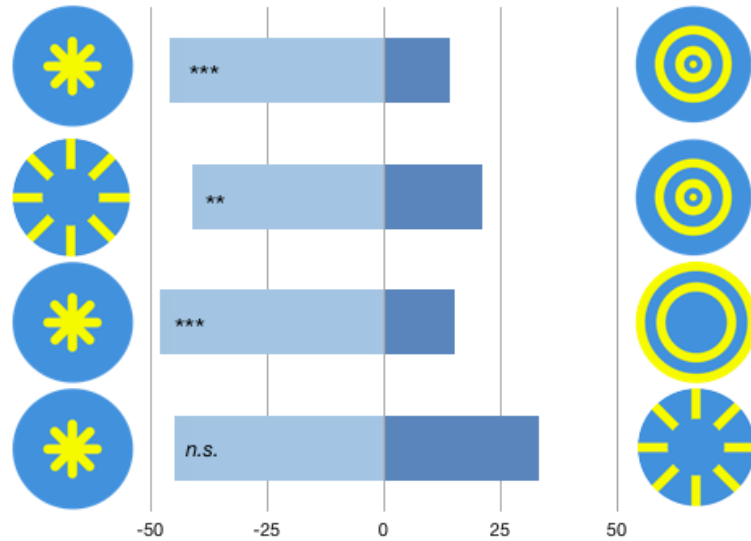


Figure 3.3: Shows the total number of choices made by workers at each pattern combination. Whenever a radial pattern was pitted against a concentric one, workers chose the radial, except in the last case where two radial patterns of different positioning were displayed in which case bees showed no preference for either pattern. Note: ** $p < .05$, *** $p < .001$

ICA and FEBAM networks show similarities and differences: overall, radial patterns are better reconstructed than concentric patterns (see Figure 3.4). Best quality reconstruction is achieved by the peripheral radial pattern using ICA, and the central radial

pattern using FEBAM. In between are the peripheral radial pattern and the central concentric pattern. The quality differences in FEBAM are very small, but ICA shows substantial quality differences that correspond with behavioural findings. More specifically, the central and peripheral radial patterns show a statistically non-significant difference, which matches behavioural findings. Central and peripheral concentric patterns are each reconstructed in significantly lower quality, consistent with behavioural findings.

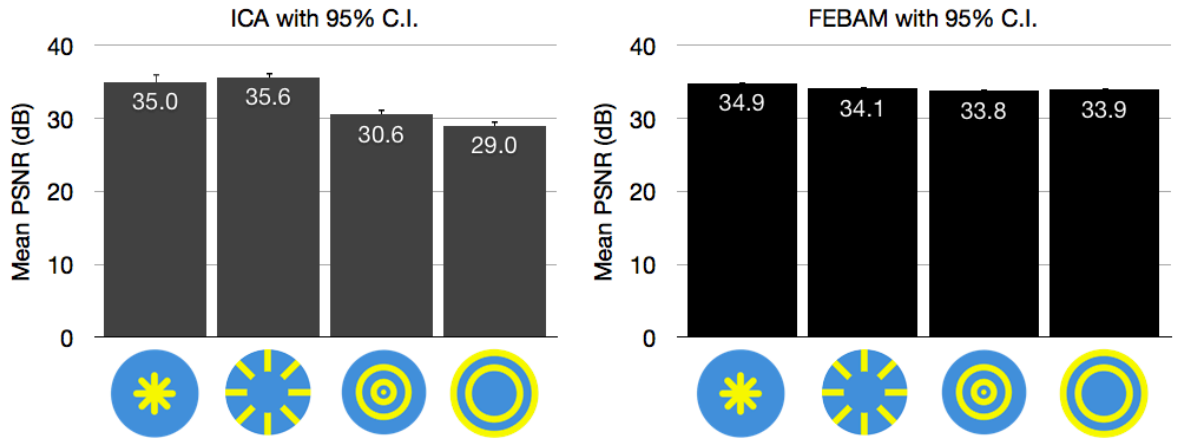


Figure 3.4: PSNR reconstruction values for pattern type and positioning. Results are based on 100 simulations. Error bars show 95% confidence interval

3.4.2 Foliage Background

The role of background in floral preferences has been documented with one recent study showing preferences towards foliage backgrounds (Forrest and Thomson, 2009). *B. impatiens* workers showed no preference for either red or blue patterns when displayed on a simple green background, but showed significant choice deviations towards blue when presented on background image of foliage patterns. This was not a pure test of unlearned preferences because the workers received reward at each pattern, but this result is nevertheless surprising because bees are known to have difficulty seeing red (Lunau

et al., 2011). Given equal amounts of reward at each colour disc, is there an information processing bias that could account for the increase in preference for blue?

As with pattern type, ICA and FEBAM also show similar results for red and blue artificial flowers with simple green background or with foliage background. Grayscale images were generated by retaining all information in green and blue channels, and 70% of the red channel (See Figure 3.5). The rationale for decreasing information in the red channel was to reflect physiological ability of honeybees and bumblebees (Peitsch et al., 1992). Overall, colour patterns with green background were reconstructed in better quality by both algorithms compared with colour patterns with foliage background. More importantly, the quality of the red pattern has decreased significantly more than the blue pattern when the green foliage background was added. Behaviourally, this can be interpreted as red becoming significantly less detectable on green foliage background. The ICA model is again perhaps more reflective of the underlying process where the reconstruction quality of blue did not differ significantly from simple green to foliage background.

The ICA model also indicates an untested novel hypothesis: if a complex pattern background pattern was pitted against a simple background pattern, the simple background pattern would be preferred regardless of blue or red pattern colour.

3.4.3 Symmetry and Spatial Frequency

Behavioural studies are mixed in terms of symmetry preferences. For example, Rodríguez et al. (2004) found innate bilateral symmetry preference by *B. terrestris* workers, but the methodology of this study has been criticized (Plowright et al., 2011). The feed-forward networks of Enquist and Johnstone (1997) and Johnstone (1994) imply that bilateral symmetry preference is a learning by-product of the visual system.

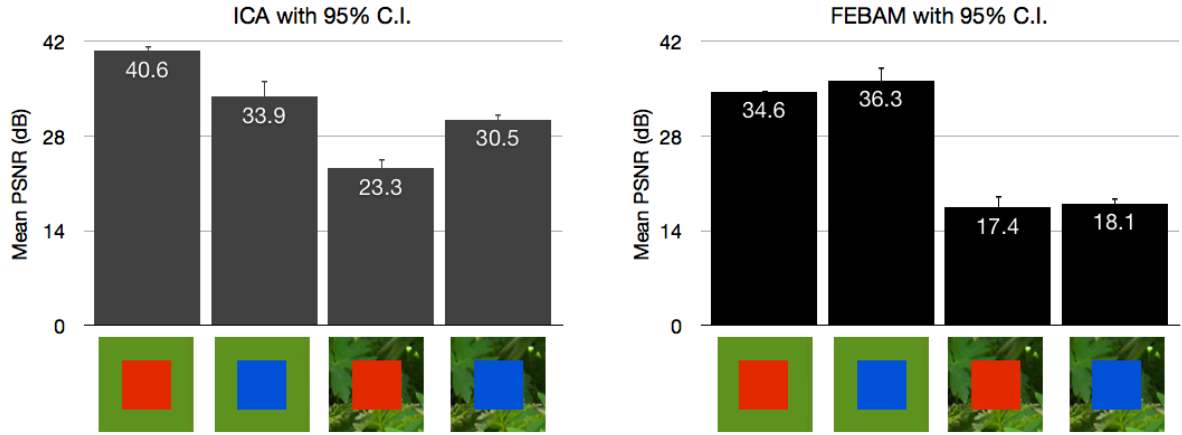


Figure 3.5: PSNR values for red and blue simple and complex background patterns, as tested by Forrest and Thomson (2009). Results are based on 60 simulations. Error bars show 95% confidence interval.

Six kinds of simulations were performed: asymmetric (ie, random), 1-axis (ie, bilateral) and 4-axis symmetric patterns in low and high spatial frequency variations were used. Low spatial frequency was generated to have 4000 pixels, and high spatial frequency to have 9000 pixels of black and white perimeter. Perimeter lengths were verified using an edge detection and pixel counting algorithm and allowed to vary by up to 5%. Further, area of black and white was set to 50% and allowed to vary by up to 5%.

1-axis symmetry ICA and FEBAM models produce corresponding but unexpected results when we manipulated symmetry and spatial frequency. The test pattern of high spatial frequency show better reconstruction for the bilaterally symmetric pattern when compared with high spatial frequency asymmetric pattern (see Fig. 3.7). However, the low spatial frequency patterns do not show this trend: the asymmetric and symmetric patterns had comparable reconstruction qualities (see Fig. 3.6).

Four-axis symmetry The increased redundancy in 4-axis symmetric patterns compared to bilaterally symmetric patterns resulted in a substantial change in quality of

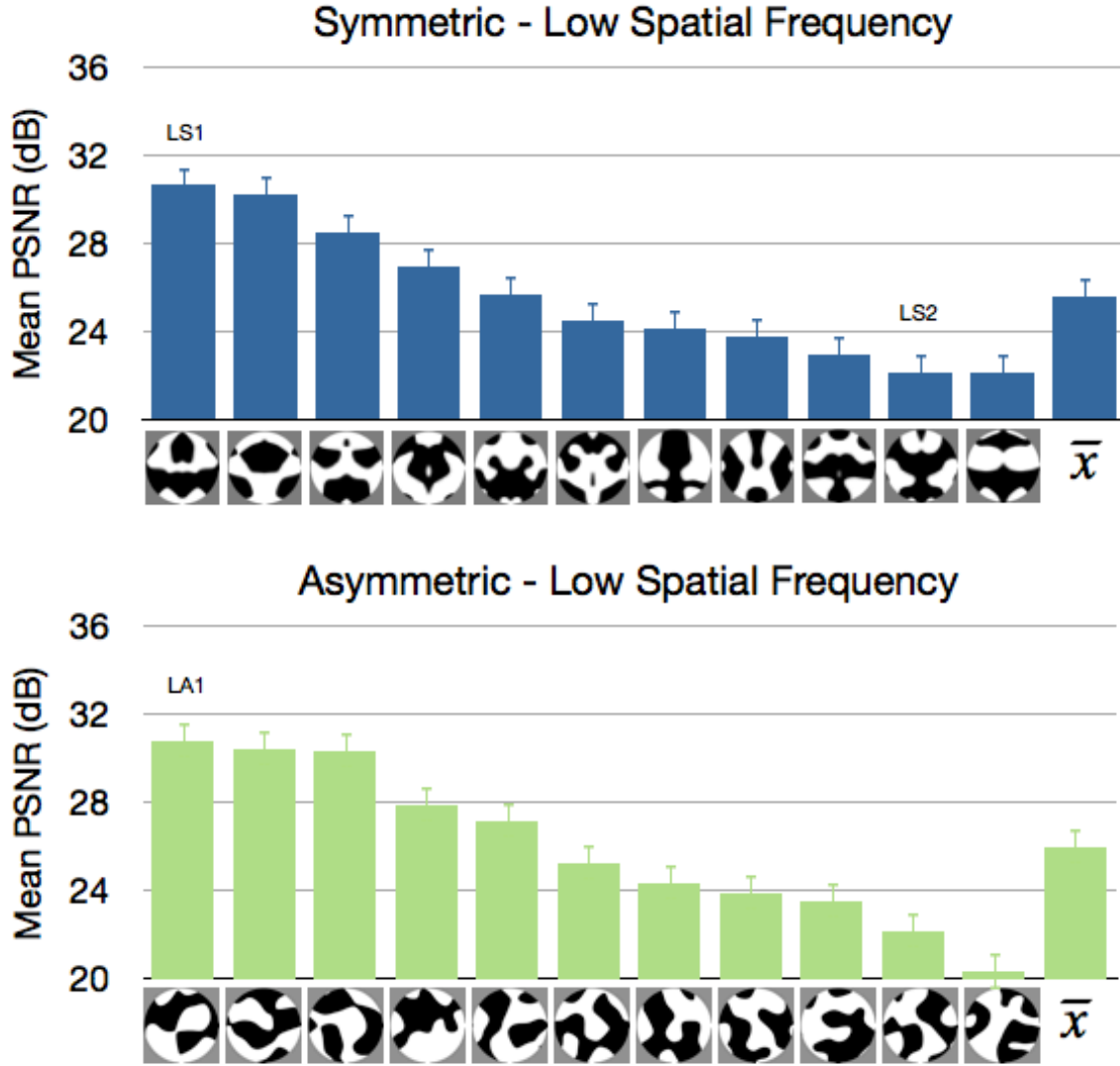


Figure 3.6: PSNR reconstruction values for low and high spatial frequency symmetric and asymmetric patterns. Results are based on 300 simulations. Error bars show 95% confidence interval. The patterns labelled “LS1”, “LS2” and “LA1” were selected for a behavioural experiment testing the predictions of this model.

image reconstruction. The effect of symmetry is now observed even in the low spatial frequency domain. Four-axis symmetric patterns were significantly better reconstructed regardless of spatial frequency (See Figure 3.8).

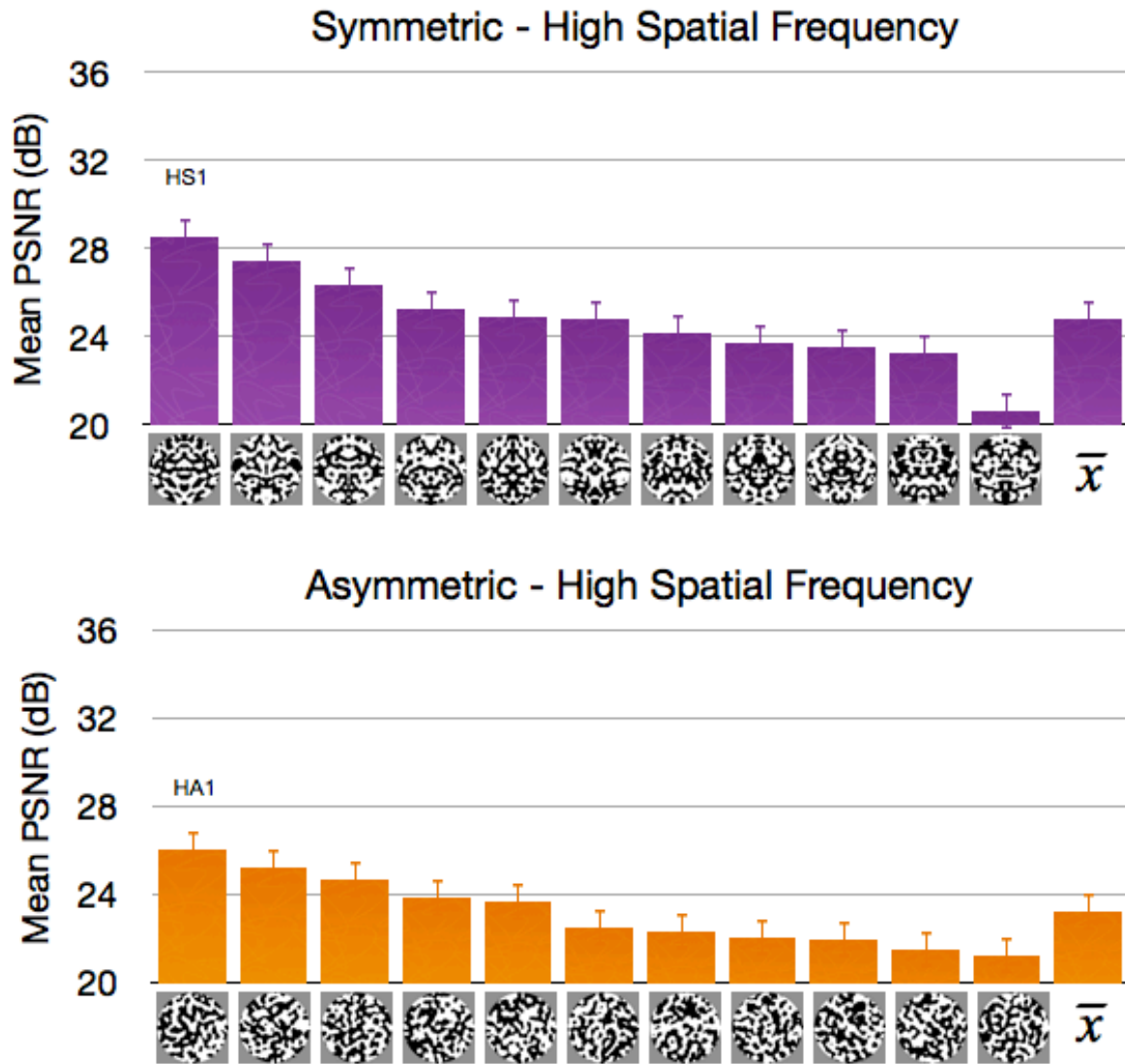


Figure 3.7: PSNR reconstruction values for high-spatial frequency symmetric and asymmetric patterns. Error bars show 95% confidence interval. The patterns labelled “HS1” and “HA1” were selected for a behavioural experiment testing the predictions of this model.

Spatial Frequency FEBAM and ICA diverge in results relating to spatial frequency. ICA captures behavioural findings that indicate a preference for low spatial-frequencies, though spatial-frequency was never specified beyond "low" or "high" (see Figures 3.9). FEBAM shows the opposite result where high spatial frequency patterns were recon-

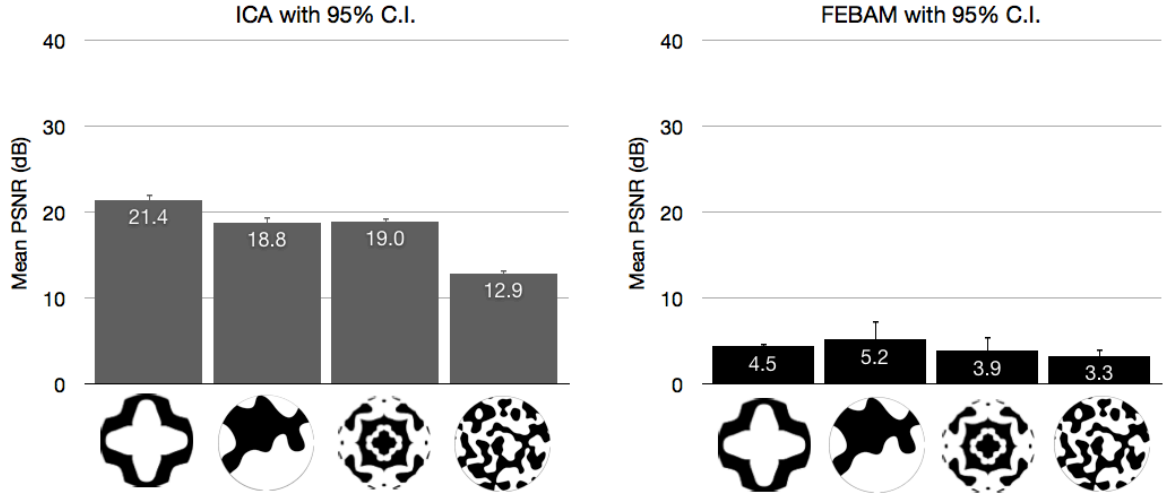


Figure 3.8: PSNR reconstruction values for 4-axis symmetric and asymmetric patterns. Error bars show 95% confidence intervals

structed in better quality.

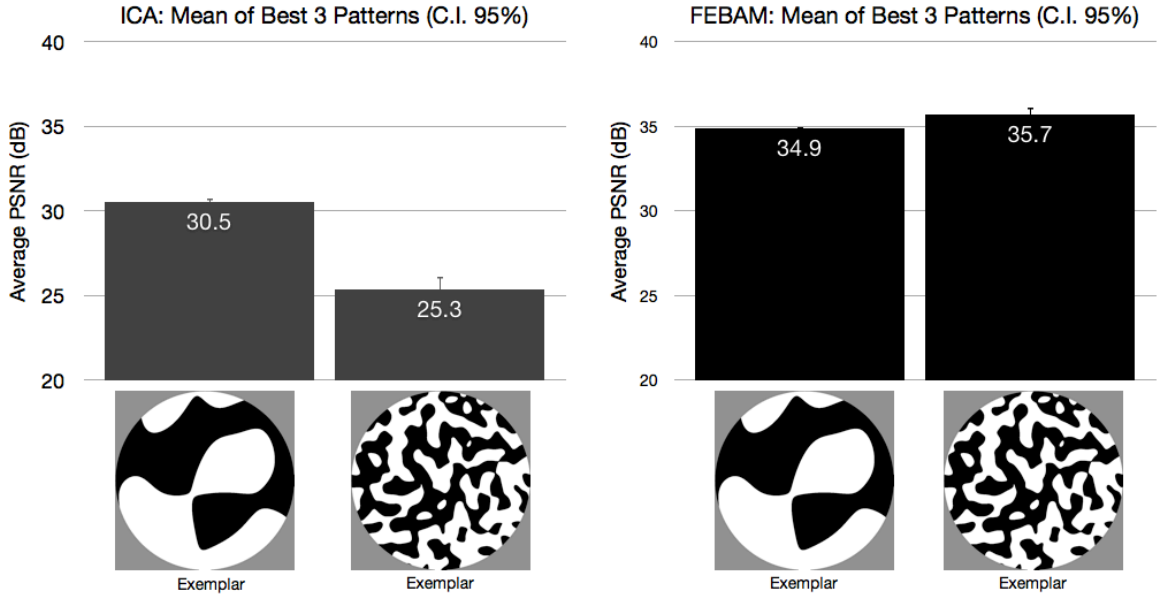


Figure 3.9: PSNR reconstruction values for high and low spatial frequency asymmetric patterns. Results are based on the three patterns reconstructed in best quality, each pattern simulation performed 50 times. Error bars show 95% confidence interval.

3.5 Discussion

Both unsupervised non-linear neural networks show a consistency with behavioural results of unlearned floral choice by bumblebees and honeybees. The results of ICA are more robust because this model captured more subtle findings related to floral positioning, and more consistency for findings related to spatial-frequency. This may be an indication that the ICA model captures a key biological element in the way these pollinators process and respond to visual information. The results lend support to the idea that bumblebees and honeybees show a behavioural preference toward the tested patterns as a result of a by-product in their visual information processing systems.

3.5.1 Differences between ICA vs FEBAM

Differences in the performance of ICA and FEBAM may lie in how features are extracted: FEBAM is based on the second moment about the mean, covariance, while ICA maximizes of non-gaussianity, which is the fourth moment about the mean. Neuroscientific data indicates that covariance information may be useful when receiving redundant information from multiple organs of the same modality. For example, the Jeffress model suggests that the auditory system uses covariance structure to extract Interaural Time Difference in order to localize the source of stimuli in space (Fitzpatrick et al., 2000; Joris et al., 1998). One of the features of many natural signals, especially visual signals of natural scenes is that they are non-gaussian. Perhaps using non-gaussianity to extract features corresponds with the visual signal better than covariance. This may be one of the reasons why ICA consistently outperformed FEBAM in terms of capturing behavioural result.

3.5.2 Visual system may perform ICA-like factorization

It has been suggested that the visual system deals with redundancy in the input signal by performing factorial representations where features are retained as independent components and noise is discarded (Atick and Redlich, 1992; Barlow, 1989). Principal component analysis (PCA) has implemented this principle to some extent, but only for second-order linear dependencies between pixels: higher-order dependencies are not separated with this process (Bartlett et al., 2002). ICA overcame this problem by requiring statistical independence between feature vectors through the minimization of mutual information using measures like maximum entropy or Kullback-Leibler divergence (Comon, 1994). Additionally, the ICA shows correspondence with the way visual receptive fields self-organize with principles of decorrelation and sparseness. Visualization of ICA filters have been likened to edge detectors in V1 of the primary visual cortex (Bell and Sejnowski, 1997). In other ways, the algorithm is biologically implausible because the learning rule involves a global transformation of the matrix.

3.5.3 Model comparison

Enquist and Johnstone (1997) and Johnstone (1994) also proposed computational models that suggested unlearned preference to be a by-product of the visual system, but with two key differences: (1) the models could predict symmetry preferences but no other visual properties, and (2) the models implied symmetry preference to be a by-product of learning, therefore, unlearned symmetry preference should not be observed behaviourally in bees. The ICA and FEBAM models also suggest that floral preferences are a by-product of the visual system, but the models have shown substantial consistency across multiple visual properties, and also suggest that preferences should be seen in flower-naïve bees, prior to any rewarded experience. Future behavioural work may focus on evaluating the

accuracy of these models by pitting against spatial frequency and symmetry. Unlearned preference for symmetry by bees for high spatial frequency patterns would suggest that the ICA model captures visual processes better than the feed-forward networks, and the absence of flower-naïve preference towards symmetry regardless of spatial frequency would support the feed-forward networks.

3.5.4 Computational cost

The aim of this study was to test the idea that the computational cost of processing stimuli could be measured in such a way that it excludes the metabolic cost of information processing. The rate of information processing under different levels of light intensity has been measured, but it's not clear how much of this cost is due to the maintenance of the processor or the brain, and how much is due to the cost of processing the information. Here we suggest that the use of unsupervised neural networks may be used to estimate the cost of information processing minus the cost of metabolic processes. The accuracy of this model for estimating computational cost depends on the extent to which it captures behavioural data.

3.5.5 Future work

In terms of future behavioural work, partial consistency with symmetry and spatial frequency produces two hypotheses that could be tested. First, low frequency symmetric patterns should be preferred over high-frequency symmetric patterns, and even more interestingly, an interaction between symmetry and spatial frequency could be tested. Half of a symmetric pattern's information may be discarded while still coding all relevant information. The interaction may indicate a ceiling in the amount of information that bees' visual system can code.

Several aspects of the models could be adjusted or replaced to capture different visual properties or increase the precision of the current results. Spatially-sensitive unsupervised neural networks such as Self-Organizing Maps (Kohonen, 2013) and Topological Bidirectional Heteroassociative memory models could be a potential candidate for future simulations (Chartier et al., 2009). ICA and FEBAM are point-models where features are not easily ranked or prioritized in terms of importance. The spatially-sensitive models may uncover a subset of features that are especially important, and may also reveal relationships between features that are not visible from the current models.

In this model we reconstructed the images and compared them to the original version using PSNR values. Future models may include Bayesian detection and classification instead. The neural networks could also be further tuned to simulate the physiological properties of different pollinators visual systems. For example, instead of using byte level information from the image, Liu and Cheng (2003) suggests summarizing independent channels using histogram information, which is a statistically more meaningful representation.

Chapter 4

How do bees first find flowers?

Interaction of two visual properties:

Spatial frequency and symmetry

4.1 Introduction

Fluctuating asymmetry in the bilaterally symmetric flower, *Epilobium angustifolium* is a reliable indicator of nectar production: better symmetry indicates more nectar, which bumblebees learn and exploit (Møller and Sorci, 1998). Indeed *E. angustifolium* is not an exception, most flowers display some form of symmetry (Neal et al., 1998). The question is whether this preference for symmetry is learned through functional experience with flowers, or it is an unlearned preference that guides workers to their first flowers. The answer to this question could reveal key principles about how brains process information, and could have consequences on the co-evolutionary relationship between pollinators and flowers.

In a study where honeybees were trained to bilaterally symmetric (ie, symmetry along

1-axis) and asymmetric (ie, random) patterns, the authors concluded that the preference for symmetric patterns is not only possible, but pre-formed (Giurfa et al., 1996). Two groups of honeybees were trained to differentiate between bilaterally symmetric or asymmetric patterns. The difference between the two groups was that one group was rewarded only on symmetric patterns whereas the other group was rewarded only on asymmetric patterns. Choice frequencies were more amplified towards symmetry-trained symmetric patterns than for asymmetry-trained asymmetric patterns, leading the authors to conclude a predisposition to symmetric patterns. But do bees show an unlearned preference towards symmetric patterns?

One study concluded that bees likely have an unlearned preference for symmetry (Rodríguez et al., 2004). Choice behaviour by *B. terrestris* workers was tested in a flight cage where bilaterally symmetric or asymmetric black and white patterns were displayed. Prior to testing, bumblebee workers were pre-trained on sucrose solution on a black disc or white disc in order to encourage landing behaviours during the testing periods. The results showed a preference for bilateral symmetry. These findings correspond with another study that used *A. mellifera* workers (Lehrer et al., 1995a), also using pre-training. However, as Giurfa et al. (1999) pointed out, testing spontaneous behaviour of truly-naïve animals may be key to understanding innate choice behaviour.

Plowright et al. (2011) tested the symmetry hypothesis with truly flower-naïve and untrained *B. impatiens* workers. Choice behaviour was measured in a twelve-arm radial-maze, and was defined by entering a maze arm at least half-way. Artificial floral stimuli were located at the end of each arm. Flower-naïve *B. impatiens* workers were compared to ones pre-trained on 100% black discs, and 100% white discs. A manipulation of symmetry and spatial frequency showed that only pre-trained workers displayed a preference for bilaterally symmetric test patterns, but not untrained workers.

These findings are consistent with another experiment, which also tested *B. impatiens* workers prior to rewarded experience (West and Lavery, 1998) and found no unlearned preference towards either radially or bilaterally symmetric patterns. However, the study by West and Lavery (1998) showed that bumblebees handled flower-like stimuli with bilaterally symmetric nectar guides 45% better than radially symmetric nectar guides. This suggests that the preference for symmetry may be more specifically focussed on some types of symmetric flowers.

Unlearned preferences have been almost exclusively studied in bilaterally symmetric patterns but this is not the only kind of symmetry. Some experiments investigating learning dynamics by bees have used radially symmetric patterns, although this is not very well defined (eg, Horridge, 2007). Symmetry can be quantified more concretely based on the number of axes of symmetry. A bilaterally symmetric pattern is defined as the simplest type of symmetry, along a single axis. A radially symmetric pattern would have multiple axes of symmetry, but it is not defined exactly how many axes are needed before a pattern can be labelled as radially symmetric. Increasing the number of axes of symmetry increases the informational redundancy of the display, thereby maximizing the experimental manipulation. Previous studies used mostly 1-axis symmetric displays may have been mixed on the question of symmetry due to this weak form of experimental manipulation.

The results of investigations into *spatial frequency* preferences by bees has also been mixed. Spatial frequency refers to the “busyness” of a pattern; it is defined as the number of features encountered along a line of a surface area (Dafni et al., 1997). A study by Lehrer et al. (1995a) showed preferences for patterns of low spatial frequencies across multiple types of patterns: radial and concentric patterns, and vertical and horizontal gratings. In each of these tests *A. mellifera* workers were pre-trained on 50% black

patterns (ie, gray). This is in contrast to previous studies that found a preference for higher spatial frequency patterns (Anderson, 1977). One distinct difference between these studies is that Lehrer et al. (1995a) presented stimuli on the vertical plane whereas Anderson (1977) presented patterns on the horizontal plane. A problem with these studies is that the spatial frequency of patterns is not quantified. Characterization such as “low” or “high” not sufficiently operationalized to allow comparisons between the results of different experiments. For example, a “low” spatial frequency pattern in one study may be a “medium” spatial frequency pattern in another study.

Most recently, unsupervised neural networks were implemented to explain aspects of floral property preferences by bumblebees (Orbán and Chartier, 2013). Independent Component Analysis (ICA) and Feature-Extracting Bidirectional Associative Memory (FEBAM) are point-models used to simulate different aspects of cognitive mechanisms. The connection of the models to behaviour is that bees should prefer patterns that the model judged to be “cheap to process”, provided flower-naïve bees are used. The cost of information processing by the models was estimated using peak-signal-to-noise ratio (PSNR) that compared the quality of the original floral property with the one reconstructed from features the models generated. The computational model was found to be consistent with behavioural findings in relation to radial *vs* concentric pattern preferences (Orbán and Plowright, 2013; Lehrer et al., 1995a), and the presence *vs* absence of background foliage (Forrest and Thomson, 2009) . The models also tested patterns in which symmetry and spatial frequency was manipulated the same way as in Plowright et al. (2011). The neural networks generated a novel prediction as explained in the previous chapter: patterns with low spatial frequency should not produce a preference for symmetry, but high spatial frequency patterns should produce an unlearned preference for symmetry.

This aim of this experiment is to behaviourally test hypotheses generated by the unsupervised neural networks and compare them to predictions that could generated from previous behavioural experiments. There are multiple predictions including differential predictions for FEBAM and ICA:

First, ICA shows a great amount of variability between low spatial-frequency patterns, but FEBAM results are homogeneous in comparison. Therefore, we will compare choice proportions between two low spatial frequency symmetric patterns. It is conceivable that ICA captures additional variable of patterns of the same category that are not obvious or visible to human vision or experimentally controlled by us.

Second, ICA predicts that overall, low spatial frequency patterns should be preferred over high spatial frequency patterns while FEBAM predicts the opposite preference: high spatial frequencies preferred over low spatial frequency patterns. Most behavioural experiments would predict a preference for a moderately low spatial frequency so this part of the result could pull apart FEBAM from ICA and behavioural predictions.

Third, ICA predicts an interaction between spatial frequency and bilateral symmetry: no effect of bilateral symmetry for low spatial frequency patterns, but high spatial-frequency bilateral symmetric patterns should elicit a preference over asymmetric high spatial-frequency patterns. FEBAM and behavioural experiments predict a main effect of spatial frequency, but no preference for symmetry. Therefore, a result confirming an interaction between symmetry and spatial frequency would validate the prediction of the ICA model, and contradict predictions by the FEBAM model and the previous literature.

Fourth, when the level of symmetry is increased to be present along four axes, ICA predicts a preference for symmetric patterns should be amplified and we should observe a preference for symmetry regardless of spatial frequency. FEBAM does not predict this amplification of preference for symmetry, and behavioural experiments have no predic-

tions relating to manipulation of the level of symmetry.

The fifth and final prediction is one specifically relating to hovering behaviour: If the ICA model is correct, and high spatial frequency patterns are computationally intensive to process, workers may hover closer to the patterns as they assess them. As a result, differences in hovering distance may exist between low and high spatial frequency patterns.

4.2 Methods

All workers in four *B. impatiens* Cresson colonies were tagged with coloured Opalith Plättchen numbered tags and tested in a flight room. Workers were fed sugar-water solution and pollen directly inside the colony, but rewards were never present in the testing environment. Workers had unlimited access to the testing environment for the full duration of the study. The testing environment was kept at constant temperature, humidity and lighting conditions 24 hours a day.

4.2.1 Apparatus

Two nest-boxes were connected simultaneously to the flight room (389 x 455 cm) using two 10 cm long and 2.5 cm diameter wire-mesh tubes. The flight room was illuminated by 12 fluorescent daylight bulbs (Sylvania FO32/841/XP/SS/EC03) powered by 40 KHz electronic ballasts (Sylvania Quicktronic T8 InstantStart). Light intensity was constant at 2200 lux.

Each of two motion-sensitive camcorders were zoomed in at each of two simultaneously presented stimuli, each at a distance of 5 m (Vivotek IP8161; Chung-Ho, Taiwan). The camcorders were triggered to record up to 15 s long colour video clip at 15 frames

per second and at 1600 x 1200 pixel resolution. The sensitivity of the motion detection algorithm was set to record only if at least 85% of two adjacent frames changed and at least 5% of a single frame's area changed. This configuration produced many false positives, but minimized the occurrence of missing a landing behaviour.

While the distance between the bumblebee and the pattern cannot be controlled, it can be observed: foraging workers are known to examine flowers by hovering in front of them (Horridge and Zhang, 1995). For this reason, hovering is chosen as a reference point for the estimation of spatial frequency. A third camcorder was positioned above the stimuli to measure hovering distance prior to landing. The motion sensitivity of this camcorder was set to record if at least 95% of two adjacent frames changed, and at least 1% of a single frame's area changed.

4.2.2 Stimuli

Stimuli consisted of symmetric and asymmetric "blobs" that were generated using an algorithm identical to patterns used in Plowright et al. (2011) (see Figure 4.1). The blobs were created by first generating a random white noise pattern, which is then low-pass filtered and discretized. Bilaterally symmetric patterns were created by mirroring one side of the pattern. Four axes symmetric patterns were created by a series of rotation and mirroring of one side of the pattern. Several dozen patterns were generated but only those were retained that did not deviate from 50% black-to-white proportion by more than 5%. Two types of patterns were created in the *high spatial frequency* domain: patterns having a contour density (ie, total length of edges) of 9,000 or 11,000 pixels and *low spatial frequency* had a contour density of 4,000 pixels. Patterns with contour density deviations of no more than 5% were retained.

Symmetry of a pattern is measured by halving a pattern vertically and correlating

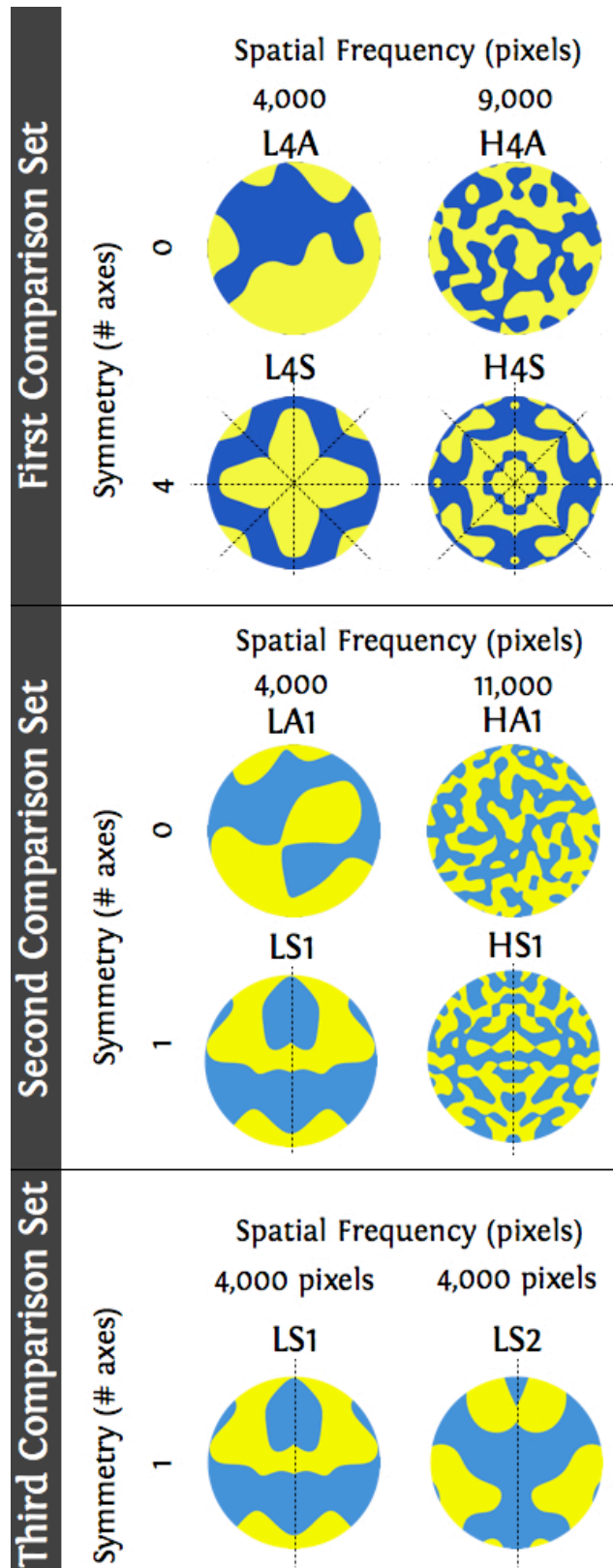


Figure 4.1: Exemplars of stimuli used in the experiment. The label for each pattern was generated by the appearance of the stimulus. “L” and “H” refer to the frequency of the stimulus (low or high); “A” or “S” refer to whether or not symmetry is present (asymmetric or symmetric); in the second and third sets the numbers “1” or “2” refer to the variant selected from the computational model described in the previous chapter. Variant “1” refers to patterns that had the best quality image reconstructed by ICA, and variant “2” refers to patterns that had the worst reconstruction quality by ICA. In the first comparison set, “4” refers to the number of axes of symmetry.

pixel values of each side. symmetric patterns were retained if level of symmetry exceeded 99%, and asymmetric patterns were retained if their level of symmetry fell below 1%. Patterns that Orbán and Chartier (2013)'s ICA model reconstructed in the best and worst quality within each category were selected.

4.2.3 Procedure

Testing commenced shortly after arrival of the commercial colonies. Stimulus location was counterbalanced: if a pattern appeared on one side, the subsequent presentation would be on the other side. Stimuli were presented in combinations of two. Pattern combinations were switched until each combination received a sufficient number of choices.

Video clips were analyzed frame-by-frame using QuickTime player 10.2. Visits from untagged workers, and workers with tags that could not be identified due to very quick movements, poor orientation or poor camcorder focus were only used in aggregate statistical analyses. In these cases, it is impossible to determine whether the visit was from a single bee, or multiple bees, potentially leading to analytical errors. Landings on stimuli occupied by another worker were also discarded because the displayed pattern was altered by the presence of the other worker.

Statistical Analysis Video data were analyzed with replicated goodness of fit tests (G-test) for categorical data (Sokal and Rohlf, 2012). Replicated G-tests are designed to analyze repeated measurements from distinct bumblebees, thereby avoiding pseudo-replication. G_h values are a test for heterogeneity of choices (ie, individual differences), and G_p values test for the deviation between a group choice proportion (ie, pooled data) and a theoretical value of chance (50:50). The G value is compared to the χ^2 distribution, but the test is not a χ^2 test. Four planned tests were performed that might justify the use of Bonferroni-correction, but due to increased criticism of this statistical technique in

many fields (Lieberman and Cunningham, 2009; García, 2004; Nakagawa, 2004; Perneger, 1998), we report precise p -values in place of Bonferroni-correction.

Independence of choices at a pair of pattern combinations was evaluated using a Generalized Linear Model (GLM) specifying a binomial distribution with Logit link and using a Type I likelihood estimation.

4.3 Results

We tagged a total of 935 *B. impatiens* workers across five colonies, and 149 (15.9%) landed on at least one artificial flower. We recorded the date of tagging and death, which allows estimating the duration of a worker's life: an average of 73.9 days and a median of 86 days. Workers were tagged in their callow form to make sure the life duration estimate was correct. All untagged adult workers were removed from the colony and killed. Only those choices of workers that could be uniquely identified were used in the replicated G-test, but all choices were used in aggregate analyses. Figure 4.2 shows a visual summary of all observed choice events including hovering (light blue), antennation (medium blue), and landing (dark blue).

To allow a visual comparison between the behavioural results and the ICA model, the results of the model are drawn for each condition along with the behavioural results. The ICA bar chart indicates peak-signal-to-noise (PSNR) ratio between the reconstructed image and the original image (see Figures 4.3, 4.4, 4.5, 4.6). PSNR values are measured in decibels (dB) and indicate the quality of a signal. The higher values indicate higher quality that is more like the original signal.

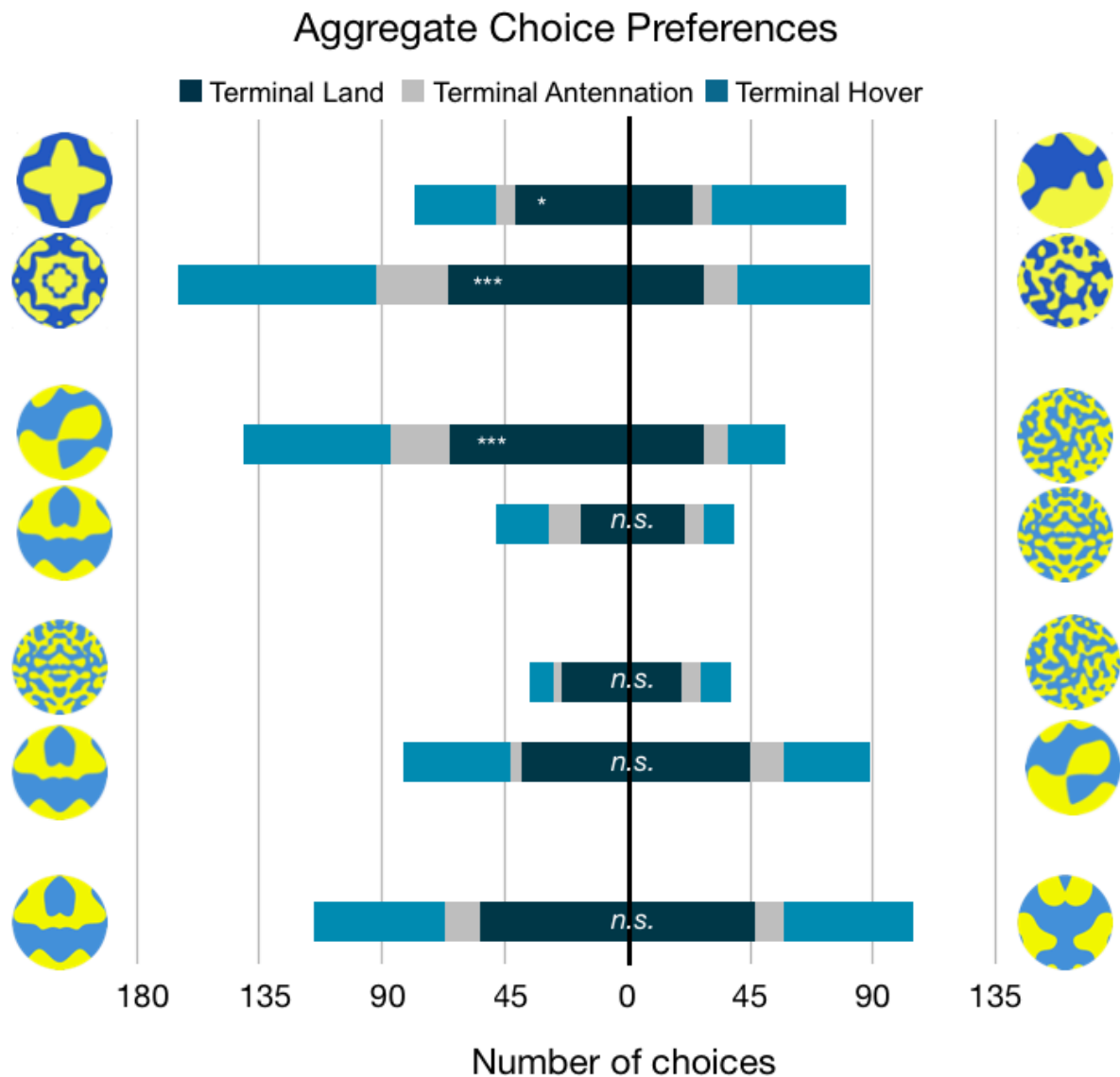


Figure 4.2: Worker preferences in aggregate form for all tested hypotheses. This figure includes workers that were not part of the replicated G-test because their identity could not be associated with a tagged bee. Behaviour Labels: dark blue: landing; medium blue: antennating; light blue: hovering. A “terminal” behaviour means that the bee terminated its choice with a particular behaviour. For example a terminal hovering is one that was not followed by antennation and landing. Note: *** $p < .001$, ** $p < .05$, n.s. $p > .05$; significance values refer to replicated goodness of fit tests on uniquely identifiable workers’ terminal landing behaviour.

4.3.1 Effect of 4-axis symmetry

Two sets of combinations were presented to assess the effect of symmetry of choice behaviour. The first combination consisted of 9,000 pixel patterns that either displayed 4-axis symmetry or were asymmetric. Thirty-three workers contributed 94 choices in the 9,000 pixel condition and made an average of 2.7 choices. Out of the 94 choices, 90 (95.7%) could be associated with a tagged worker. Twenty-seven of the 33 workers (81.8%) showed a preference towards the 4-axis symmetric pattern. The pooled G-test is significant, $G_p(1) = 18.41, p < 0.001$, and heterogeneity of choices are not significant, $G_h(32) = 40.37, p = 0.147$ (See Figure 4.3).

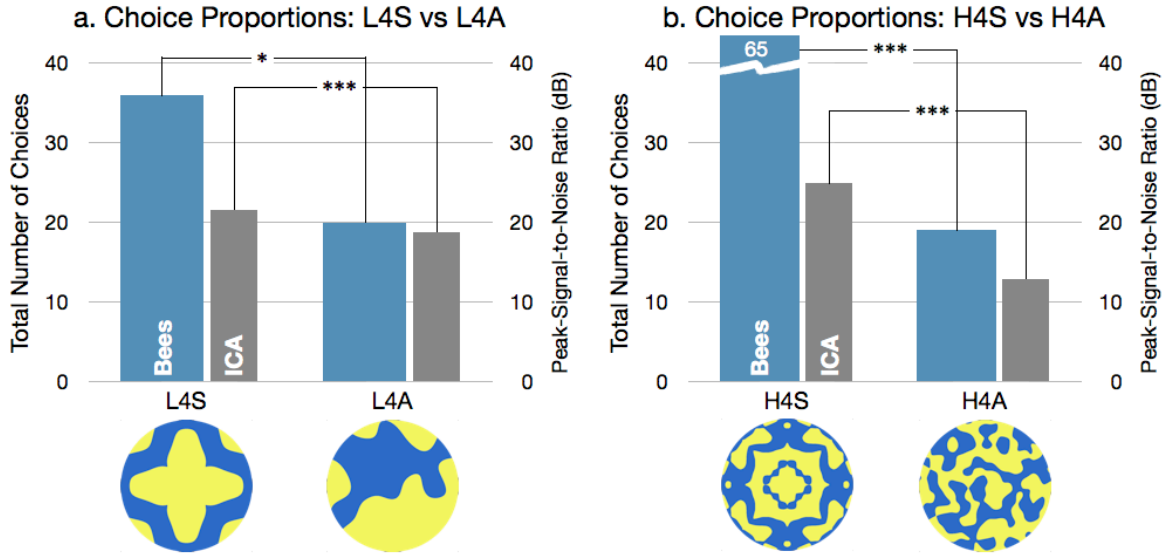


Figure 4.3: (a) Low spatial frequency patterns, manipulating 4-axis symmetry. (b) High spatial frequency patterns manipulating 4-axis symmetry. Data represents only those choices that were associated with unique workers. Note: *** $p < .001$, ** $p < .05$

Twenty-seven workers contributed 65 choices in the 4,000 pixel condition and made an average of 1.7 choices. Out of 65 choices, 56 (86.2%) could be associated with a tagged worker. Fifteen of the 27 workers (55.6%) showed a marked preference towards the symmetric pattern. The pooled Goodness of fit test shows a significant result ($G_p(1) =$

4.64, $p = .031$), but an also significant heterogeneity indicates substantial individual variation in preferences ($G_h(26) = 41.45, p = .028$) (See Figure 4.3).

4.3.2 Effect of spatial frequency

Effect of spatial frequency with respect to random patterns The effect of spatial frequency on random patterns was observed using 4,000 and 11,000 pixel perimeter, asymmetric patterns. Seventeen unique workers landed a total of 93 times on the presented stimuli. Each worker made an average of 1.9 choices, and 33 (35.5%) choices could be uniquely identified. Twelve of the seventeen workers (70.5%) showed a marked preference towards the low spatial frequency pattern. A significant replicated G-test underscores this finding: $G_p(1) = 11.64, p < 0.001$. Heterogeneity of choices is non-significant, suggesting that most bees had similar individual preferences ($G_h(16) = 20.81, p = 0.186$) (See Figure 4.4b).

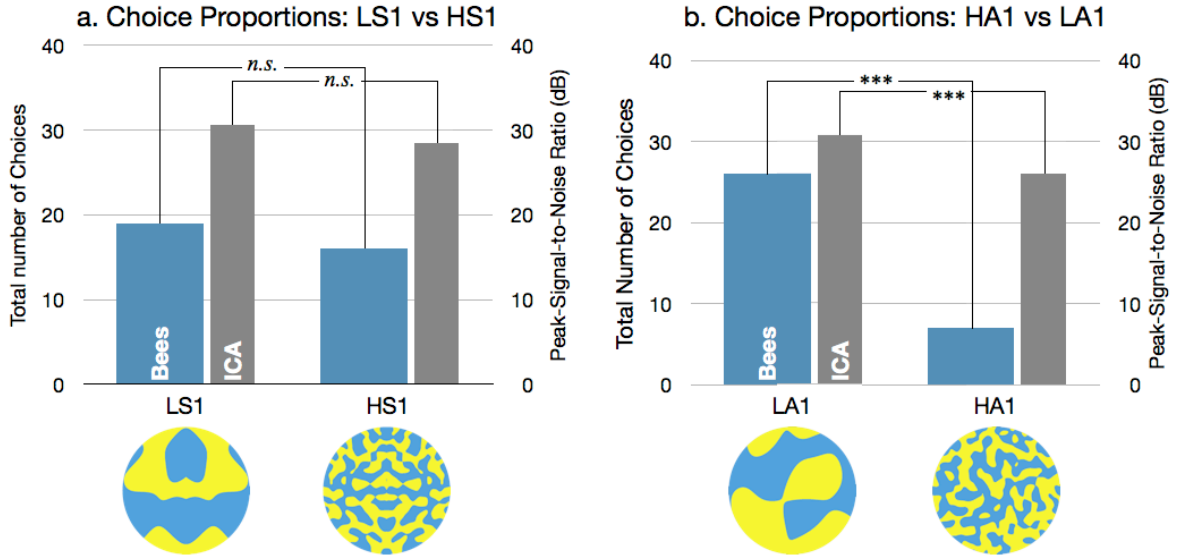


Figure 4.4: Choice proportions at low and high spatial frequency patterns displaying (a) bilaterally symmetric or (b) random, asymmetric patterns. Data represents only those choices that were associated with uniquely identified workers. ICA model results are shown for comparison. Note: *** $p < .001$, n.s. $p > .05$

Effect of spatial frequency with respect to bilaterally symmetric patterns

Choice proportions at two combinations were examined. One combination consisted of 1-axis symmetric patterns displaying 4,000 or 11,000 pixel perimeter patterns (LA *vs* HA), and the other combination consisted of asymmetric patterns displaying the same combination of spatial frequencies (LS *vs* HS). The earlier described GLM was used to test choice behaviour characteristics between the LA - HA and LS -HS. The overall Chi-square test shows a significant result ($\chi^2 = 31.12, p < 0.001$) indicating bees choice characteristics at the LA *vs* HA combination were independent from the LS *vs* HS combination (See Figure 4.4a, b).

4.3.3 Effect of bilateral (1-axis) symmetry

The effect of 1-axis symmetry was examined using 4,000 and 11,000 pixel perimeter patterns displaying bilateral symmetry or asymmetry. Twenty-six workers contributed a total of 84 choices in the 4,000 pixel condition. Workers made an average of 2.7 choices, and 70 (83.3%) choices could be uniquely identified. Eight of the 26 (30%) workers showed a clear preference for the symmetric pattern. A non-significant replicated G-test underlines the absence of preference for symmetry: $G_p(1) = 0.92, p = 0.339$ (See Figure 4.5). However, heterogeneity of choices was significant, indicating significant variation in individual preferences ($G_h(25) = 53.97, p < 0.001$).

Eighteen workers made a total of 44 choices in the 11,000 pixel condition. Each worker made an average of 2.1 choices, and 38 (86.4%) choices could be associated with a unique worker. Six of the 18 workers (33%) showed a marked preference for the symmetric pattern, but a non-significant replicated G-test shows an absence of preference for either pattern: $G_p(1) = 0.11, p = 0.746$. However, significant individual variation exists in choice preference as evidenced by significant heterogeneity of choice: $G_h(17) = 29.35, p =$

0.032 (See Figure 4.5).

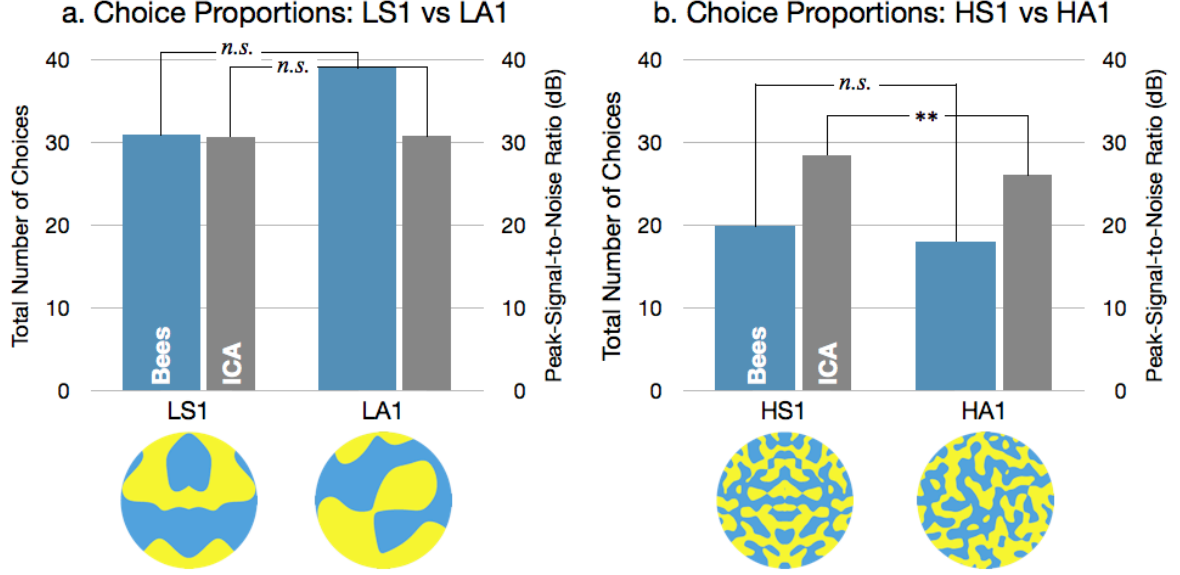


Figure 4.5: Choice proportions at (a) low spatial frequency patterns differing in level of symmetry, and (b) high spatial frequency patterns differing in level of symmetry. Data represents only those choices that were associated with uniquely identified workers. ICA model results are shown for comparison. Note: ** $p < 0.01$; n.s. $p > .05$

4.3.4 Variations in ICA model predictions

Two 4,000 pixel perimeter symmetric patterns were compared to evaluate the possibility that variations in ICA image reconstructions indicated difference in behavioural preferences. Thirty-three distinct workers made a total of 84 choices. Each worker made an average of 2.0 choices, and the choice of 66 (78.5%) workers could be uniquely identified. Seventeen of the 33 workers (51.6%) showed a preference for the ICA predicted pattern, but the results of the replicated G-test were non-significant: $G_p(1) = 1.52, p = 0.218$ (See Figure 4.6). Heterogeneity of choices was also non-significant ($G_h(32) = 35.78, p = 0.295$).

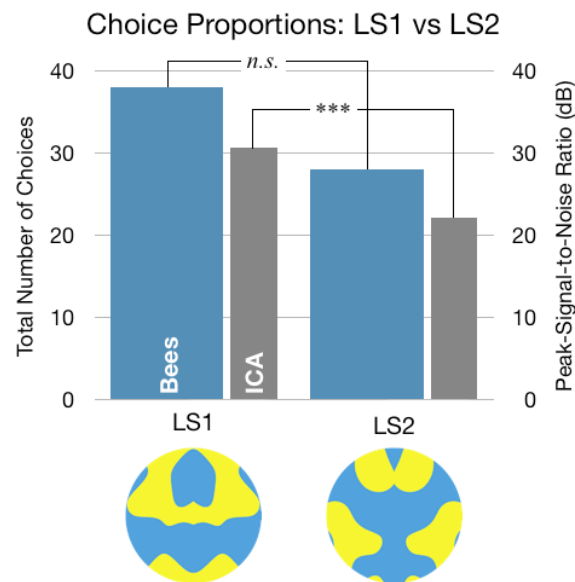


Figure 4.6: Choice proportions at two low spatial frequency bilaterally symmetric patterns. Blue bars indicate choice proportions by bees, and grey bars indicate ICA reconstruction quality as measured by peak-signal-to-noise ratio. Note: *** $p < .001$; n.s. $p > .05$

Hovering distance analysis Quantifying hovering distance was determined by the following behavioural markers: the furthest point from the stimulus at which the worker displays a pause, and orients towards the pattern. At the same time the flight pattern displays a slowdown and change in lateral direction, and movement begins to move in the anterior direction, towards the stimulus (See attached video for an exemplar). Only hovering behaviour that terminated in landing was examined. Observations of distance and angle of approach were recorded between the centre point of the stimulus and the head of the worker. Observations were discarded if these inflection points indicating hovering behaviour could not be observed.

Approach angles were also measured. 0 degrees is defined as the direction pointing away from the front of a pattern. Angles between -90° and $+90^\circ$ (ie, behind the pattern) were discarded because clear hovering behaviour could not be observed in these cases. Workers that approached visibly from a position much higher or lower than the height of the stimulus were also discarded because distance estimates could not be reliably obtained.

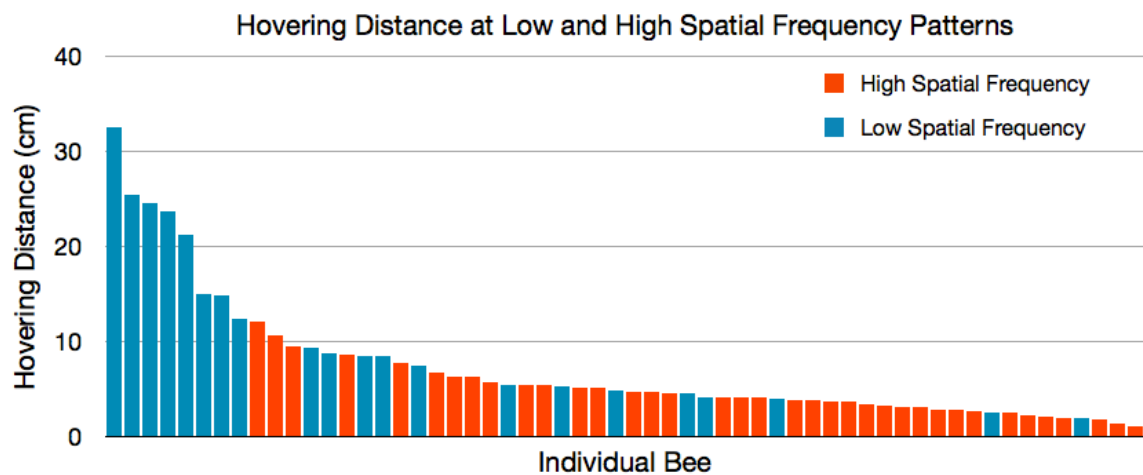


Figure 4.7: Hovering distance rank ordered for individual workers at low and high frequency patterns.

A Kruskal-Wallis test shows a significant difference between hovering distance as a function of spatial frequency: $K(1) = 12.84, p < .0001$ (See Figure 4.7). Bees approached low spatial frequency patterns from a significantly greater distance than they approached high spatial frequency patterns: the mean approach distance for low frequency patterns was 11 cm *vs* 5 cm for high spatial frequency patterns. Therefore, bees inspected high spatial frequency patterns at an average resolution of 1.08 cycles per degree, and low spatial frequency patterns at an average resolution of 0.53 cycles per degree. The behaviours were qualitatively very different too: workers displayed substantially more lateral movements (ie, zig-zag) as they approached the high spatial frequency patterns. In comparison, workers' approach to low spatial frequency patterns is best characterized by a nearly straight line to the pattern.

4.4 Discussion

The purpose of this experiment was to test the predictions of two unsupervised neural networks, and clarify the ambiguity in the literature with regards to the effect of symmetry and spatial frequency on choice behaviour. Testing unlearned and untrained choice behaviour in free-flying bumblebees ensures that results of this study can provide conclusive answers that were previously difficult to judge due to methodological inconsistencies.

4.4.1 Variations in ICA reconstruction are noise

The ICA model showed significant variations in the reconstruction of low spatial frequency symmetric patterns. The range of PSNR ratios substantially varied, from 22.2 dB to 30.6 dB. We first tested whether these variations are due to noise in the algorithm, or workers perceive the differences in patterns. The results here indicate that the variations are due to noise, and not an unexplained or uncontrolled element of the visual properties. The results of the ICA algorithm are unusual because the amount of variation decreased with increasing spatial frequency of test patterns from 8.4 dB in low spatial frequency patterns to 4 dB in high frequency patterns. While the minimum values did not differ significantly, the maximum quality of reconstruction did decrease significantly.

4.4.2 Spatial frequency result corresponds with ICA model

Choice behaviour at asymmetric patterns of different spatial frequencies corresponds with model's predictions, and are consistent with previous literature. Workers showed a significant preference towards random or asymmetric low spatial frequency patterns when compared with high spatial frequency patterns. The spatial frequency of these patterns

is operationally defined in multiple ways: the perimeter of blue and yellow lines on the artificial flower, taking into consideration the average hovering distance of workers.

4.4.3 Symmetry *vs* spatial frequency result supports ICA model

Model predictions support symmetry findings in multiple ways. First, the behavioural results in relation to four-axis symmetry are consistent with the ICA model’s predictions. The ICA model predicted that four-axis symmetry should be preferred regardless of spatial frequency, and this is what we found. In terms of bilateral symmetry, the model’s predictions are also supported. The GLM’s results indicate that choice proportions between low- and high asymmetric and symmetric patterns (LA-HA *vs* LS-HS) were independent. In other words, the presentation of LA-HA combination elicited choices proportions that are independent from choice proportions presented in the LS-HS condition. Additionally, choice proportions between the four-axis symmetric and asymmetric patterns along the low spatial frequency dimensions showed a significant amount of heterogeneity, suggesting a weaker overall preference towards symmetry. These findings support the notion that increasing spatial frequency also increases the “computational load” on bees’ visual system, but increasing levels of symmetry mitigate this additional computational load. In other words, the relevance of symmetry becomes more important for computationally expensive patterns.

Differences between 1-axis and 4-axis symmetry comparisons with asymmetric patterns indicate that bees have an unlearned preferences towards increased redundancy in information. Bilateral symmetry did not elicit a direct preference towards symmetry, but workers behaved independently between asymmetric and bilaterally symmetric patterns of varying spatial frequencies. Perhaps bilateral symmetry did not provide a sufficient amount of computational incentive to “draw” bees to these patterns. The fourfold in-

crease in redundancy of 4-axis symmetry patterns provided sufficient incentive to prefer these patterns even for low spatial frequency patterns.

4.4.4 Limits to generalizability, and future directions

There are a number aspects that limit the generalizability of this study. First, the lab environment is highly artificial, which may give rise to changes in choice behaviour: the static temperature, continuous lighting may have affected bee behaviour in unexpected ways. For example, workers activity levels appear to “even out” in the absence of circadian rhythm cues (Stelzer et al., 2010). Second, while workers did not receive reward in the testing environment, feeding had to take place in some shape and form. During testing sessions, workers received injections of sugar-water directly into empty wax pots, but during transport and prior to the testing session a small proportion of workers received sugar-water solution through a transparent glass feeder and a feeder built into the nest. While this is not foraging behaviour, and this type of feeding behaviour took place under very low light intensity conditions, it is unclear what if any learning has taken place.

Prior literature has been mixed on the effect of symmetry on choice behaviour. Some experiments appear to suggest that an unlearned preference is a possibility (Rodríguez et al., 2004; Giurfa et al., 1996) while others suggest symmetry to be an effect of learning dynamics (Plowright et al., 2011; West and Laverty, 1998). Experiments examining the flower displays highlight the relevance of symmetry although inferences about pollinators’ information processing biases are not possible to interpret from this perspective (Møller and Sorci, 1998; Neal et al., 1998). Computational models based on feed-forward networks also indicated a symmetry preference that is a result of learning dynamics rather than visual processing (Enquist and Johnstone, 1997; Johnstone, 1994).

Two possible reasons for the disagreement in results between empirical studies may

have been the lack of an operational definition for spatial frequency in the context of behavioural pattern preferences, and the weak manipulation of symmetry. Here, we used a quantifiable definition for the patterns (ie, total perimeter length), which can easily be compared with future studies. In terms of symmetry, we used the number of axes of symmetry as a definition for symmetry, which created possibilities of testing more patterns than just bilaterally or radially symmetric.

Also, previous studies examined symmetry independently of spatial frequency because there was no indication of a relationship between the two properties. The interaction between symmetry and spatial frequency was a prediction of the *computational load hypothesis*, tested and generated by the ICA and FEBAM models, not obvious from behavioural studies or even the feed-forward network models.

Our findings underline the usefulness of alternating behavioural experiments and computational models in an iterative fashion where the results inform one another to produce new data and novel hypotheses. Broadly, the implications of this standard are that behavioural experiments should be designed with the computational constraints of mathematical models in mind, and computational models should be designed with testability and methodological constraints of behavioural experiments in mind.

Chapter 5

General discussion

This thesis set out to test several novel hypotheses, utilize advancements in technology and explain results through computational modelling. The guiding question throughout the thesis was how bees first find flowers. It is a deceptively simple question that covers some of the most interesting problems modern sciences face today. For an inexperienced bee to find its first flower, it needs to emerge from larval form with a brain that includes specific features to enable detecting relevant aspects of the environment. This question can be explored (a) genetically: studying the genes or gene-complexes that are responsible for the expression of proteins, or different types of ribonucleic acids that contribute to the formation of alleles that affect information processing structures in the bee brain (eg, Dias and Ressler, 2013). Scientists are still puzzled by the question of how genetic processes give rise to behaviour. Fragments in between genotypes and phenotypes can be explained, but the whole picture is far from clear. (b) Neuroscientifically: studying the wiring configuration of nerve cords, the brain structures where particular types of information processing may be pinpointed, and the activation patterns during the presentation of visual properties (eg, Pouget et al., 2000). Logical positivists would argue that complex behaviours, thinking, conscious thought, or soul are a result of the

emergent properties of billions of neural processes. However, no satisfactory explanation exists about how these features arise. The relatively simpler brains of bees could help look for fundamental rules in neural functioning. Studying unlearned behaviour further simplifies this problem by removing complexities resulting from learning dynamics and memory processes that most cognitive systems possess. (c) Behaviourally: studying the absolute and difference thresholds of visual properties such as the colours, electromagnetic intensities, or motion cues that insect brains are capable of processing (eg, Orbán and Plowright, 2013). Behavioural approaches are also useful in identifying the mechanism by which the organism preferentially selects its environment. Behavioural experiments are also useful in connecting the previously described approaches. (d) Computationally: studying the language by which the brain works (eg, Orbán and Chartier, 2013). One of the greatest problems in interpreting neuroscientific results is that we often don't know the specific activation patterns' meaning, or how they are caused and how they cause behaviour. Computational models attempt to simulate the function of small regions in the brain that perform specific computations. (e) Evolutionarily: studying the evolutionary context the problems the organism faced, and the selection pressures that produced the solutions (Glover, 2014). Answering the question of why particular structures or functions exist helps narrow the long list of possible answers to a shorter list of likely answers, and offers an organizing principle for the often seemingly disparate pieces of evidence. Knowing the function of an object (eg, chair) helps to understand how it works and guide exploration to discover the mechanisms. For example, one can sit on on a chair, but also stand on it, attach it to a ceiling, or use it as an exercise weight, but useful answers are narrowed if we consider that the problem it is designed to solve is sitting.

The scope of this thesis was constrained to behavioural explorations, studying how the

organism responds to flower-like stimuli, thereby also filling in gaps on the evolutionary problem of co-evolution; and computational explorations, studying how brain centres may translate information recorded by the ommatidial array to be used by other brain centres for learning, memory and action.

Our answer progressed in three components. First, we tested the relative importance of two visual properties: the positioning of shapes on an artificial flower, and the type of shape on an artificial flower. While each of these visual properties are known to affect choice proportions, it was not known how the two visual properties interact. Further, it was also not clear at which point in the behavioural sequence of a choice, different visual properties matter. One of the novelties here was operationalizing choice to mean one of four behaviours: hover, antennation, land or floral exploration. With the exception of floral exploration, the three behaviours have been used in previous literature to refer to choice, but were used unsystematically, sometimes choice referring to hovering, sometimes to landing and rarely to antennating. Another novelty in this part of the thesis was the use of advanced tracking technology to replace human observation and various maze designs, both of which constrain the scale of the experiments, and the external validity of the methods. For the first time, radio-frequency identification readers were built into clay moulded artificial flowers. This allowed the tracking of uniquely identifiable individual choices of hundreds of bees that explored the testing environment *ad libitum*. The strength of this method was to enable recording hundreds of thousands of choices in the matter of a month. However, the choice criterion was defined as floral exploration, which is a very strict criterion of choice. To overcome this limitation, we used motion-sensitive camcorders that retained the strength of radio-frequency identification experiments, but with the feature that hovering, antennation and landing could also be observed. All of this allowed us to reach a conclusion: different visual properties are relevant for guiding

preference and different stages of choice behaviour.

Second, we sought computational models that could explain the behavioural findings from the first component of this thesis, and other results in the literature. The goal was to find a model that could explain the types of computations that may take place in something analogous to a mammalian *sub-cortical* region: intermediary processing that occurs after an electrochemical signal is generated from the sensory neurons, but before the information is used in integrated cognitive computations for learning, memory and action. Our choice fell on two unsupervised neural networks that have been suggested to capture aspects of biological processing, although in some ways they are not biological at all (eg, due to a small number of global transformations of matrices). Each of these models have an assumption about the way choices are elicited: prior to floral experience, bees will choose floral properties that are computationally more affordable, while maintaining certain constraints (eg, visual property must stand out from the background). The cost of computation of multiple test patterns was measured by attempting to reconstruct the original test pattern from the features that each of the unsupervised neural networks extracted. The two models, Independent Component Analysis, and Feature-Extracting Bidirectional Associative Memory were both partially successful in capturing existing behavioural results. However, Independent Component Analysis was more accurate by capturing subtle effects, including the position *vs* pattern type effect from the first thesis study. The model also produced a novel hypothesis. An interaction between symmetry and spatial frequency should exist: as spatial frequency increases, symmetrical pattern should be more preferred.

The third component tested the symmetry *vs* spatial frequency hypothesis generated by the Independent Component Analysis. The novelty of this hypothesis emerges from the relationship between the two visual properties. While each property has been

studied separately, it is the first time we operationalized spatial frequency beyond “low”, “medium” and “high” by measuring the number of perimeter pixels. We also operationalized symmetry beyond “bilateral” and “radial” by manipulating the axes of symmetry, and studied the preference for symmetry in function of spatial frequency. This study shows preference behaviour manipulated by both spatial frequency and symmetry: (1) as the axes of symmetry increased, the preference towards symmetric patterns also increased. (2) Preference also increased as the spatial frequency of the pattern decreased. This finding underlies the importance of studying multiple properties at once in function of one another.

5.1 Studying multiple visual properties

Many studies have focussed on a single property, but using this method it is impossible to assess and rank the relative contribution of any one visual property to choice behaviour. Just because bees are forced to make a choice between different levels of a single visual property, it does not mean that any particular level of a property has any relation to floral choice behaviour. To put it a different way, it is not clear whether strength of preference or weakness of aversion is measured. The solution to this problem has two parts: (a) using test paradigms where non-choice is an option (eg, flight-cage), and (b) testing multiple properties and systematically manipulating them allows ranking by strength of preference. Over the course of multiple experiments and multiple properties, a set of visual properties can be generated that are most important to initial floral choice.

There are complicating factors to studying multiple visual properties. One of these factors is that the importance of a visual property may be specific to a particular portion of the choice behaviour sequence. For example, a particular colour may only be relevant to “catch” a bee’s attention as it flies by. Once hovering is elicited, other visual properties

may become more important in eliciting the next behaviour in the sequence that ends with floral exploration. The search for relevant visual properties may also become more effective using computational models that can explain current data and can produce novel hypotheses.

5.2 The use of technology in bee studies

In terms of technological advancements, we implemented existing technology in novel ways. For example, while RFID has been used in social insect research, an RFID reader was never incorporated into artificial flowers. This enabled the study to not only record worker movement at certain checkpoints (eg, entering and exiting the test environment), but to record choice behaviour at different visual properties. The future of insect research will certainly involve increased reliance on RFID, and the management and analysis of large scale data will become necessary.

Multiple motion sensitive camcorders in different positions (ie, above and in front of stimuli) also enabled the observation of multiple kinds of behaviours at once: hovering, antennation and landing observed for each worker, and in substantially larger numbers than by human observations. The use of high resolution camcorders also allowed uniquely identifying workers that landed by its Opälth Plattchen tag. In the future, high frame-rate high-definition camcorders may allow uniquely identifying hovering and antennating workers as well, which may uncover individual differences between worker foraging characteristics.

5.3 Future directions and conclusion

The future study of unlearned behaviours by pollinators could greatly benefit from using more realistic visual patterns, possibly even real flowers. For example, the use of isogenic flowers can increase the realism of visual properties while maintaining a high level of experimental control (eg, Whitney et al., 2013, 2009). It is now possible to manipulate the expression and colour of corolla venation patterns in isogenic flowers. Increasing the number and types of properties that can be expressed could turn the study of unlearned visual preferences from using paper discs and plastic objects to using precise but real visual properties.

In terms of modelling, unsupervised neural networks that maintain topological organization would be a next logical step. For example, two unsupervised neural network models, Kohonen's Self-Organizing Maps and Bidirectional Associative Memory based architectures with topological features are good candidates (Kohonen, 2013; Chartier et al., 2009). These models could provide new insights about the way different visual properties may be spatially encoded by artificial neural networks. The point-models implemented as the second component of this thesis do not visualize low dimensional features very well, and more importantly do not reveal any relationships between features.

The principal contribution of this thesis is the use of computational models and behavioural experiments in an iterative and integrated manner, and the exploitation of technological advancements in tracking and observation. This thesis could hopefully be considered as a useful example of how computational models and behavioural experiments can co-exist, and inspire new ideas into far more complex and accurate explanations of unlearned cognitive biases.

Appendix A

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