SHORT COMMUNICATION

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Symmetry is in the eye of the 'beeholder': innate preference for bilateral symmetry in flower-naïve bumblebees

Abstract Bilateral symmetry has been considered as an indicator of phenotypic and genotypic quality supporting innate preferences for highly symmetric partners. Insect pollinators preferentially visit flowers of a particular symmetry type, thus leading to the suggestion that they have innate preferences for symmetrical flowers or flower models. Here we show that flower-naïve bumblebees (*Bombus terrestris*), with no experience of symmetric or asymmetric patterns and whose visual experience was accurately controlled, have innate preferences for bilateral symmetry. The presence of color cues did not influence the bees' original preference. Our results thus show that bilateral symmetry is innately preferred in the context of food search, a fact that supports the selection of symmetry in flower displays. Furthermore, such innate preferences indicate that the nervous system of naïve animals may be primed to respond to relevant sensory cues in the environment.

Introduction

Bilateral symmetry has been considered as an indicator of phenotypic and genotypic quality, supporting innate preferences for highly symmetric partners (Møller 1990; Møller and Thornhill 1998; Watson and Thornhill 1994).

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A different explanation for symmetry preference posits that symmetrical stimuli are more easily processed by the visual system and that preference for symmetry is a by-product of visual object recognition following specific experiences (Enquist and Arak 1994; Enquist and Johnstone 1997; Jansson et al. 2002). From this viewpoint, perceptual experience produces symmetry preference, which is therefore absent in naïve subjects (Jansson et al. 2002). In deciding between these options, it is therefore critical to control the subjects' prior visual experience.

Different taxonomic groups of pollinators have been found to preferentially visit radially as well as bilaterally symmetric flowers (Neal et al.1998; Giurfa et al. 1999; Møller 2000). It has thus been suggested that some pollinators have innate preferences for symmetric flowers or flower models (Free 1970; Møller 1995; Lehrer et al. 1995; Lehrer 1999), consistent with reports on floral symmetry being a distinctive cue for recognizing phenotypic and genotypic quality in angiosperms (Møller and Eriksson 1994, 1995; Møller 2000). Up until now, experiments reporting innate preferences for symmetry have failed to achieve a sufficiently accurate control of the visual experience of the animals prior to the relevant tests with symmetric and asymmetric stimuli. It is thus difficult to decide whether animals under such circumstances do indeed base their choices on spontaneous preferences for symmetry or whether they simply rely on their previous experience with natural flowers in the field. For instance, experienced honeybees trained to choose bilateral symmetric stimuli transfer their preference to novel symmetric stimuli more frequently than bees trained with asymmetric stimuli do when confronted with novel asymmetric stimuli (Giurfa et al. 1996). Such a predisposition for symmetry may reflect an innate predisposition to learn better about symmetric flowers (Møller and Eriksson 1994, 1995) or may be based on the transfer of past experience with such flowers in the field. The best method for overcoming this problem is thus to study naïve animals in their first encounters with symmetric and asymmetric stimuli.

We investigated whether flower-naïve bumblebees show innate preferences for symmetric or asymmetric patterns when they first encounter them in an appetitive context. To this end, flower-naïve bumblebees were first trained on plain, homogeneous disks and were subsequently tested with vertically presented bilaterally symmetric and asymmetric patterns. The amount of reinforced visual experience before the tests was accurately controlled. 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 (the axis defining bilateral symmetry in the vertical plane), especially because the disk was presented horizontally during training. We also studied the effect of color on symmetry choice. For honeybees, color seems to be more important than pattern (Menzel 1985), such that it could override symmetry, thus minimizing the actual role of symmetry as a natural cue guiding the animals' choice.

Materials and methods

We studied flower-naïve bumblebees (*Bombus terrestris*) during their first encounters with symmetric and asymmetric stimuli under absolute control of their reinforced visual experience. A colony of bumblebees was housed in a flight cage and situated in a glass house without any contact with colored, patterned or scented nectar sources. Bees were individually marked and studied during their first flights. The flight cage was connected to an experimental arena by means of a tunnel that could be closed. Both compartments measured 120×120×100 cm. In the experimental arena we presented a vertical round plate, 50 cm in diameter, displaying a single training stimulus at a time. The plate could be rotated to change the relative positions of the stimulus. During the tests, the training stimulus was hidden and two symmetric and two asymmetric stimuli were presented simultaneously (Fig. 1).
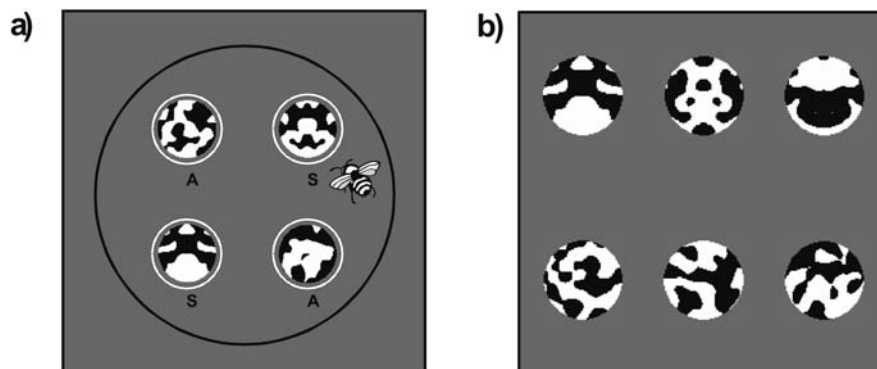
Stimuli

The *training stimulus* was a plain disk, 7 cm in diameter. To the human eye, it was either black or white (first experiment) or black or yellow (second experiment). Colors were printed with a Canon color printer on good quality copy paper. The *test stimuli* were symmetric or asymmetric and were randomly generated by a computer program. They were identical in their statistical properties (e.g. amount of black and white—or yellow—pixels, contour density, contour length, total energy, etc) but differed in the spatial distribution of the black and white (or black and yellow) pixels (Fig. 1). The neutral gray background on which stimuli were presented was provided by a HKS-92 N cardboard (K+E, Stuttgart, Germany). The white paper did not reflect UV light. Chromatic contrast, as measured in the honeybee color space (Backhaus 1991), was reduced in the black-and-white stimuli and important in the black-and-yellow stimuli. Symmetric and asymmetric stimuli varied from bee to bee. A bee entering the arena could reliably resolve their spatial details. Before the experiments, bees may have been exposed to bilaterally symmetrical stimuli, i.e., other bees, the nest structure, etc., but these were never reinforced in an appetitive context of food search, which is the context of our experiments.

Procedure

Training and testing was done with one single individually-marked bee at a time. During *pre-training*, the bee was rewarded with 5 µl of 50% sucrose solution on a plain black or white disk (first experiment) or on a black or yellow disk (second experiment) presented horizontally in the flight cage and successively moved through the tunnel into the experimental arena. Thirty rewards were provided in this way to the bee: 15 with the disk lying flat on the ground and 15 with its position being progressively varied until reaching the vertical on the round plate. Black and white (first experiment) or black and yellow (second experiment) disks were randomly alternated in a balanced way (15:15).

Training began once the bee learned to fly toward the vertical round plate to get a drop of sucrose solution on the plain disk, visible now on the plate. Thirty rewards were provided to the bee (15 on the black disk and 15 on the white—or on the yellow—disk). The alternation between colors was also randomized and balanced during training. At the other three visible positions of the plate, only the grey background was visible. The position of the training stimulus was constantly changed to avoid positional learning.

During the *tests*, two symmetric and two asymmetric stimuli were presented simultaneously. None was rewarded. We recorded

Fig. 1 a Frontal view of the vertical stimulus plate during the test situation: four test stimuli, two symmetric (*S*) and two asymmetric (*A*) were simultaneously presented. None was rewarded. **b** Examples of test stimuli. *Upper row*: symmetric; *lower row*: asymmetric. Stimuli were 7 cm in diameter and were presented vertically, lying flat on a gray background. They were generated by a computer program (Matlab, The MathWorks, Natick, Mass.) by convolving random arrays with circular Gaussians. The outputs were constrained so that the ratio of black to white pixels was 1:1. For symmetric patterns, the starting array was bilaterally symmetrical. In the first experiment, stimuli were black and white as shown; in the second experiment they were black and yellow (not shown)

the choices that ended with the bees touching the stimuli with the antennae or the legs for a period of 2 min. Four tests were performed interspersed with training phases identical to the previous one but with ten instead of 30 rewards on the rewarding stimulus (five on one colored disk and five on the other). From the first to the fourth test, the procedure was kept constant and only the relative positions of the symmetric and asymmetric stimuli were changed.

To avoid pseudoreplication, a single score (percent of choices for bilaterally symmetrical patterns) was derived from each 2-min test for each bee. For each test, the mean %±SE from all bees tested could be obtained. ANOVAs for repeated measures were performed to detect whether performance significantly improved along tests. This analysis allows verifying the lack of effect of the plain disks on the bees' test performance. As blocks of ten training trials with the plain disks were interspersed between the tests, if performance had improved along the four tests, one would have to conclude that the disks themselves provided information about bilateral symmetry. The value of α was set to 0.05 for all analyses.

Results

In the first experiment, we recorded 286 touches from eight bumblebees along the four tests with black and white symmetric and asymmetric patterns. The mean number of touches per foraging bout was 8.9±1.0 ($n=32$). The bees had a significant preference for the symmetric stimuli. This preference remained constant along the four tests (ANOVA for repeated measurements: $F=0.56$, $df=3,21$, $P=0.65$). Therefore, the data for the four tests were pooled. Figure 2 (black bar) shows that the bees clearly preferred the symmetric stimuli (72.14±4.72% choices, $n=32$). The performance significantly differed from a theoretical mean value of 50% (two-sided t test: $t=4.69$, $df=62$, $P<0.0001$). These results therefore show that flower-naïve bumble-

bees, with no experience of symmetric or asymmetric patterns and whose prior visual experience was accurately controlled, have innate preferences for bilateral symmetry.

Innate preference for symmetry may be irrelevant if it is easily overridden by more relevant sensory cues present on flowers such as color. We thus repeated the experiment with a new group of flower-naïve bees using black and yellow disks and patterns. Symmetric and asymmetric patterns had the same high chromatic contrast such that if bees used this information preferentially they should choose randomly between them. In this second experiment, we recorded 299 touches from eight bumblebees along the four tests with black and yellow patterns. The mean number of touches per foraging bout was 9.6±0.9 ($n=32$). As in the first experiment, these values remained constant along the four tests. Bees again preferred the symmetric stimuli. This preference remained constant along the four tests ($F=0.6$, $df=3,21$, $P=0.62$). Thus, the data of the four tests were pooled. Figure 2 (white bar) shows that the bees clearly preferred the symmetric stimuli (74.51±3.50% choices, $n=32$). The performance significantly differed from a theoretical mean value of 50% ($t=7.00$, $df=62$, $P<0.0001$).

Discussion

Our results show that flower-naïve bumblebees have innate preferences for bilateral symmetric stimuli in the context of food search and that such preferences are unaffected by the presence of salient color cues. These results constitute the first clear demonstration of an innate preference for symmetry in insects, because the animals' absolute appetitive experience was carefully controlled before their first foraging choices of bilateral symmetric and asymmetric stimuli. Previous results pointing out a reduction in the visitation rate of natural flowers with damaged corollas, and therefore with an impaired degree of symmetry (Møller 1995), are not conclusive, since petal cutting may imply a change in alternative cues like flower size or chemical signature. As flower-constant pollinators foraging on a particular floral species learn to visit the same kind of untreated flower on the basis of a compound of sensory cues (Menzel 1985; Chittka et al. 1999), changing one or more of these cues will result in a trivial reduction of the visitation rate because the modified flower no longer coincides with the learned one. The same problem is present in experiments performed with bumblebees presented with artificial flowers in which the degree of bilateral symmetry was changed by cutting off parts of the corolla (West and Laverty 1998). Preferences for symmetry exhibited by experienced foragers (as opposed to flower-naïve foragers) in tests with controlled artificial symmetric stimuli (Lehrer et al. 1995; Giurfa et al. 1996) are also inconclusive, because the insects' visual experience in the field prior to the experiment was uncontrolled. Therefore, only studies controlling the type and amount of experience of naïve animals can uncover
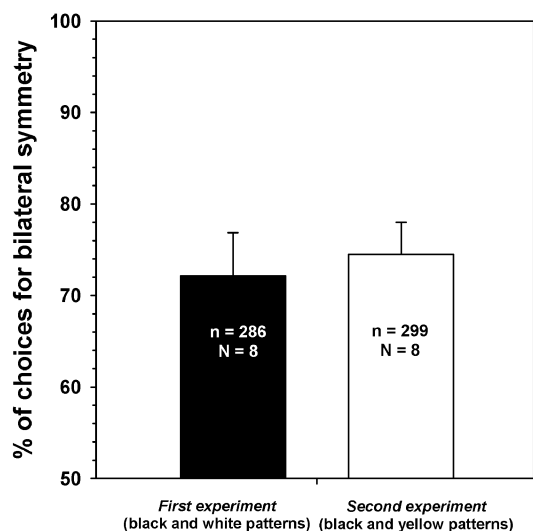
Fig. 2 Percentage of choices for symmetric stimuli (mean+SE) in the first (black and white test patterns: *black bar*) and in the second experiment (black and yellow test patterns: *white bar*). In both cases, the *bar* represents the pooled data of the four tests. Baseline is set at 50% (random choice between symmetric and asymmetric stimuli). *n*: total number of choices recorded; *N*: number of bees tested

innate predispositions if any (Giurfa et al. 1995; Gumbert 2000).

The fact that bees are already prepared to respond to symmetry in their first foraging flights, despite the presence of important orientation cues such as color, underlines the role of floral symmetry as a cue guiding the animals' choice. It is also consistent with findings in humans in which color cues do not affect symmetry preference (Morales and Pashler 1999). Contrary to previous evidence (Giurfa et al. 1996), the preference for bilateral symmetry did not arise after an explicit training session. It could not be viewed, therefore, as a by-product of visual object recognition following specific experiences (Enquist and Arak 1994; Enquist and Johnstone 1997; Jansson et al. 2002). Rather, our results support the hypothesis that considers symmetry as a signal that com-municates the quality of the signal's sender and that may therefore promote innate biases (Møller 1990; Møller and Thornhill 1998; Watson and Thornhill 1994). Perfectly symmetrical flowers might signal a high quality and/or quantity of nectar or pollen to pollinators that, in turn, might exert strong selection pressure on symmetric features (Møller and Eriksson 1994, 1995). Additionally, an innate preference for symmetry indicates that particular sensory processing pathways in the nervous systems of naïve animals are primed to respond to relevant sensory cues in the environment. However, innate preferences in bees can easily be overridden or inhibited by information acquired through individual experience (Giurfa et al. 1995; Gumbert 2000), thus underlining the joint action of pre-wired and plastic behavior in the animals' foraging choice.

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