Spring 4-29-2015

Visual choice behaviour by bumblebees (Bombus impatiens) confirms unsupervised neural network's predictions

Levente L. Orbán PhD
Kwantlen Polytechnic University

Catherine M. S. Plowright
University of Ottawa

Sylvain Chartier
University of Ottawa

Emma Thompson PhD
University of Ottawa

Vicki Xu
University of Ottawa

Original Publication Citation
Orbán, L.L., Plowright, C.M.S., Chartier, S., Thompson, E., & Xu, V. (2015). Visual choice behaviour by bumblebees (*Bombus impatiens*) confirms unsupervised neural network's predictions. *Journal of Comparative Psychology, 129*(3), 229 - 236. doi: 10.3791/52033

Journal of Comparative Psychology

0735-7036/15/$12.00 http://dx.doi.org/10.1037/a0039227

AQ: 1

Visual Choice Behavior by Bumblebees (*Bombus impatiens*) Confirms Unsupervised Neural Network's Predictions

AQ: au
AQ: 2

Levente L. Orbán, Catherine M. S. Plowright, Sylvain Chartier, Emma Thompson, and Vicki Xu
University of Ottawa

The behavioral experiment herein tests the *computational load* hypothesis generated by an unsupervised neural network to examine bumblebee (*Bombus impatiens*) behavior at 2 visual properties: spatial frequency and symmetry. Untrained "flower-naïve" bumblebees were hypothesized to prefer symmetry only when the spatial frequency of artificial flowers is high and therefore places great information-processing demands on the bumblebees' visual system. Bumblebee choice behavior was recorded using high-definition motion-sensitive camcorders. The results support the computational model's prediction: 1-axis symmetry influenced bumblebees' preference behavior at low and high spatial frequency patterns. Additionally, increasing the level of symmetry from 1 axis to 4 axes amplified preference toward the symmetric patterns of both low and high spatial frequency patterns. The results are discussed in the context of the artificial neural network model and other hypotheses generated from the behavioral literature.

Keywords: bumblebees, symmetry, visual preferences, neural networks, unlearned behavior

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 & Sorci, 1998). Indeed, *E. angustifolium* is not an exception; most flowers display some form of symmetry (Neal, Dafni, & Giurfa, 1998). The biological relevance of symmetry is still debated (Citerne, Jabbour, Nadot, & Damerval, 2010; Endress, 1999): Symmetry has been shown to be a good indicator of floral reward in some species, though workers preferred symmetric patterns even if a particular flower did not produce any nectar (Møller & Eriksson, 1995), and handling times are lower on symmetrical artificial flowers compared with asymmetrical ones (West & Laverty, 1998). The question is whether pollinator preference for symmetry is learned through functional experience with flowers or if it is an unlearned preference that guides workers to their first flowers (Orbán & Plowright, 2014a). This question is answered by AQ: 3 testing a novel hypothesis generated by an unsupervised neural network that suggests a trade-off in *computational load* between symmetry and spatial frequency. 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, Lehrer, & Kevan, 1997). *Computational load* refers to the information content of a visual property.

We implemented an unsupervised neural network to explain aspects of floral property preferences by bumblebees (Orbán, 2014). Independent component analysis (ICA) is a point model (i.e., does not model a spatial relationship) used to mimic reduction of a high-dimensional signal into low-dimensional features (i.e., a principal component analysis for non-Gaussian signals/visual signals). ICA's origins lie in the processing of natural scenes and, mathematically, ICA components are based on the fourth moment about the mean (maximization of non-Gaussianity). The model is AQ: 4 not used here with the intention to describe neural structures and processes in the bee brain. It is used as a high-level cognitive model designed to capture the product of a particular cognitive function. The idea that dimensionality reduction may take place in the visual system has been suggested in the past (Barlow, 1992; Field, 1994). The connection of these models to the choice behaviors hypothesized here is that bees may prefer patterns that the model reconstructed in high quality. Given that all parameters are fixed in the model, patterns that are reconstructed in higher quality using the same number of features are "cheaper to process." Flower-naïve bees prior to rewarded experience may be sensitive to these differences in computational load.

The ICA was found to be consistent with behavioral findings in relation to radial versus concentric pattern preferences (Lehrer, Horridge, Zhang, & Gadagkar, 1995; Orbán & Plowright, 2013) and the presence versus absence of background foliage (Forrest & Thomson, 2009). The models also tested patterns in which symmetry and spatial frequency were manipulated the same way as the

---

Levente L. Orbán, Catherine M. S. Plowright, Sylvain Chartier, Emma Thompson, and Vicki Xu, School of Psychology, University of Ottawa.

Levente L. Orbán is now at Department of Psychology, Kwantlen Polytechnic University.

We are grateful for Charles Collins' help with producing the experimental stimuli and are grateful to Michael Richards for helping with constructing MySQL queries. We also thank Emily Sibbald and Amandeep Bassi for their helpful comments on the manuscript, Kinga Orbán for help with data analysis, and Koppert Canada for their bumblebee colony donations. Research grants to Catherine M. S. Plowright and Sylvain Chartier from the Natural Sciences and Engineering Research Council of Canada supported this work.

Correspondence concerning this article should be addressed to Levente L. Orbán, Kwantlen Polytechnic University, Department of Psychology, 12666 72nd Avenue, Surrey, Brit. Col., V3W 2M8, Canada. E-mail: llo@kpu.ca

test patterns in a recent empirical study (Plowright, Evans, Leung, & Collin, 2011). The neural networks generated a novel prediction, as explained in the previous paper: Patterns with low spatial frequency should not produce a preference for symmetry, but high spatial frequency patterns should.

The empirical literature has been mixed on whether symmetry is an unlearned or a learned preference. For example, one study concluded that bumblebees (*Bombus terrestris*) likely have an unlearned preference for symmetry (Rodríguez, Gumbert, Hempel de Ibarra, Kunze, & Giurfa, 2004). Choice behavior 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 pretrained on sucrose solution on a black disk or white disk in order to encourage landing behaviors during the testing periods. The results showed a preference for bilateral symmetry. These findings correspond with another study that used *Apis mellifera* workers (Lehrer et al., 1995), also using pretraining. However, as Giurfa and colleagues (Giurfa, Dafni, & Neal, 1999) pointed out, testing the spontaneous behavior of truly naïve animals is key to understanding innate choice behavior. Plowright et al. (2011) tested the symmetry hypothesis with truly flower-naïve and untrained *B. impatiens* workers but found no preferences. However, *B. impatiens* workers pretrained on 100% black disks and 100% white disks in this same study displayed a preference for bilaterally symmetric test patterns, suggesting that symmetry preference is indeed learned.

Unlearned preferences have been almost exclusively studied at bilaterally symmetric patterns, but this is not the only kind of symmetry. Some experiments investigating learning dynamics by bees (*A. mellifera*) have used radially symmetric patterns, although this is not very well defined (Horridge, 2007). A radially symmetric pattern has multiple axes of symmetry, though it is not clear exactly how many axes are needed before a pattern can be labeled as radially symmetric. For example, two circles within one another (Møller & Sorci, 1998) and six-axis (Wignall, Heiling, Cheng, & Herberstein, 2006) or even three-axis (West & Laverty, 1998) symmetric patterns have been called radially symmetric. A bilaterally symmetric pattern is defined as the simplest type of symmetry along a single axis. Increasing the number of axes of symmetry increases the informational redundancy of the display, thereby maximizing the experimental manipulation. Previous studies that used mostly one-axis symmetric displays may have been mixed on the question of symmetry due to weak experimental manipulation. Here, we propose using the more precise definition of symmetry by the number of axes rather than labels used previously. Our intention was not to design biologically plausible symmetries but to maximize symmetry differences while staying within bumblebees' visual capabilities.

The results of investigations into *spatial frequency* preferences by bees have also been mixed. A study showed preferences for low spatial frequency patterns across multiple types of shapes: radial and concentric configurations and vertical and horizontal gratings (Lehrer et al., 1995). Though *A. mellifera* workers were pretrained on 50% black patterns (i.e., gray), this finding is in contrast to another study that found a preference for higher spatial frequency patterns (Anderson, 1977). One distinct difference between these two studies is that the former (Lehrer et al., 1995) presented stimuli on the vertical plane, whereas the latter (Anderson, 1977) presented patterns on the horizontal plane. An additional inconsistency with these studies is that the spatial frequency of patterns is not quantified. Characterization such as "low" or "high" is not sufficiently operationalized to allow comparisons between the results of different experiments.

The aim of this experiment is to behaviorally test hypotheses generated by the ICA model and compare them to predictions generated by previous behavioral experiments. First, ICA predicts that, overall, low spatial frequency patterns should be preferred over high spatial frequency patterns.

Second, 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. The behavioral 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. Behavioral studies do not provide a prediction about how the two visual properties influence choice behavior.

Third, when the level of symmetry is increased to be present along four axes, ICA predicts that a preference for symmetric patterns should be amplified and that we should observe a preference for symmetry regardless of spatial frequency. Behavioral experiments have no predictions relating to manipulation of symmetry.

The fourth and final prediction is one specifically relating to hovering behavior: If the ICA model captures reality more accurately, 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 should exist between low and high spatial frequency patterns.

Method

Subjects

All workers in five *B. impatiens* Cresson colonies were tagged with colored Opalith Plätchen numbered tags and tested in a 3.82 m wide × 4.55 m deep × 2.55 m high flight room (Orbán & Plowright, 2014b). 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 hr a day. The uninterrupted lighting in and of itself did not likely affect the foraging activity of the bumblebees. Under naturally occurring continuous daylight, north of the Arctic Circle, bumblebees maintain a diurnal rhythm of foraging activity (Stelzer & Chittka, 2010). AQ: 5

Flower naïveté. "Flower naïveté" refers to the level of experience bumblebee workers had with artificial stimuli in the testing environment. First, workers never received any reward in the testing environment. Second, bumblebees were tagged in their callow form, which ensured that all stimuli choices made by the workers were recorded. In other words, there was no way for workers to have uncontrolled prior experience in the testing environment. This enabled the observation of workers' first unrewarded floral choice and allowed us to compare this to their subsequent unrewarded floral choices. AQ: 6

Apparatus

Two nest boxes were connected simultaneously to the flight room (389 × 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 2,200 lux.

Each of two motion-sensitive camcorders was zoomed in at each of two simultaneously presented stimuli, each at a distance of 5 m (Vivotek IP8161, Chung-Ho, Taiwan) (principle of operation similar to that in Lihoreau et al. [2012]). The camcorders were triggered to record up to a 15-s-long color video clip at 15 frames per second and at 1,600 × 1,200 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—for example, if a worker represents 5% of the frame's area, this area had to have changed at the sensitivity level of 85% or 95% (i.e., very little) to ensure that a recording was made. This configuration produced many false positives but minimized the occurrence of missing a landing behavior.

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 & 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.

Stimuli

F1

AQ: 7

Stimuli consisted of symmetric and asymmetric patterns that were generated using an algorithm identical to patterns used in Plowright et al. (2011) (see Figure 1). The patterns 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-axis 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 that did not deviate from 50% black-to-white proportion by more than 5% were retained. Two types of patterns were created in the *high spatial frequency* domain: patterns having a contour density (i.e., total length of edges) of 9,000 or 11,000 pixels and *low spatial frequency* having a contour density of 4,000 pixels. Patterns with contour density deviations of no more than 5% were retained.

The symmetry of a pattern is measured by halving a pattern vertically and correlating the pixel values of each side. Symmetric patterns were retained if their level of symmetry exceeded 99%, and asymmetric patterns were retained if their level of symmetry fell below 1%. Patterns that the ICA model reconstructed in the best and worst quality within each category were selected (Orbán & Chartier, 2013).

Procedure

Testing commenced within a week of arrival of the commercial colonies (Koppert Canada, Scarborough, Ontario, Canada). 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. Choices made in the presence of another worker (i.e., socially influenced choices) were discarded. The rule of discarding data included any worker that landed or hovered within 1 m of the stimulus at the time of a conspecific's arrival. There were no positions within 2 m of the stimuli for workers to observe conspecifics from a resting position. The role of social influence has been shown in previous studies (Plowright et al., 2013; Worden & Papaj, 2005). Learning can

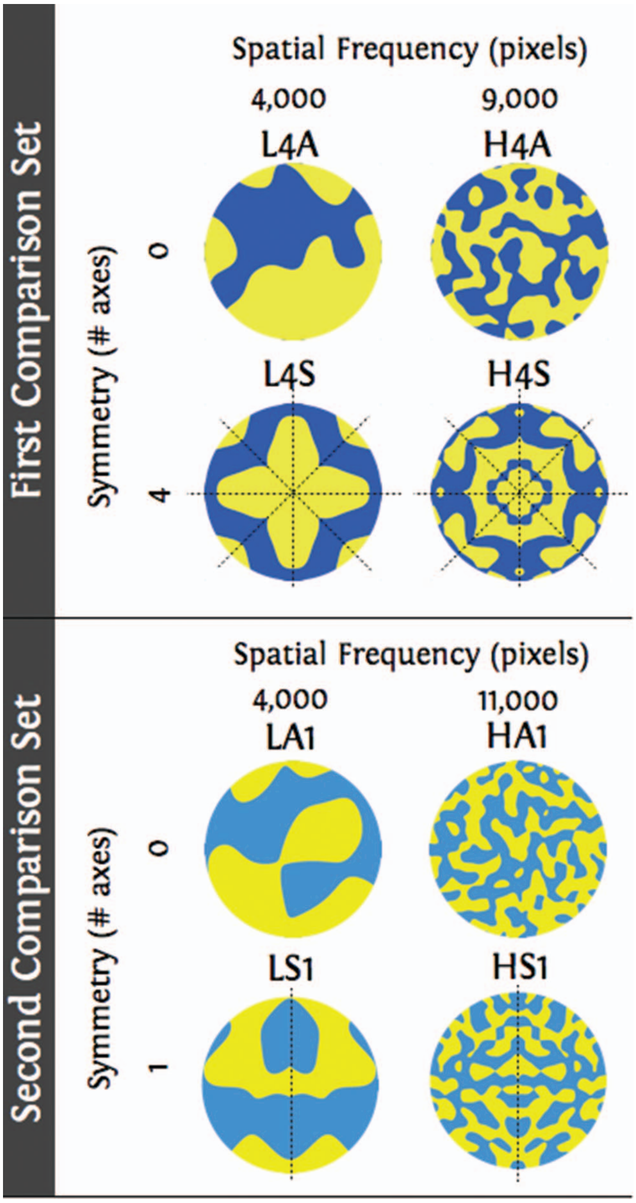


Figure 1. Exemplars of stimuli used in the experiment. The label for each pattern was generated by the appearance of the stimulus: "L" and "H" refer to the frequency of the stimulus (low or high); "A" or "S" refer to whether or not symmetry is present (asymmetric or symmetric); values of 1 or 4 refer to the number of axes of symmetry.

ONLINE COLOR

take place in the presence of a conspecific on an unrewarding flower, but not unless they have had rewarded experience on other low-quality flowers, which was not the case in this study (Jones, Ryan, & Chittka, 2015).

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 that did not consider individual differences. 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-tests) for categorical data (Sokal & 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 (i.e., individual differences), and G_p values test for the deviation between a group choice proportion (i.e., pooled data) and a theoretical value of chance AQ: 8 (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 (García, 2004; Lieberman & Cunningham, 2009; 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.

Finally, hovering data were analyzed using a Kruskal–Wallis one-way analysis of variance (ANOVA) because the data did not meet assumptions of normality and homogeneity. This test is the nonparametric equivalent of one-way ANOVA.

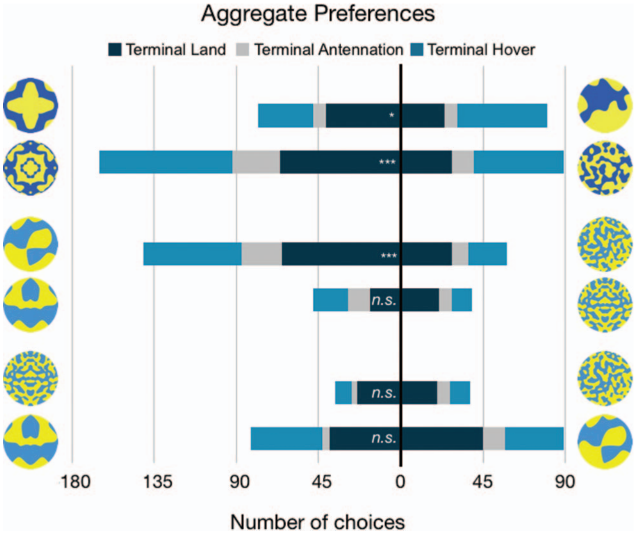
Results

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. Only those choices that could be associated with a tagged worker were used in the replicated G-test, but all choices were used in aggregate analyses. A figure has been compiled to summarize overall choices across the conditions (see F2 Figure 2).

To allow a visual comparison between the behavioral results and the ICA model, the results of the model are drawn for each condition along with the behavioral results. The ICA bar chart indicates the peak signal-to-noise (PSNR) ratio between the reconstructed image and the F3-F5 original image (see Figures 3, 4, and 5). PSNR values are measured in decibels (dB) and indicate the reconstruction quality of a signal. The higher values indicate higher quality that is more like the original AQ: 9 signal.

Effect of Four-Axis Symmetry

Two sets of combinations were presented to assess the effect of symmetry of choice behavior. The first combination consisted of 9,000-pixel patterns that either displayed four-axis symmetry or were asymmetric. Thirty-three workers contributed 90 choices in the 9,000-pixel condition. The pooled G-test, which compares the differences between the observed choice proportion for the group and a theoretical value of chance, is significant, $G_p(1) = 18.41$, $p < .001$, and individual differences are not significant, $G_h(32) = 40.37$, $p = .147$ (see Figure 3).



ONLINE COLOR

Figure 2. Summary of choice deviations across all conditions. Colors indicate "terminal behavior," meaning the last observed behavior at the stimulus. A terminal hover behavior indicates that a worker did not proceed to antennate or land on the stimulus. A terminal antennation indicates that the worker hovered and antennated but did not land on the stimulus. Finally, terminal land behavior indicates that a worker hovered, antennated, and landed on the stimulus. All inferential statistics are based on landing behavior (i.e., most stringent criterion for preference), except for analysis of hovering data. * $p < .05$. *** $p < .001$. *ns* $p > .05$. AQ: 20

Twenty-seven workers contributed 65 choices in the 4,000-pixel condition and made an average of 1.7 choices. 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 3).

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 workers landed a total of 93 times on the presented stimuli. A significant replicated G-test showing a preference toward the low-frequency patterns underscores this finding: $G_p(1) = 11.64$, $p < .001$. Heterogeneity of choices is nonsignificant even though workers from several different colonies were used [$G_h(16) = 20.81$, $p = .186$] (see Figure 4a).

Effect of spatial frequency with respect to bilaterally symmetric patterns. Choice proportions at two combinations were examined. One combination consisted of asymmetric patterns displaying 4,000- or 11,000-pixel-perimeter patterns (low spatial frequency asymmetric, or LA, vs. high spatial frequency asymmetric,

ONLINE COLOR

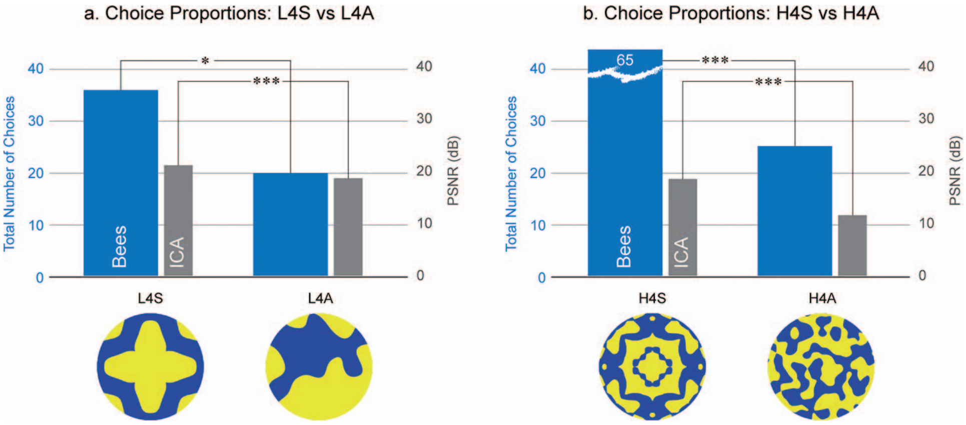


Figure 3. Low spatial frequency patterns manipulating four-axis symmetry (a). High spatial frequency patterns manipulating four-axis symmetry (b). Data contain only those choices that were associated with identified workers. The left vertical chart axis refers to the behavioral results (blue bars), and the right vertical chart axis refers to the ICA model (gray bars). * $p < .05$. *** $p < .001$.

AQ: 10-11 or HA), and the other combination consisted of bilaterally symmetric patterns (i.e., one-axis symmetry) displaying the same combination of spatial frequencies (low spatial frequency symmetric, AQ: 12-13 or LS, vs. high spatial frequency symmetric, or HS). A GLM was implemented on the count data to test choice behavior characteristics between the LA–HA and LS–HS. The overall χ^2 test shows a significant result [$\chi^2(3) = 31.12$, $p < .001$, $n = 131$] indicating that the bees' choice characteristics depended on whether the patterns were symmetric or not (see Figures 4a and 4b).

Effect of One-Axis (Bilateral) Symmetry

The effect of one-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. A replicated G-test did not detect preference for symmetry: $G_p(1) = 0.92$, $p = .339$ (see Figure 5). However, heterogeneity of choices was significant, indicating significant variation in individual preferences [$G_h(25) = 53.97$, $p < .001$].

Eighteen workers made a total of 44 choices in the 11,000-pixel condition. A nonsignificant replicated G-test shows an absence of preference for either pattern: $G_p(1) = 0.11$, $p = .746$. However, significant individual variation exists in choice preference, as evidenced by significant heterogeneity of choice: $G_h(17) = 29.35$, $p = .032$ (see Figure 5).

Hovering Distance Analysis

Quantifying hovering distance was determined by the following behavioral markers: the farthest point from the stimulus at which the worker displays a pause and orients toward the pattern (i.e., begins a "zig-zag" motion). At the same time, the flight pattern displays a slowdown and change in lateral direction, and the bee begins to move in the anterior direction toward the stimulus. Only AQ: 14 hovering behavior that terminated in landing was examined. Ob-

ONLINE COLOR

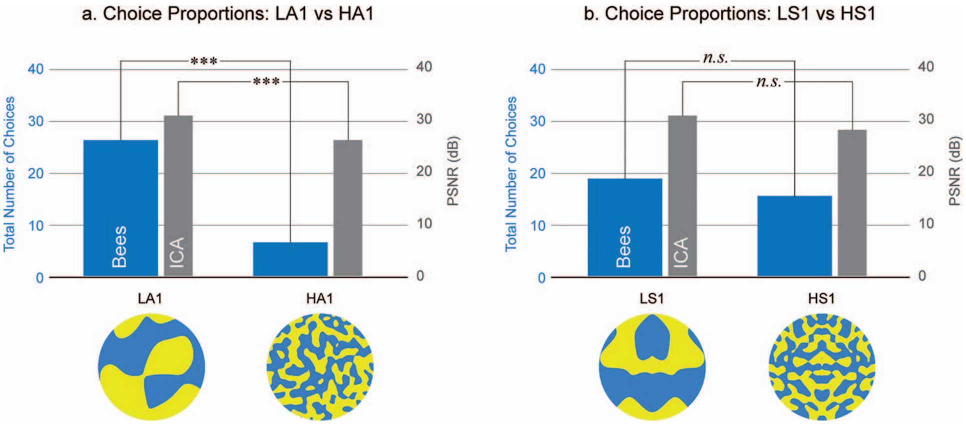


Figure 4. Choice proportions at low and high spatial frequency patterns displaying bilaterally symmetric (a) or random asymmetric patterns (b). Data represent only those choices that were associated with identified workers. ICA model results are shown for comparison. The left vertical chart axis refers to the behavioral results (blue bars), and the right vertical chart axis refers to the ICA model (gray bars). *** $p < .001$. *ns* $p > .05$.

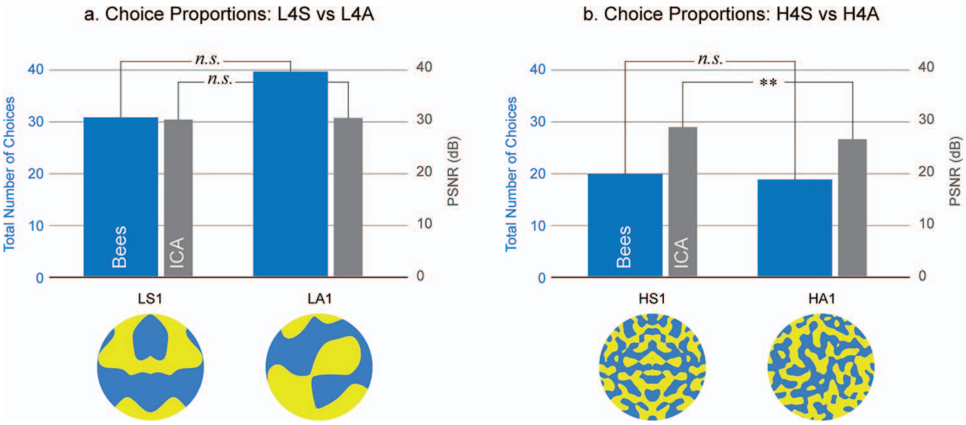


Figure 5. Choice proportions at low spatial frequency patterns differing in level of symmetry (a) and high spatial frequency patterns differing in level of symmetry (b). Data represent only those choices that were associated with identified workers. ICA model results are shown for comparison. ** $p < .01$. *ns* $p > .05$.

servations of distance and angle of approach were recorded between the center point of the stimulus and the head of the worker. Observations were discarded if these inflection points indicating hovering behavior could not be observed.

Approach angles were also measured. Zero degrees is defined as the direction pointing away from the front of a pattern. Approach angles between −90 °C and +90 °C (i.e., behind the pattern) were discarded. 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.

A Kruskal–Wallis test shows a significant difference in hovering distance between low and high spatial frequency patterns: $K(1) = 12.84$, $p < .0001$ (see Figure 6). Bees approached low spatial frequency patterns from a significantly greater distance than they approached high spatial frequency patterns: The mean approach distance for low spatial frequency patterns was 12 cm versus 5 cm for high spatial frequency patterns. Therefore, workers 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.

Discussion

The purpose of this experiment was to clarify the ambiguities in the literature with regard to the effect of symmetry and spatial frequency on choice behavior. Testing unlearned and untrained choice behavior in free-flying bumblebees ensures that the results of this study provide conclusive answers that were previously difficult to judge due to methodological inconsistencies.

Spatial Frequency Result Corresponds With ICA Model

Choice behavior at asymmetric patterns of different spatial frequencies corresponds with the ICA model's predictions, consistent with previous literature. Workers showed a significant preference toward low spatial frequency patterns (even if asymmetric) when compared with high spatial frequency patterns. The spatial frequency of these patterns is defined by the perimeter of blue and yellow lines on the artificial flower and the average hovering distance of workers at each pattern type. In free-flight conditions, it is not possible to manipulate spatial resolution in

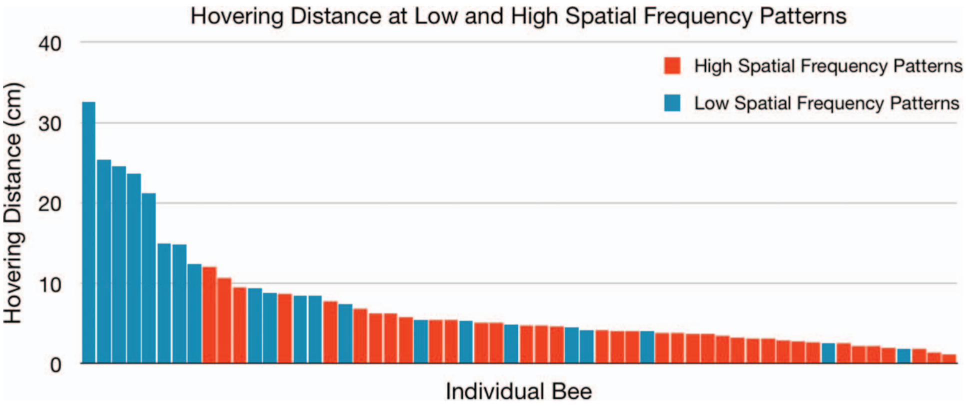


Figure 6. Hovering distance rank ordered for individual workers at low- and high-frequency patterns.

absolute terms because workers adjust object viewing distance according to object characteristics. The spatial frequency manipulations were designed to be within the boundaries of the bumblebees' visual ability. The goal was not to capture the spatial frequency of any particular floral species in nature, but rather to harness the variable experimentally and disentangle it from symmetry.

Symmetry Versus Spatial Frequency Result Supports ICA Model

The predictions derived from the ICA model were confirmed by our findings on preference for symmetry. First, our behavioral 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 not independent: Preference for frequency depended on symmetry. In other words, the presentation of the LA–HA combination elicited choice 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 significant heterogeneity, suggesting a weaker overall preference toward symmetry. Previous literature did not make a prediction to this effect.

Methodological Considerations and Future Directions

These findings support the notion that increasing spatial frequency also increases the computational load on the bees' visual system but that 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 one-axis and four-axis symmetry comparisons with asymmetric patterns indicate that bees have an unlearned preference toward increased redundancy in information. Bilateral symmetry did not elicit a direct preference toward symmetry, but workers' behavior was not independent between asymmetric and bilaterally symmetric patterns. Perhaps bilateral symmetry did not provide a sufficient amount of computational incentive to "draw" bees to these patterns. The fourfold increase in redundancy of four-axis symmetry patterns provided sufficient incentive to prefer these patterns, even for low spatial frequency patterns. An interesting future direction would be to dissociate levels of symmetry from levels of informational redundancy. While increasing the axes of symmetry necessarily increases information redundancy, the opposite is not necessarily true.

Prior literature has been mixed on the effect of symmetry on choice behavior. Some experiments appear to suggest that an unlearned preference is a possibility (Giurfa, Eichmann, & Menzel, 1996; Rodríguez et al., 2004), while others suggest symmetry to be an effect of learning dynamics (Plowright et al., 2011; West & 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 & 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 & Johnstone, 1997; Johnstone, 1994).

Two possible reasons for the disagreement in the literature between empirical studies may have been the lack of an operational definition for spatial frequency in the context of behavioral pattern preferences and the weak manipulation of symmetry. In this study, we used a quantifiable definition for the patterns (i.e., 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 patterns. In the future, degree of symmetry or degree of asymmetry may also be manipulated by comparing the level of similarity across different axes. AQ: 15

With respect to hovering behavior, our findings correspond with a study in which *B. terrestris* workers were found to fly significantly slower and closer to the ground, thereby increasing the angle subtended by the stimuli (Spaethe, Tautz, & Chittka, 2001). Indeed, what we found here was that bumblebees adjusted their hovering distance for high spatial frequency patterns, though they never reached the angle subtended by low spatial frequency patterns.

Conclusion

Previous studies examined symmetry independently of spatial frequency because there was no indication of a relationship between the two properties. With the help of two unsupervised neural network models, we have confirmed that an interaction between symmetry and spatial frequency exists. These findings support the *computational load hypothesis* and explain why the literature has been mixed in relation to the choice behavior of honeybees and bumblebees at patterns manipulating symmetry and spatial frequency. More broadly, we have shown that initial foraging choices are highly sensitive to the information-processing characteristics of floral displays and that biologically inspired computational models can be useful to examine fundamental questions about the nature of cognition.

References

Anderson, A. M. (1977). The influence of pointed regions on the shape preference of honey bees. *Animal Behaviour, 25,* 88–94.

Barlow, H. B. (1992). The biological role of the neocortex. In A. Aertsen & V. B. Braitenberg (Eds.), *Information processing in the cortex: Experiments and theory* (pp. 54–80). Heidelberg, Germany: Springer-Verlag.

Citerne, H., Jabbour, F., Nadot, S., & Damerval, C. (2010). The evolution of floral symmetry. *Advances in Botanical Research, 54,* 85–137.

Dafni, A., Lehrer, M., & Kevan, P. G. (1997). Spatial flower parameters and insect spatial vision. *Biological Reviews, 72,* 239–282.

Endress, P. K. (1999). Symmetry in flowers—Diversity and evolution. *International Journal of Plant Sciences, 160,* S3–S23.

Enquist, M., & Johnstone, R. A. (1997). Generalization and the evolution of symmetry preferences. *Proceedings of the Royal Society B: Biological Sciences, 264,* 1345–1348.

Field, D. J. (1994). What is the goal of sensory coding? *Neural Computation, 6,* 559–601.

Forrest, J., & Thomson, J. D. (2009). Background complexity affects colour preference in bumblebees. *Naturwissenschaften, 96,* 921–925.

García, L. V. (2004). Escaping the Bonferroni iron claw in ecological studies. *Oikos, 105,* 657–663.

Giurfa, M., Dafni, A., & Neal, P. R. (1999). Floral symmetry and its role in plant-pollinator systems. *International Journal of Plant Sciences, 160,* S41–S50.

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

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

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

Johnstone, R. A. (1994). Female preference for symmetrical males as a by-product of selection for mate recognition. *Nature, 372,* 172–175.

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

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

Lieberman, M. D., & Cunningham, W. A. (2009). Type I and Type II error concerns in FMRI research: Re-balancing the scale. *Social Cognitive and Affective Neuroscience, 4,* 423–428.

Lihoreau, M., Raine, N. E., Reynolds, A. M., Stelzer, R. J., Lim, K. S., Smith, A. D., . . . Chittka, L. (2012). Radar tracking and motion-sensitive cameras on flowers reveal the development of pollinator multi-destination routes over large spatial scales. *PLoS Biology, 10,* e1001392.

Møller, A. P., & Eriksson, M. (1995). Pollinator preference for symmetrical flowers and sexual selection in plants. *Oikos, 73,* 15–22.

Møller, A. P., & Sorci, G. (1998). Insect preference for symmetrical artificial flowers. *Oecologia, 114,* 37–42.

Nakagawa, S. (2004). A farewell to Bonferroni: The problems of low statistical power and publication bias. *Behavioral Ecology, 15,* 1044–1045.

Neal, P. R., Dafni, A., & Giurfa, M. (1998). Floral symmetry and its role in plant-pollinator systems: Terminology, distribution, and hypotheses. *Annual Review of Ecology and Systematics, 29,* 345–373.

Orbán, L. L. (2014). *Behavioural studies and computational models exploring visual properties that lead to the first floral contact by bumblebees* (Unpublished PhD dissertation). University of Ottawa, Ottawa, Ontario. http://hdl.handle.net/10393/30917. AQ: 17

Orbán, L. L., & Chartier, S. (2013). Unsupervised non-linear neural networks capture aspects of floral choice behaviour. In *21st Proceedings of the European Symposium on Artificial Neural Networks, Computational Intelligence and Machine Learning* (pp. 149–154). AQ: 18

Orbán, L. L., & Plowright, C. M. S. (2013). The effect of flower-like and non-flower-like visual properties on choice of unrewarding patterns by bumblebees. *Naturwissenschaften, 100,* 621–631.

Orbán, L. L., & Plowright, C. M. S. (2014a). Getting to the start line: How bumblebees and honeybees are visually guided towards their first floral contact. *Insectes Sociaux, 61,* 325–336.

Orbán, L. L., & Plowright, C. M. S. (2014b). Radio frequency identification and motion-sensitive video efficiently automate recording of choice behaviour by bumblebees. *Journal of Visualized Experiments, 93,* e52033.

Perneger, T. V. (1998). What's wrong with Bonferroni adjustments. *British Medical Journal, 316,* 1236–1238.

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

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

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

Sokal, R. R., & Rohlf, F. J. (2012). *Biometry* (4th ed.). New York, NY: WH Freeman and Co.

Spaethe, J., Tautz, J., & Chittka, L. (2001). Visual constraints in foraging bumblebees: Flower size and color affect search time and flight behavior. *Proceedings of the National Academy of Sciences of the United States of America, 98,* 3898–3903.

Stelzer, R. J., & Chittka, L. (2010). Bumblebee foraging rhythms under the midnight sun measured with radiofrequency identification. *BMC Biology, 8,* 93–100.

West, E. L., & Laverty, T. M. (1998). Effect of floral symmetry on flower choice and foraging behaviour of bumble bees. *Canadian Journal of Zoology, 76,* 730–739.

Wignall, A. E., Heiling, A. M., Cheng, K., & Herberstein, M. E. (2006). Flower symmetry preferences in honeybees and their crab spider predators. *Ethology, 112,* 510–518.

Worden, B. D., & Papaj, D. R. (2005). Flower choice copying in bumblebees. *Biology Letters, 1,* 504–507. AQ: 19

Received May 22, 2014
Revision received March 9, 2015
Accepted March 9, 2015 ■

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES

AQau—Please confirm the given-names and surnames are identified properly by the colors.
🟥= Given-Name, 🟦= Surname
The colors are for proofing purposes only. The colors will not appear online or in print.

AQ1—Author: Please note that Latin names for all animals, including humans, must be included in the **article title**, **abstract**, and **first mention of Method section**.

AQ2—Author: Please be sure to provide the name of the department(s) with which you and your coauthors are affiliated at your respective institutes if you have not already done so. If you are affiliated with a governmental department, business, hospital, clinic, VA center, or other nonuniversity-based institute, please provide the city and U.S. state (or the city, province, and country) in which the institute is based. Departments should be listed in the author footnote only, not the byline. If you or your coauthors have changed affiliations since the article was written, please include a separate note indicating the new department/affiliation: [author's name] is now at [affiliation].

AQ3—Author: Should this be 2014a or 2014b? Please clarify.

AQ4—Author: Should this be "model" (singular) or "models" (plural) throughout? Please clarify.

AQ5—Author: Should this be 2014a or 2014b? Please clarify.

AQ6—Author: The last sentence of this paragraph was revised. Please confirm that it is correct as written.

AQ7—Author: Please confirm that "four-axis symmetric patterns" is correct as written here.

AQ8—Author: Should "G" in "G value" be italicized?

AQ9—Author: The figure callouts are out of order in the text. Please note that APA style dictates that all callouts must be in numerical order throughout the text. Please revise the callouts or renumber the figures accordingly.

AQ11—Author: Please spell out HA.

AQ13—Author: Please spell out HS.

AQ14—Author: Please note that this sentence was revised (changed from "movement begins to move"). Please confirm that it is correct as written.

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES

AQ15—Author: Please note that the word "patterns" was added at the end of this sentence. Please confirm OK.

AQ17—Author: Please provide the university name and location for the reference Orbán (2014).

AQ18—Author: Please spell out the proceedings name in the reference Orbán & Chartier (2013).

AQ19—Author: Please provide complete mailing address for corresponding author, including street address and postal code.

AQ20—Author: Please consider revising the caption for Figure 2, as the figures will only appear in color online.