The preference for symmetry in flower-naïve and not-so-naïve bumblebees

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A B S T R A C T

Truly flower-naïve bumblebees, with no prior rewarded experience for visits on any visual patterns outside the colony, were tested for their choice of bilaterally symmetric over asymmetric patterns in a radial-arm maze. No preference for symmetry was found. Prior training with rewarded black and white disks did, however, lead to a significant preference for symmetry. The preference was not specific to symmetry along the vertical axis: a preference for horizontal symmetry was found as well. The results challenge the notion that a preference for bilateral symmetry is unlearned. The preference for symmetry was the product of non-differential conditioning.

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This study investigates the development of preferences for bilateral floral symmetry in bumblebees (reviewed in Goulson, 2003). For example, in a field study on fireweed (Epilobium angustifolium), naturally occurring symmetric flowers were preferentially visited by Bombus terrestris and this was also true of experimentally manipulated flowers that affected symmetry (Møller, 1995; see also Møller & Sorci, 1998). The functional significance of this preference is that symmetric flowers are in general more rewarding (Møller & Eriksson, 1995). More recently, asymmetries in plants, petals and leaves have been shown to be an index of developmental instability as they are caused by environmental factors such as radiation and pollutants and genetic factors such as mutations and homozygosity (Møller, 2000; Møller & Shykoff, 1999).

One way in which the preference could develop in bumblebees is through experience with rewarding flowers. Indeed, Giurfa, Eichmann, and Menzel (1996) have shown that in honeybees, discrimination training between symmetric and asymmetric patterns transfers to novel stimuli (rewarded experience with symmetric stimuli generalized to novel symmetric stimuli, and, similarly, rewarded experience with asymmetric stimuli generalized to novel asymmetric stimuli). The preference for bilaterally symmetric stimuli in honeybees is reviewed by Horridge (2009). Another possibility would be that the preference develops prior to floral encounters: perhaps flower-naïve bumblebees selectively approach symmetric stimuli. The evidence on this point is mixed. No preference for bilateral symmetry was found by West and Laverty (1998). In contrast, it has recently been reported that flower-naïve bumblebees do indeed have such an “innate” preference (Rodríguez, Gumbert, Hempel de Ibarra, Kunze, & Giurfa, 2004). In this paper, we revisit the question as to the behavior of truly flower-naïve bumblebees.

To study the behavior of bees prior to their first floral encounter, researchers occasionally resort to pre-training on rewarding, ostensibly neutral patterns (see Hudon & Plowright, 2010). This practice was adopted in the study by Rodríguez...
Table 1
The number of bees (trained or untrained) used from each colony. The duration of training was a minimum of 4 rewarded visits to the black and 4 to rewarded visits to the white disks, or a minimum of 12 rewarded visits to each. For the symmetric testing stimuli, the axis of symmetry was vertical or horizontal.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Number bees</th>
<th>Duration of training</th>
<th>Testing stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24 untrained</td>
<td>N/A</td>
<td>Vertical</td>
</tr>
<tr>
<td>2</td>
<td>8 untrained</td>
<td>N/A</td>
<td>Vertical</td>
</tr>
<tr>
<td>3</td>
<td>8 untrained</td>
<td>N/A</td>
<td>Vertical</td>
</tr>
<tr>
<td>4</td>
<td>10 trained</td>
<td>Min. 12</td>
<td>Vertical</td>
</tr>
</tbody>
</table>

et al. (2004); “flower-naïve bumblebees were first trained on plain, homogeneous disks and were subsequently tested with vertically presented bilaterally symmetric and asymmetric patterns ... A disk was chosen as a 'neutral' training stimulus because it presents infinite possible symmetry axes, including radial symmetry. There was no reason, therefore, for the bees to prioritize only one of these possibilities.” (p. 375). Following training on both plain black disks and white disks, both of which were rewarding, the bumblebees were subsequently tested on novel, unrewarding, black and white patterns that were symmetric or asymmetric.

We have recently challenged the assumption that prior training is neutral and shown that in fact it can influence subsequent choices; a preference for radial over concentric patterns by truly flower-naïve bumblebees (i.e. no rewarded experience whatsoever outside the colony) was amplified by prior training on a variety of ostensibly neutral patterns such as a grid of squares (Séguin & Plowright, 2008). We had suggested that the question as to whether prior training on plain disks was neutral or whether it biased subsequent choice for symmetry should not be pre-judged by experimenters but should be put to the bees, as we did here.

Three hypotheses were tested: (1) The first was the null hypothesis that bees with no prior history of rewards on visual patterns outside the colony (i.e. truly flower-naïve bumblebees) would have no preference for symmetric over asymmetric stimuli. Spatial frequency of the patterns, which is known to be an important variable in pattern perception (e.g. Lehrer, Horridge, Zhang, & Gadagkar, 1995) was unconfounded with symmetry and explicitly manipulated. (2) The second was that such a preference for symmetric stimuli could be created by prior reward on black and white disks (i.e. in not-so-naïve bumblebees), where the bees were reinforced equally for approaching black and white disks. Such a finding would falsify the assumption that the training was neutral. Moreover, it would reveal a new route for learning floral symmetry: it would show that it could be learned through non-differential conditioning (both black and white disks were rewarding), rather than differential conditioning, where a discrimination is learned between a rewarding and an unrewarding stimulus (the importance of differential conditioning in color discriminations in bees has recently been highlighted by Dyer & Chittka, 2004; Giurfa, 2004). (3) The third hypothesis was that if there was an effect of training, it was to create a specific preference for vertical symmetry (i.e. one side of a vertical axis is the mirror image of the other side). Perhaps the vertical axis was most salient given the vertical positioning of the patterns. Accordingly, we manipulated the axis of symmetry of the novel unrewarding test stimuli.

Methods

Subjects

Four colonies of commercially reared bumblebees (Bombus impatiens Cresson) were purchased from Biobest Biological Systems (Leamington, ON). The number of bees used from each colony and how they were treated is given in Table 1. Outside of training and testing phases, the colonies were given access to sugar solution from a reservoir underneath the colony box. They were also fed pollen ad libitum. The reservoir of sugar solution was capped one or two days before testing to motivate search for food. Workers that were trained were individually identified with a colored numbered label glued to the thorax.

Apparatus

The bees were trained in a 12-arm radial maze (Fig. 1) modeled after Lehrer et al. (1995) and described in Simonds and Plowright (2004), Plowright, Simonds, and Butler (2006) and Séguin and Plowright (2008). The colony box was attached to the end of one of the corridors (14 cm × 15 cm × 15 cm, W × L × H) of the maze by a 30.5 cm long wooden walkway covered with glass plates. Since one of the corridors functioned as the entrance and did not contain a stimulus, the corridor across from it was also blocked off, as were the two corridors at 90° to the entrance corridor, so that only 8 corridors were used. The central area of the maze was 22 cm wide, and the entrance to each corridor was 6 cm wide. The walls of the maze were made of opaque gray Plexiglas® while both the ceiling and floor were transparent. The maze was placed on a gray turntable. Feeding troughs (9.6 cm long, holding 0.5 mL) projected inside the corridor from the center of the back wall of each corridor.
Fig. 1. Photograph of training and testing apparatus. Eight of the corridors were used while the remaining four were blocked at the entrance from the central area. (a) Trained bees were rewarded with sugar solution in a feeding trough placed below black disks and white disks. (b) The apparatus used for testing for preference for symmetry contained no feeding troughs.

A syringe containing sugar solution (2:1 sugar and water by volume) could be inserted from the outside through a small hole located directly above each feeding trough.

A second radial arm maze was used for the testing procedure. It was identical to the training apparatus, except that it did not contain any feeding troughs on the back wall of each corridor.

The apparatus was illuminated by eight fluorescent bulbs on the ceiling (Sylvania SuperSaver Daylight Deluxe 34 W bulbs, model # F34DXSS) mounted on a 60 Hz ballast as well as two 500 W twin-head halogen work lights.

Stimuli

Both training and testing stimuli were laminated paper disks, 7 cm in diameter, fixed on the back walls of the corridors with Velcro®. During training, 4 black and 4 white disks were used. Because the patterns used by Rodríguez et al. (2004, Fig. 1b) seem to vary in spatial frequency, during testing, symmetry and spatial frequency were manipulated factorially to create four types of testing stimuli (for details see Appendix A) diagrammed in Fig. 2. Both testing and training stimuli were printed using an HP Color LaserJet CM10165 PCL6 printer.

Fig. 2. Examples of each of the types of test patterns used: Asymmetric and symmetric patterns of both high and low spatial frequency.
Table 2
Arrangement of stimuli within the radial-arm maze. Four exemplars (arbitrarily numbered 1–4) of each of four stimulus types (LS, HS, LA, HA) were used. Two copies of each were used to make eight stimuli for eight corridors. Corridors are labeled 1–8 clockwise from the entrance. L: low frequency; H: high frequency; S: symmetric; A: asymmetric.

<table>
<thead>
<tr>
<th>Bee number</th>
<th>Corridor</th>
<th>Stimulus numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-2-3-4</td>
<td>Asymmetric</td>
</tr>
<tr>
<td>1</td>
<td>LS</td>
<td>HS</td>
</tr>
<tr>
<td>2</td>
<td>HA</td>
<td>LA</td>
</tr>
<tr>
<td>3</td>
<td>HS</td>
<td>HA</td>
</tr>
<tr>
<td>4</td>
<td>LA</td>
<td>HS</td>
</tr>
<tr>
<td>5</td>
<td>LS</td>
<td>HA</td>
</tr>
<tr>
<td>6</td>
<td>HA</td>
<td>LS</td>
</tr>
<tr>
<td>7</td>
<td>HS</td>
<td>LA</td>
</tr>
<tr>
<td>8</td>
<td>LA</td>
<td>HS</td>
</tr>
</tbody>
</table>

For Colony 2, where 16 trained bees were tested, the sequence was repeated. For Colony 4, where 10 bees were tested, the arrangement for Bees 1 and 2 was repeated.

Procedure

Training

Four black and four white disks were placed, alternately, on the back wall of each corridor of the maze, directly above the feeding troughs (Fig. 1a). The troughs were filled with 0.5 mL of sugar solution (2:1 water/sugar by volume) and were refilled when empty. Bees were allowed to forage freely (for Colony 2: approximately 25 h over 5 days; for Colonies 3 and 4 for which training was extended: approximately 50 h over 5–10 days). Once it was observed that most bees were drinking from the feeding troughs, we began to record the number of visits made by each individual bee, where a visit was defined as a bee extending its proboscis into the feeding trough. For Colony 2, once a bee made at least 4 visits to the black and 4 visits to the white disks, it was immediately tested. Training was extended for Colonies 3 and 4: bees were required to make at least 12 visits to the black and 12 to the white disks before they were tested.

Testing of trained and untrained bees

In Colony 1, two groups of eight bees were tested in a ‘pure’ manipulation of symmetry: one group was tested with only low spatial frequency patterns, and the other was tested with only high spatial frequency patterns. For all the rest of the bees in this study, eight stimuli were presented simultaneously in the maze and none was rewarded: two copies of each of four types (2 symmetric vs. asymmetric × 2 spatial frequencies, high vs. low). The stimuli were alternated in such a way that the same pattern was never present in more than two adjacent corridors (Table 2). Between testing sessions, the arrangement of the patterns was changed and the maze itself was rotated by 180° in order to control for any inconsistencies in lighting, corridor preference, etc. To ensure that any possible preferences were not attributable to a particular pattern or a particular combination, four exemplars of symmetric and four exemplars of asymmetric patterns of each spatial frequency were used. Across eight bees, each of the four exemplars of symmetric patterns (of each spatial frequency) was presented in combination with two different exemplars of asymmetric patterns (Table 2).

For Colony 4, the axis of symmetry of the test stimuli was manipulated between two groups of 10 bees (Table 1). Bees in each group were paired so that after one bee was tested with vertically symmetric stimuli, the next bee was tested with the same stimuli except that the vertically symmetric stimuli were rotated by 90° to produce horizontally symmetric stimuli.

To test a trained bee, following training it was captured in a vial and placed into a wooden box (10 cm × 10 cm × 5 cm, W × L × H) attached to a wooden walkway through which it entered the second radial-arm maze (Fig. 1b). The testing of untrained bees proceeded in the same way except that it was the colony box that was attached to the maze by a walkway. Bees were tested individually and their first 16 choices were recorded, where a choice was defined as walking/flying across an imaginary line halfway through the corridor. Each bee was tested once, and then removed from the colony.

Statistical analyses

Because the data were binary (e.g. choice of symmetric vs. asymmetric patterns), with replication within bees (16 choices per bee), a replicated test of goodness of fit test using the G-statistic (Sokal & Rohlf, 1995) was used. Two G values were obtained: (1) The \( G_p \) value (P for Pooled) tests for whether the pooled data (i.e. group choice proportions) deviate significantly from a theoretical value of chance (50:50), and (2) the \( G_h \) value (H for Heterogeneity) tests for individual differences. In tests of significance, the obtained \( G \) values were compared to a \( \chi^2 \) value.
Table 3
Mean choice frequencies out of 16 choices for bees in each of three colonies. The patterns are categorized according to symmetry and frequency (high and low).

<table>
<thead>
<tr>
<th>Colony</th>
<th># Bees</th>
<th>Mean choice frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Symmetric</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>4.75</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>4.00</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>3.875</td>
</tr>
<tr>
<td>Grand mean frequency</td>
<td>7.33 (SE = 0.42)</td>
<td>8.67</td>
</tr>
<tr>
<td>Grand mean proportion</td>
<td>.46</td>
<td>.54</td>
</tr>
</tbody>
</table>

The G-test compares a choice proportion to a theoretical value. To make comparisons among groups (e.g., different colonies, different training durations, different axes of symmetry), a logistic model, which specifies a binomial error term, was fit to the choice frequencies using GLIM (Generalized Linear Interactive Modeling; Francis, Green, & Payne, 1993).

Results

Do the choice proportions of untrained bees differ from chance?

Symmetric vs. asymmetric patterns

Table 3 shows the mean choice frequencies for symmetric and asymmetric patterns for eight bees in each of three colonies. No colony differences were detected ($\chi^2 = 0.59$, df = 2). The overall choice proportion of .46 (7.33 choices out of 16) did not differ from a chance value of 50:50 ($G_P = 3.01$, df = 1) and no individual differences were found ($G_H = 30.53$, df = 23).

To address the possibility that there might have been a preference for symmetry in the first few choices that was subsequently “washed out” with repeated exposure to unrewarding stimuli, the comparison was repeated on the first four choices. The proportion of .42 (1.7 out of 4 choices) did not differ from chance ($G_P = 2.68$, df = 1), and individual differences were not significant ($G_H = 26.51$, df = 23).

To address the possibility that there may have been a preference for symmetry that was masked by the manipulation of spatial frequency, we examined the choice proportions in two groups of eight bees where symmetry was manipulated within bees and spatial frequency was manipulated between groups. In neither group was there a significant preference for symmetry (high spatial frequency group: mean 6.87 out of 16 choices, or 43% of the symmetric stimuli; $G_P = 2.54$, df = 1; low spatial frequency group: mean 8 out of 16 choices of the symmetric stimuli; $G_P = 0$, df = 1). In the high spatial frequency group, however, individual differences were significant ($G_H = 16.06$, df = 7, $p = .003$). Partitioning the $G$ value into individual contributions revealed a significant value ($G = 4.19$, df = 1, $p = .04$) for one bee that showed a 12:4 choice proportion for the symmetric stimuli.

High vs. low spatial frequency patterns

Given that the comparison between high and low spatial frequency patterns was the second comparison on the same data set, the Bonferroni approach to correcting $\alpha$ level was taken, and a significance level of .05/2 = .025 was adopted. A significant difference in choice proportions among colonies was found ($\chi^2 = 8.38$, df = 2, $p = .016$).

Table 3 shows that Colony 1 had a choice proportion of .63 ((4.75 + 5.375)/16) for high spatial frequency patterns that was significantly higher than chance ($G_P = 9.14$, df = 1, $p = .003$). In contrast, no preference for high spatial frequency patterns was found for the other two colonies (Colony 2: $G_P = 1.13$, df = 1; Colony 3: $G_P = 0.78$, df = 1). For all three colonies, individual differences were non-significant (Colony 1: $G_H = 6.54$, df = 7; Colony 2: $G_H = 9.42$, df = 7; Colony 3: $G_H = 9.17$, df = 7).

Does rewarded experience on black and white disks lead to a preference for symmetry?

Table 4 shows the mean choice frequencies for symmetric and asymmetric patterns for the bees in each of three colonies that had been rewarded on black and white disks prior to testing. Though the preference for symmetric patterns seems stronger for the bees that were trained on the black and white disks for longer, the effect of duration of training was not significant ($\chi^2 = 2.43$, df = 1) and so the data were collapsed across colonies. The choice proportion for symmetric patterns was significantly higher than chance ($G_P = 8.52$, 1 df, $p = .004$). Individual differences were not significant ($G_H = 29.74$, df = 33).

Is only vertical symmetry preferred following prior rewarded experience on the disks?

Table 5 shows the mean choice proportion for the trained bees in Colony 4 that were tested for their preference of symmetric patterns for which the axis of symmetry was either vertical or horizontal. No difference between the two groups of bees was detected ($\chi^2 = 0.21$, df = 1). The preference for horizontal symmetry over asymmetry was just as strong as...
Table 4
Mean choice frequencies out of 16 choices for trained bees in each of 3 colonies. The bees in Colony 2 were rewarded a minimum of four times on the black and four times on the white disks. The bees in the other two colonies were trained for longer, with a minimum of 12 rewarded visits on the black and 12 on the white disks.

<table>
<thead>
<tr>
<th>Colony</th>
<th># Bees</th>
<th>Training duration</th>
<th>Mean choice frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Symmetric</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>Min. 4</td>
<td>8.4</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>Min. 12</td>
<td>9.6</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>Min. 12</td>
<td>9.4</td>
</tr>
<tr>
<td>Weighted mean</td>
<td></td>
<td></td>
<td>9.0 (SE = 0.32)</td>
</tr>
<tr>
<td>Mean proportion</td>
<td></td>
<td></td>
<td>.56</td>
</tr>
</tbody>
</table>

Table 5
Mean choice frequencies for symmetric and asymmetric patterns out of 16 choices for trained bees (minimum of 12 rewarded visits on black and 12 on white disks) in Colony 4. For 10 bees, the axis of symmetry was vertical and for 10 other bees the axis of symmetry was horizontal.

<table>
<thead>
<tr>
<th>Axis of symmetry</th>
<th>Mean choice frequency</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symmetric</td>
<td>Asymmetric</td>
<td></td>
</tr>
<tr>
<td>Vertical</td>
<td>9.4</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Horizontal</td>
<td>9.8</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.6 (SE = 0.41)</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Mean proportion</td>
<td>.60</td>
<td>.40</td>
<td></td>
</tr>
</tbody>
</table>

the preference for vertical symmetry over asymmetry. Collapsing across groups of bees, the preference for symmetry was significant ($G_p = 12.89$, df = 1, $p = .0003$). Individual differences were once again non-significant ($G_{HI} = 16.50$, df = 19).

Discussion

In this study on truly flower-naïve bumblebees, no evidence for any preference for symmetric patterns was obtained. Our sample size, however, was large enough to detect such an effect. The sample size of 40 untrained bees (three groups of eight bees in Colony 1, one in Colony 2 and one in Colony 3) is larger than that in the study by Rodríguez et al. (2004) where only eight bees were used in their Experiment 1 to detect a preference of 72%, though repeated observations on their bees resulted in a sample size of almost 300 choices. In another study using the same maze (Perreault & Plowright, 2009), a significant learned pattern preference of 60% was found using 12 bees given 10 choices. Moreover, in Table 5, a preference of 60% in trained bees was found with only 20 bees.

The significant preference for high over low spatial frequency patterns in one of our colonies underscores the importance of harnessing this variable in studies of symmetry preference. Preferences for both low spatial frequency (Lehrer et al., 1995) and high spatial frequency (Dafni, Lehrer, & Kevan, 1997) have been reported in the literature, but since high and low are relative terms, what we have termed high spatial frequency may not be comparable.

In contrast to the untrained bees, the trained bees showed a choice proportion for symmetric patterns that was significantly higher than chance. Hence training on black and white rewarding disks is not neutral but affects subsequent choice. This conclusion is not a methodological quibble as it speaks to the very mechanism underlying a preference for symmetry. Our results lead us to re-interpret the results and challenge the conclusions of Rodríguez et al. (2004) that flower-naïve bumblebees have an “innate preference” for bilateral symmetry: the bees were not-so-naïve and we have shown that their preferences may well have been learned. The learning is all the more noteworthy since it was in the context of non-differential rather than differential conditioning which is sometimes necessary for subtle discriminations.

We extended the question of whether the preference for vertical symmetry was learned to whether the preference was selective. Given that the black and white disks were symmetrical along every possible axis, we asked whether training would nonetheless lead to a specific preference for vertical symmetry. Our results in Table 5, however, show a preference for horizontal symmetry as well. Though all the patterns were presented vertically at the end of the corridors in the maze, the learning during training did not result in preferences limited to vertical symmetry.

While we have shown that the preference for symmetry is the result of a non-differential conditioning procedure, our results do raise the issue, for further research, of the mechanism underlying the effect. Perhaps during training, it is not so much the rewarded visitation of visual patterns that was at work as the general experience of spending time in the maze or obtaining rewards in the maze. This possibility seems improbable in light of our previous work (Séguin & Plowright, 2008) on the effects of training on subsequent pattern preferences in the same maze. It was only training with specific patterns (grids of squares, grids of circles and grids of diamonds) that influenced subsequent choice of unrewarding radial vs. concentric patterns. Such training was unlikely to just lead to an increase in familiarity with the maze, an increase in motivation or an increase in attention to the ends of the corridors, because training with a ring of circles had no effect. Perhaps the bees extracted both vertical and horizontal symmetry from the disks for association with reward, and this associative learning lead to the subsequent preference for symmetry. Another possibility is more speculative, being suggested...
by research on exposure learning in different species and different procedures (reviewed by Hall, 1980). As suggested by the work of O’Malley, Arnone, and Ziegenfus (1969) on non-differential reinforcement, perhaps the trained bees did not learn that symmetrical flowers were rewarding, but rather learned during training to ignore brightness differences (since both black and white disks were rewarding), and thus were less susceptible to distraction by the blackness and whiteness of the unrewarding test stimuli and more able to attend to symmetry vs. asymmetry. These post-hoc explanations admit of various possible states of pre-experimental knowledge of symmetry that is activated by experience.

Given that the study of preferences for symmetry was undertaken in the context of adaptations to cues as to floral reward, further research should be aimed at exploring the possibility of species differences between B. impatiens and B. terrestris and the flora with which they evolved. Conspicuously lacking from the discussion on the adaptive significance of a preference for symmetry, however, is any guidance as to what possible advantage it could be, to the insects, whether the preference was learned or unlearned. The question “Why learn?” awaits attention in this area though it has begun to receive serious consideration in other areas of bee cognition (Ings, Raine, & Chittka, 2009) and cognition in other animals (Dukas & Ratcliffe, 2009; Shettleworth, 2010).

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Appendix A. How the testing stimuli were created

The testing stimuli were created via MATLAB (themathworks.com, Natick, MA). Matrices of random 8-bit grayscale pixels drawn from a flat distribution from 1 to 256 were generated. The matrices were 828 × 828 bits in size so that the diameter of the stimuli would be 7 cm when printed at 118 pixels/cm. The matrices were then passed through a circularly symmetrical low-pass Butterworth filter of order 5. The resulting images were then reduced to 1 bit according to the average pixel value of the grayscale array. This generated images with only pure black and pure white pixels. All pixels outside of the circular area bounded by the square matrix were set to 0 (black) in order to create circular stimuli. At the bee’s viewing distance of 13.5 cm, the low-pass filter was set to 0.2 cycles/degree and 0.1 cycles/degree for the high frequency and low frequency patterns, respectively, which is above the resolution threshold of visual acuity for bumblebees (Macuda, Geggear, Laverty, & Timney, 2001).

From the stimuli that were generated for each of the four categories, four of each type were selected (arbitrarily numbered patterns 1–4) that had the same total length of boundaries between light and dark pixels, as well as the same total number of white and black pixels within the circular area. The proportion of white to black pixels was held at 50% for both the high frequency and low frequency stimuli. The total perimeter length was 6260 pixels (53 cm) ± 5% and 4000 pixels (34 cm) ± 5% for the high spatial frequency and low spatial frequency stimuli, respectively. To generate the symmetric patterns, the left half of the stimulus was replaced by the mirror reversal of the right half.

References


