

Learning of Abstract Concepts and Rules by the Honeybee

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Despite the tiny brain of the honeybee, some remarkable higher cognitive functions have emerged from this assembly of about one million neurons. Work on the honeybee over the past decade is beginning to suggest that insects may not be the simple, reflexive creatures that they were once believed to be. Bees display perceptual and “cognitive” capacities that are surprisingly rich, complex and flexible. This article reviews the recent progress on the honeybee’s ability to learn and use abstract rules and concepts, to categorize visual objects in various ways, and to memorize task-specific information while navigating through their environment. This review is not intended to be exhaustive. Rather, it highlights important advances in our understanding of the processes underlying the bee’s remarkable behaviors.

The worker honeybee possesses a tiny brain that occupies a volume of about one cubic millimeter, weighs about one milligram and contains not more than a million neurons. Nevertheless, this creature, by virtue of its lifestyle, is a spectacularly suitable organism for studying the principles of pattern recognition and navigation, as well as of learning in a more general sense (von Frisch, 1971, 1993; Menzel, 1990). Gathering nectar and pollen is the *raison d’être* of a forager’s existence. Since the species of flowers that are in bloom, say, this week are likely to be replaced by a different species at a different location next week, the bee needs, and has indeed evolved, an impressive ability to learn colors, odors, shapes and routes quickly and accurately (Chittka, Vorobyev, Shmida, & Menzel 1993; Lehrer, Horridge, Zhang, & Gadagkar, 1995; Vorobyev & Menzel, 1999; Wehner, 1981). A bee can learn a new odor in just a few visits, a new color in about half an hour (after it has made about five visits to collect a food reward), a new pattern in about half a day (after 20-30 rewarded visits), and a new route to a food source in about 3-4 visits (provided the route is not through a complex labyrinth). The underlying navigational skills that make this learning possible require extensive, perceptual, information processing, and storage mechanisms which, in turn, allow bees to display perceptual and “cognitive” abilities that are surprisingly rich, complex and flexible (Collett & Collett, 2002; Giurfa, 2003; Menzel & Müller, 1996; Srinivasan & Zhang, 1998, Zhang & Srinivasan, 2004a, 2004b).

The present article reviews recent studies that have challenged the perceptual capacities of honeybees by asking if bees can, for instance, learn abstract rules by which to navigate mazes, learn and generalize abstract concepts, categorize complex visual objects, or acquire and memorize task-specific information when faced with a complex problem. This review discusses some key examples of recent

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advances made toward answering these questions.

Learning Rules to Negotiate Complex Mazes

The ability to learn mazes has been investigated extensively in a number of vertebrates, notably rats, mice and pigeons (Dale, 1988; Pick & Yanai, 1983). Comparatively few studies, however, have explored the ability of invertebrates to do so.

Zhang, Bartsch, and Srinivasan (1996) explored this question by training bees to fly through a variety of complex mazes in search of a sugar solution reward in the presence or absence of a number of specific visual cues. Each maze consisted of a 4x5 matrix of identical cubic boxes with a hole in the center of each side wall. A path through the maze was created by leaving some holes between boxes open and blocking others. Bees had to fly through a sequence of these boxes to reach the reward. We changed the position and orientation of the maze frequently to prevent the bees from using external landmarks as navigational cues and, before each test, we swapped the boxes to eliminate the effects of any olfactory cues.

Learning to Negotiate a Maze by Following a Color Cue

One series of experiments investigated the ability of bees to find their way through such a maze by learning to follow a color mark—a 4x4 cm green square—affixed immediately below the correct exit hole in each box (Figure 1a). The bees were trained to take the correct path over a series of trials in which the feeder was moved progressively farther along the path until it reached the third box. Over these trials the bees had the opportunity to learn that the green square indicated the correct exit hole. After the bees had reached this stage, we moved the feeder to the last box until each bee had visited it once, and then to its final location in the feeder cage behind the last box.

Each bee's performance was tested immediately thereafter. Their performance was scored by assigning each flight to one of four categories, C₁- C₄. These included, respectively, flights in which the bee flew to the goal without making a mistake, flights in which the bee turned back and retraced her path one or more times but always remained on the correct path, flights in which the bee made one or more wrong turns at the decision boxes but still arrived at the goal within five minutes, and unsuccessful flights in which the bee did not reach the goal within five minutes. In some tests, we evaluated a bee's performance by measuring the time it took her to reach the goal.

Our results demonstrated that the bees initially trained to follow color marks through only a small, initial part of the maze were immediately able to follow the same cue to find their way through the rest of the maze (Test 1 in Figure 1b). Further, their performance on 'sign tracking' continued to be good when we tested them in a rearranged maze using the same color cues (Test 2 in Figure 1b). That is,

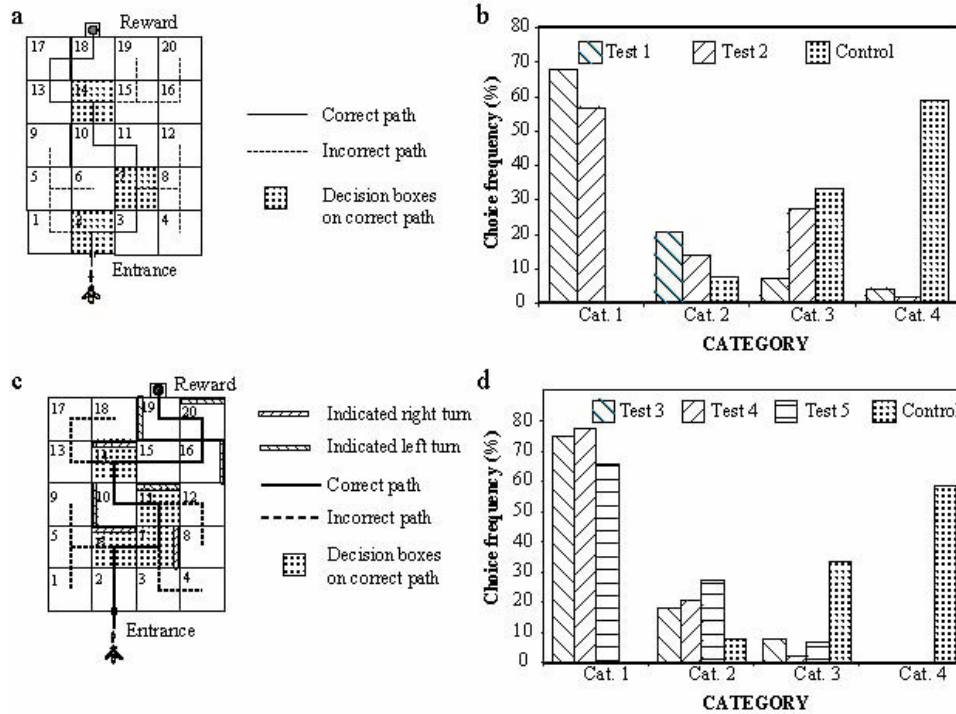


Figure 1. Learning to negotiate complex mazes by using visual cues. Details are given in the text. (Adapted from Zhang and Srinivasan, 2004b).

the trained bees were able to generalize the sign tracking rule that they had learned and used it to fly successfully through a novel maze. Their performance in both tests 1 and 2 was significantly better than a control group of similarly trained bees that were tested in an unmarked maze with an unfamiliar route to the goal.

Learning to Negotiate a Maze by Using a Symbolic Cue

In this series of maze experiments we also asked whether our bees could learn to negotiate a maze by using a symbolic cue (Zhang et al., 1996). Here, whether to make a left or a right turn in any given box was indicated by a color cue on the back wall of the box in which the turn had to be made (Figure 1c). Our bees also learned this task well. Again, bees trained to use this symbolic cue on one route were immediately able to use it to follow a novel path though another maze (Test 4 and 5, Figure 1d). The performances in all tests (Test 4, 5, and 6) were as impressive as those in the previous experiments, and significantly better than our control group (Figure 1d).

Again, in this second series of experiments, the bees had to learn a sequence of rules. The difference was that now the color cue provided route information in a less direct way than in the first series of experiments; it signaled the direction in which to turn rather than the correct exit. In a third series of experiments using similar mazes and in which no cues were given, the bees could not learn the route on their own. We had to teach them the entire route by training them in a stepwise manner how to get through each box.

Together, these studies demonstrate that bees can, in fact, learn a complex task if they are taught to do it in a series of simple steps. However, this does not necessarily require leading the bee step-by-step through each successive box in the maze (although that would certainly be a feasible procedure). Training is most rapid if the bee learns the maze by acquiring a set of “rules” that it can follow. During training for the first series of experiments, the bees learned first that the color cue signaled the presence of food, and then that the color cue indicated points along the path that led to the food. Behaving as if they knew and used such rules, the trained bees could successfully fly through novel mazes. Sign-tracking behavior of this kind has previously been reported in pigeons and rats (Hearst & Jenkins, 1974). In experiments not reported here, we have also shown that bees trained to use one color to negotiate a maze can immediately use a novel color to negotiate the same maze and bees trained to use one color in a particular maze can use a novel color to negotiate a novel maze. Consequently, it seems that bees can apply a learned rule to novel but analogous contingencies in the spatial and the chromatic domains.

Learning to negotiate a maze by a symbolic cue can be considered a special case of symbolic matching-to-sample, involving the choice of a sensorimotor-response according to the sample. Chittka and colleagues have trained bumblebees on a similar task, and demonstrated that they can learn such a sensorimotor matching to sample task as well (Chittka, 1998; Chittka & Thomson, 1997). After these maze experiments, we initiated a series of experiments to specifically investigate the use of a symbolic matching rule by honeybees, which we will describe later in this chapter.

Visual Stimuli as Navigational “Signposts” for Walking Honeybees

Zhang, Lehrer, and Srinivasan (1998a) investigated whether bees are capable of using visual stimuli as signposts along a route if they are walking rather than flying. In these experiments, bees flew from their hive to the entrance of a small Y-maze (Figure 2a). Bees entered the maze through a 20 cm long, transparent tunnel that was narrow enough to prevent them from flying and discourage them from turning around and leaving the maze through the entrance. Yellow and white, blue and white, or black and white vertical gratings lined the walls of the tunnel up to the choice point and indicated whether the bee was to continue on through the right or the left arm of the maze to reach the food reward (Figure 2a).

We alternated the yellow and blue gratings on the right wall of the entrance tunnel every 10 min over the course of training and, to encourage the bees to pay particular attention to the grating color on the right wall, the black and white grating was always on the left wall. The reward was in the left arm of the maze on trials when the blue grating was in the tunnel and in the right arm when the yellow grating was used.

Our bees easily learned that the blue and yellow gratings indicated left and right turns, respectively (Figure 2b, c). In a control test using black and white gratings on both sides of the entrance tunnel, the bees chose the two arms randomly indicating that they were not being influenced by extraneous cues (Figure 2d).

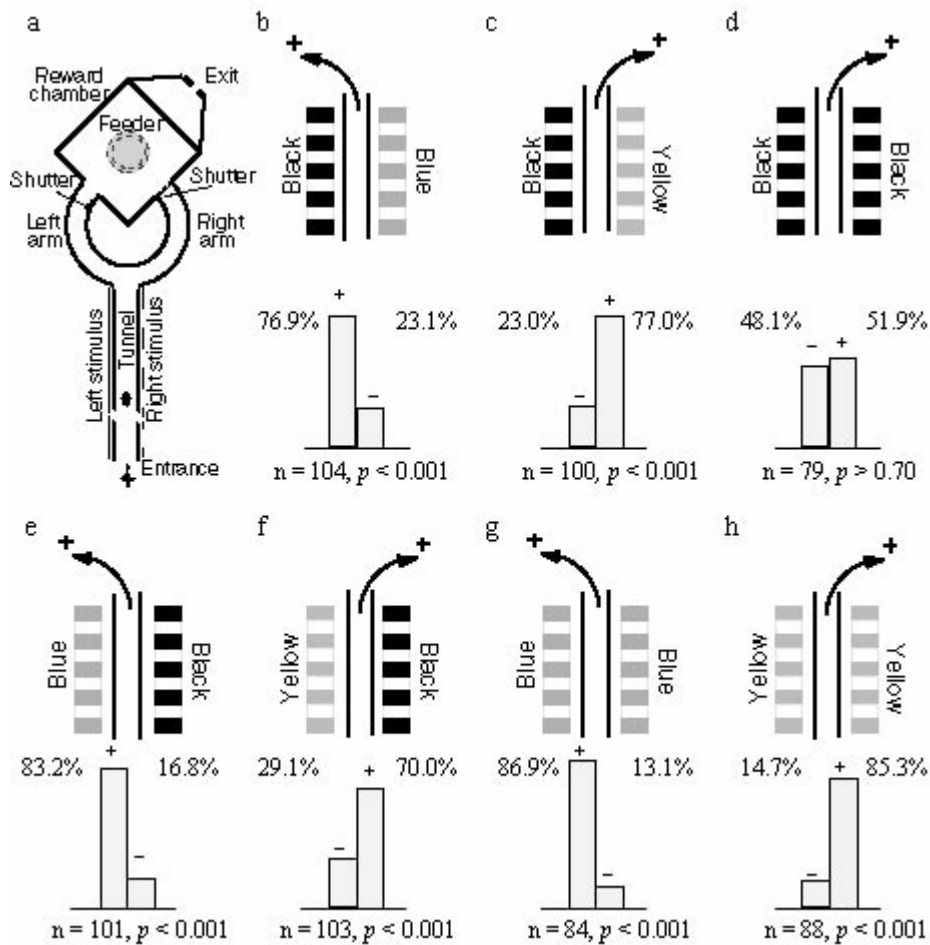


Figure 2. Visual stimuli as navigational ‘Signposts’ indicating route for walking honeybees. Y-maze apparatus used for eye-specific route training of walking honeybees. Details in text. (a) In the learning tests, the trained bees significantly prefer to choose the left arm ($n = 104, p < 0.001$) when a blue grating was presented to the right eye (b), but the right arm ($n = 100, p < 0.001$) when a yellow grating was presented to the same eye (c). The arrow labeled “+” denotes the “correct” arm. (d) shows the results of a test in which both walls of the tunnel were black and white gratings to control for the influence of extraneous cues ($n = 79, p > 0.70$). In the transfer tests, the blue grating on the left wall made the bees turn left ($n = 101, p < 0.001$) (e), whereas the yellow grating on the left wall made them turn right ($n = 103, p < 0.001$) (f). The preference in turning direction was even stronger when blue gratings were presented on both eyes ($n = 84, p < 0.001$) (g), and yellow gratings were presented on both eyes ($n = 88, p < 0.001$) (h). (Modified from Zhang et al. 1998a).

Then we tested the trained bees in transfer tests under novel conditions in which each of the colored gratings was presented on the left side of the tunnel and the black and white grating was presented on the right. Again, the bees held fast to the rule that they had learned (Figure 2e, f). Further, when the blue grating was presented on both sides of the entrance tunnel, the bees exhibited a strong tendency to turn left; when yellow was on both sides of the tunnel, they showed a strong tendency to turn right (Figure 2g, h).

Olfactory Stimuli as Navigational “Signposts”

Honeybees have a well-developed olfactory system and workers can be trained easily to visit dishes containing specific odors (von Frisch, 1993). In an extension of these findings, Zhang and colleagues carried out an experiment in which they examined whether honeybees could use scent as a symbolic navigational cue. Their maze consisted of a series of interconnected perspex covered cylindrical chambers, 25 cm in height and diameter, leading to a feeder (Figure 3a).

Essence of either mango or lemon was presented at the entrance to cylinder A, each in a different perforated plastic vial. Two small fans on the cylinder wall provided a gentle stream of scented air that greeted the bees as they entered. After entering cylinder A, bees proceeded into cylinder B via an aperture behind which stood a baffle that prevented them from seeing the interior of B until they entered it. Cylinder B had an exit on both its right and left sides each of which led to another cylinder, one of which contained a sugar-solution feeder that could not be seen until the bee entered the cylinder. After collecting its reward, each bee was released.

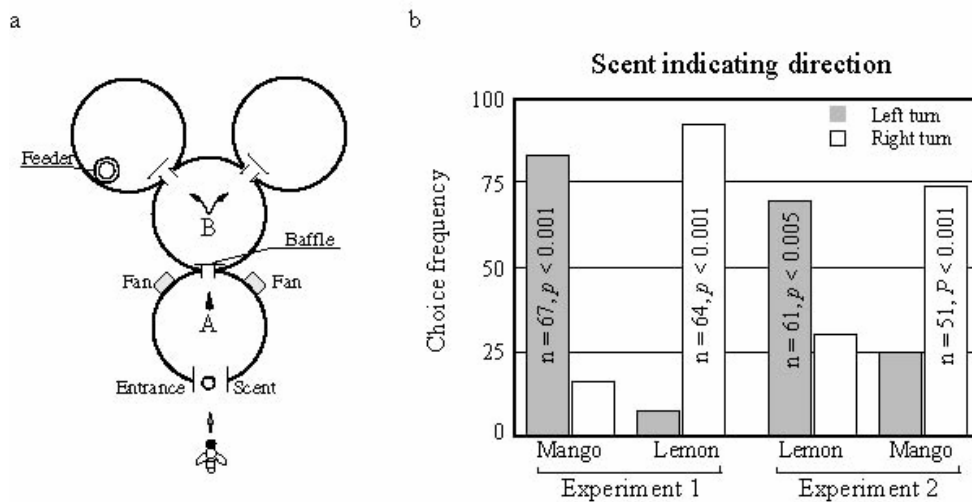


Figure 3. Scent stimuli as indicators signaling route. The apparatus consisted of a series of interconnected cylinders (A & B) leading to a feeder in one of the cylinders at the last stage. The scent stimulus was presented at the entrance of cylinder A, and bees chose one of exits at cylinder B (a). The results of the first experiment showed that the choice frequency in favor of the left exit was 83.7% ($n = 67$, $p < 0.001$) with mango, and 92.5% ($n = 64$, $p < 0.001$) with lemon at the entrance to cylinder A. The results of the second experiment showed that the choice frequency in favor to the left exit was 69.9% ($n = 61$, $p < 0.005$) with lemon, and 74.8% ($n = 51$, $p < 0.001$) with mango at the entrance to cylinder A (b).

In the experiments, groups of bees had to learn to choose the right or left exit to get the food reward depending upon which odor they encountered at the maze entrance, and choice-odor associations were changed between experiments. Once again, the bees performed very well, clearly demonstrating that an odor can be used as a cue to signal the correct route through a maze.

Learning the Principles of “Symbolic Matching”

One of the more complex tasks that has been used to investigate certain principles of learning and memory is the so-called “Delayed Match-to-Sample Task” (DMTS). This task has been put to monkeys (e.g., D’Amato, Salmon, & Colombo, 1985), dolphins (e.g., Herman & Gordon, 1974) and pigeons (e.g., Roberts, 1972) among others, using the same basic procedure. Each trial begins with the presentation of a sample stimulus. The sample is followed by a delay or retention interval and then by the presentation of two or more comparison stimuli, one of which is identical to the sample stimulus. The animal obtains a reward if it chooses the test stimulus that matches the sample (hence, the name “delayed match-to-sample”). Sometimes, a decision is required to be made immediately following the sample and without a delay, i.e., a match-to sample (MTS). Most experiments use two or three sample stimuli, which are varied randomly from trial to trial. In a more complex variant called a “Symbolic Delayed Match-to-Sample” task (SDMTS), none of the comparison stimuli physically matches the sample: the experimenter arbitrarily designates the correct choice. Here, the animal has to learn to associate the correct test stimulus with each sample stimulus. We wondered if bees could learn such tasks.

Collett and Wehner have suggested that foraging insects that repeatedly travel between a food source and their home navigate by using a series of visual images (or “snapshots”) of the environment that they have acquired en route (Collett, 1996; Collett, Fry, & Wehner, 1993; Collett & Kelber, 1988, Judd & Collett, 1998; Wehner, Bleuler, & Shah, 1990; Wehner, Michel, & Antonsen, 1996). They suggest that by comparing a currently viewed scene with the appropriate stored image, the insect can determine whether or not it is on the correct path, and make any necessary corrections.

Consequently, successful foraging may, in fact, require bees to be able to solve navigational problems that are analogous to SDMTS tasks. Researchers at the Australian National University (ANU) have carried out several projects to investigate whether bees can use such delayed symbolic matching to navigate on their foraging trips.

Navigation by Associative Grouping and Recall of Visual Stimuli

The ANU team (Zhang, Lehrer, & Srinivasan, 1998b; Zhang, Lehrer, & Srinivasan, 1999) investigated the honeybee’s ability to learn a visually based SDMTS task. The bees were trained to fly through a compound Y maze built from a group of interconnected cylinders the first of which held the sample stimulus (Figure 4a). The second and third cylinders each had two exits—each marked with a different visual stimulus—between which the bees had to choose. A bee, having made a correct choice in the second and third cylinder, would arrive in the fourth cylinder which held a feeder filled with sugar solution. Consequently, the second and the third cylinders were decision points in which the foraging bee had to decide which stimulus signaled the correct exit. The correct choice was cued by the single sample stimulus that the bee saw in the first cylinder.

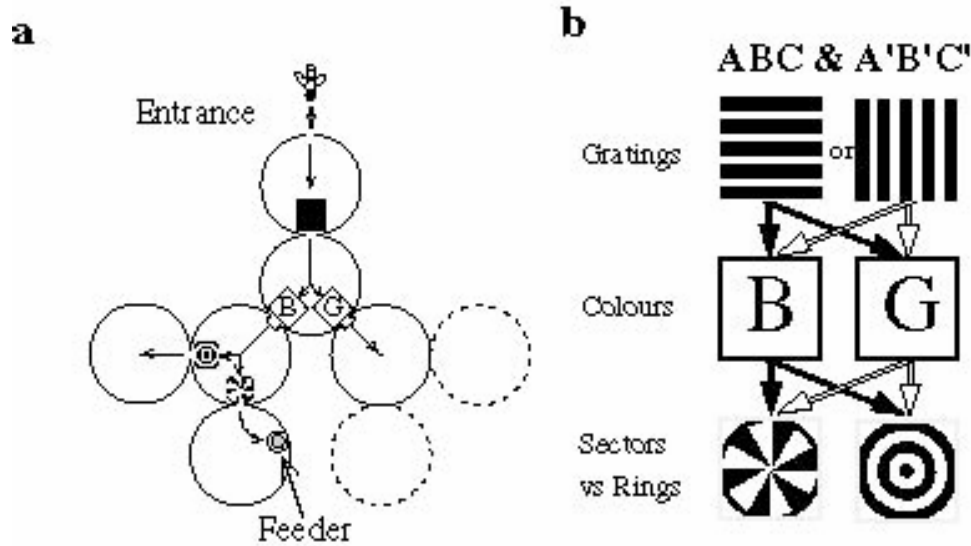


Figure 4. The apparatus and visual stimuli for the DMTS task in the visual modality. The maze apparatus (a) and visual stimuli (b). Details are given in the text. (Modified from Zhang et al., 1999).

During training, the sample stimulus was a black-and-white grating oriented either horizontally or vertically (Stimulus A or Stimulus A', respectively). The second cylinder (first decision stage) offered a choice between a blue or green square (Stimulus B and B', respectively), and the third cylinder a choice between a sectored disc or concentric rings pattern (C and C', respectively) (Figure 4b). The horizontal grating indicated that the feeder could only be reached by choosing blue in the second cylinder and the sectored disc in the third; the vertical grating meant that the correct choice was green in the second cylinder and the ring pattern in the third.

After training, the bees were tested not only on the training sequences ABC and A'B'C' (learning tests) (Figure 5a), but also in transfer tests which represented five other permutations of the training sequences. The results of tests on all of the sequences are illustrated in Figure 5a-f.

The results showed that bees are indeed capable of learning SDMTS tasks. Viewing the sample stimulus apparently triggered a recall of the stimuli that should be chosen in each of the subsequent stages along the correct foraging route (Figure 5a). Furthermore, trained bees continued to choose the correct stimuli at each stage of the maze even in transfer tests that used other permutations of the stimulus sequence (Figure 5 b-f). These findings indicate that, in general, exposure to any one of the stimuli that were encountered in the training (A, B, C, A', B', C') was sufficient to trigger an associative recall of all of the other stimuli belonging to that set. In all of the tests, changing the sample stimulus (from A to A', B to B' or C to C') caused the bees to change (and reverse) their preference for the stimuli encountered at subsequent points in the maze (Figure 5b, d & f). It should be noted that in this experiment the bees were not trained specifically to distinguish between the sample stimuli A and A', which were the sample stimuli alternately presented in the first cylinder during training. Nevertheless, they distinguished between them in

the transfer tests because they associated the two stimuli with the stimulus sets ABC and A'B'C', respectively. It is also clear from these experiments that the bees were capable of treating the stimulus pairs B, B' (Figure 5c & d), and C, C' (Figure 5e & f) as sample stimuli even though these were never encountered as sample stimuli in the training phase.

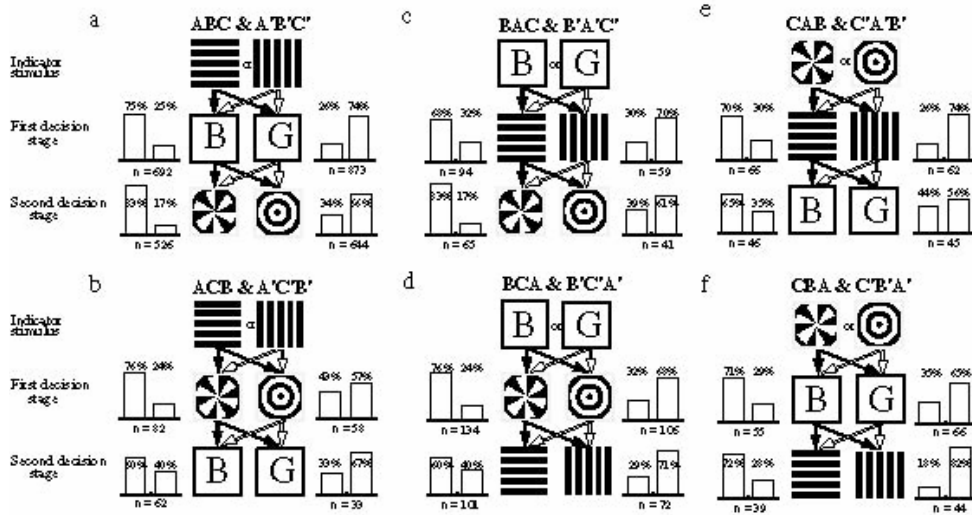


Figure 5. Performance in the learning and transfer tests. Choice behavior of bees, trained simultaneously on sequences ABC and A'B'C', in subsequent tests where they encounter training sequences (a) or altered sequences (b-f). In each panel, the top set of histograms shows the percentage of choices in favor of each of the two stimuli in the first decision stage, and the bottom set the percentage of choices in favor of each of the two stimuli in the second decision stage. The left column of histograms shows the percentage of choices in favor of the two stimuli in each stage, when the sample at the first cylinder is the left one. The right column of histograms shows the percentage of choices in favor of the two stimuli in each stage, when the sample at the first cylinder is the right one (a-f). Other details are given in the text. (Modified from Zhang et al., 1999).

These data suggest that bees solved the SDMTS task by mapping the six visual stimuli that they encountered during the training phase into two distinct sets, (A, B, C) and (A', B', C'), as illustrated in Figure 6. After training, exposure to any member of one of these sets triggered a recall of the other two members of the set.

The Colletts found that ants and bees attach to each landmark a local vector that spans the distance to the next landmark along the route (Collett & Collett, 2002) and suggested that landmarks act primarily as signposts that tell insects what particular action they need to perform, rather than telling them where they are. Kohler and Wehner suggested that landmark memories could be linked in such a way that the matching of one memorized snapshot activated the next—and the steering commands leading to it (Kohler & Wehner, 2005).

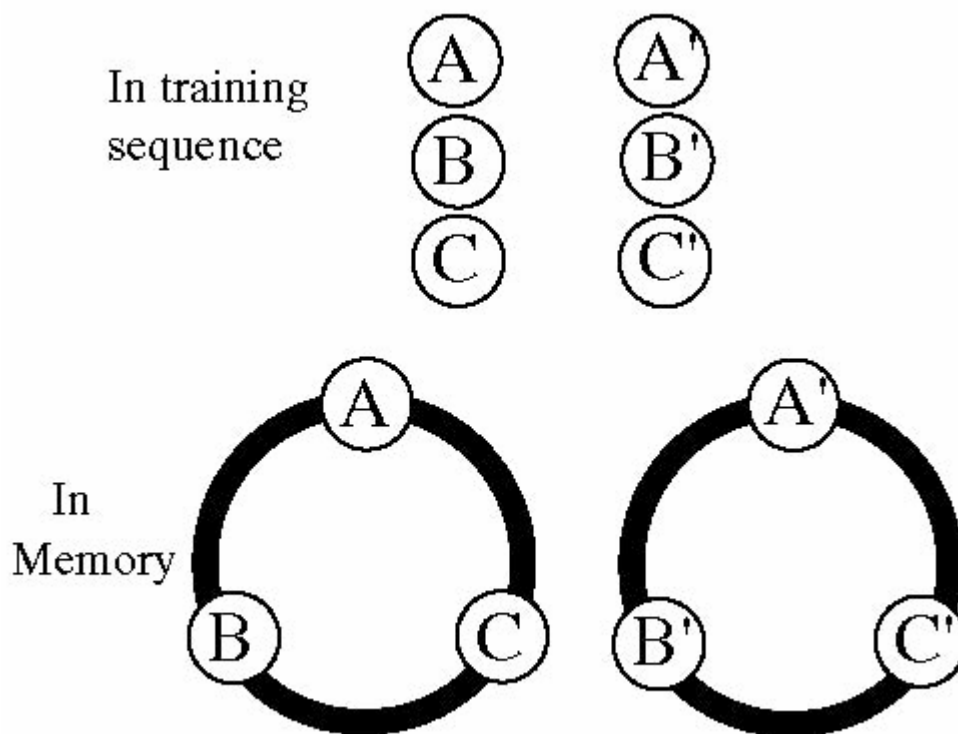


Figure 6. A model for associative grouping, derived from the results of the Symbolic-Delayed-Match-to-Sample experiments. Details are given in the text. (Adapted from Zhang & Srinivasan, 2004b).

Learning a SDMTS Task across Sensory Modalities

The previous results led us to ask if bees can learn a SDMTS task when they were required to make associations across sensory modalities. Clearly, humans display impressive cross-modal associative recall. It is a common experience, for instance, that a smell or a sound can trigger a vivid recollection of an associated long-past event even if it involves a different sensory modality (Baddeley, 1993).

The ANU team (Srinivasan, Zhang, & Zhu, 1998) explored this capacity in bees by asking whether bees could learn to associate specific scents with specific colors. Again, they used a compound Y-maze but this time with just a single decision chamber. The sample stimulus, presented in the first cylinder, was either lemon or mango scent. The decision involved choosing between a blue or yellow visual stimulus. Lemon at the entrance indicated that blue was the correct choice, mango indicated that yellow was correct (Figure 7a). After training, the bees were able to make the correct color choices in 76.6-79% of the tests (yellow correct, $n = 81$, 3 tests, and blue correct, $n = 80$, 3 tests, respectively). In both cases, the results were highly significant ($p < 0.001$). In a complementary experiment, a fresh group of bees was trained to learn the opposite task (i.e., associate mango with blue and lemon with yellow). These bees performed equally well. After training, they made the correct choices 80.6% and 88.7% of the time (blue correct, $n = 87$, 3 tests, and yellow correct, $n = 76$, 3 tests, respectively; in both cases, $p < 0.001$; Figure 7b).

The authors also trained bees to make symbolic matches in the opposite direction, that is, associate a color in the first cylinder with a scent at the decision point (Figure 7c). The bees were equally successful and the results were fundamentally the same as in the first set of experiments (Figure 7d).

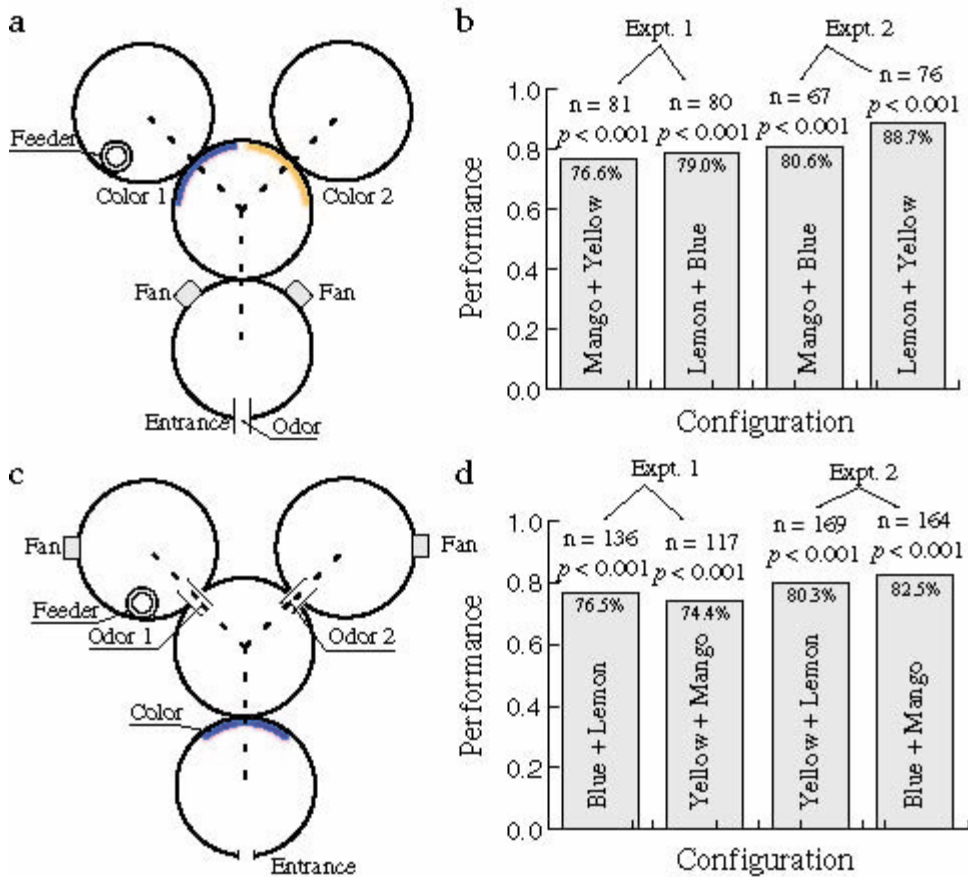


Figure 7. Learning a SDMTS task across the sensory modalities. Details are given in the text. (Modified from Srinivasan et al., 1998).

The evidence presented here clearly shows that honeybees are able to learn Symbolic-Delayed-Matching-To-Sample tasks across sensory modalities. Learning an SDMTS task requires that the bee be able, when presented with a sample stimulus, to recall other stimuli that are associated with the sample stimulus. For a foraging honeybee, cross-modal associative recall could facilitate the search for a food source. For example, detecting the scent of lavender could initiate a search for purple flowers.

Scents Triggering the Recall of Previously Associated Locations and Visual Patterns

Naturally, the next question to be answered was whether honeybees can use the principle of symbolic matching and association across modalities while forag-

ing in a natural environment. It is well known that honeybees are able to navigate rapidly and accurately to food sources that are often kilometers away. This is achieved by using visual cues, such as the location and color of nectar-bearing flowers (von Frisch, 1993; Gould, 1993; Wehner, 1981), and chemical cues such as the scent and the taste of the nectar (Frisch, 1993; Gould, 1993). The ANU team (Reinhard, Srinivasan, Guez, & Zhang, 2004; Reinhard, Srinivasan, & Zhang, 2004) investigated whether learned olfactory cues can elicit visual and navigational memories that may assist honeybees in navigating back to a known food source.

In one of the experiments (Experiment A), individually marked bees were trained to alternately visit one of two sugar feeders each of which was positioned at a different location and carried either a rose or lemon scent (scents 1 and 2, respectively; Figure 8a). After two days of training, the feeders were replaced by empty, unscented jars. Then, in turn, scent 1 and scent 2 were blown into the bees' hive for 8 min each using a small fan attached to the hive entrance. During each scent presentation, we counted the number of marked bees that arrived at each test feeder. We also noted which feeder was visited first, the number of landings made on each feeder, the number of times that bees circled the feeder, and the total number of visits (circles plus landings) made to each feeder.

The trained bees that emerged from the hive in response to a scent showed a significant preference for the location that had carried the scent during their training (Figure 8c, d). The results were very similar when we repeated the experiment with rose and almond or lemon and almond scents (Reinhard et al., 2004a, 2004b), suggesting that the scents blown into the bees' hive elicited memories (perhaps visual) of a specific location to which they had been trained.

In a second experiment (Experiment B), Reinhard et al. (2004a) investigated whether an individual bee could learn to associate a particular scent with a specific color. In this case, the training feeders were wrapped with different colored pieces of cardboard, and marked bees were trained alternately to a yellow, rose-scented feeder, and a blue, lemon-scented feeder, swapping every 20-30 min. During training, the feeders were positioned on the perimeter of a circular area 10 meters in diameter that was located about 50 meters from the hive (Figure 8b). The positions of the training feeders were varied randomly along the perimeter of the circle to ensure that the bees learned to associate the scent with a color rather than the feeder's location. During the subsequent tests, two empty, unscented, colored test feeders were placed opposite each other at a randomly chosen location on the circle's perimeter (Figure 8b). Rose and lemon scents were then blown into the bees' hive and we counted the bees' visits to the two feeders as before.

Once again, the bees mastered the task and learned to associate the scents and their matched colors. So, when feeder position is randomized during training as in this experiment, the bees learned that a scent was associated with a specific colored feeder regardless of its location (Figure 8e, f).

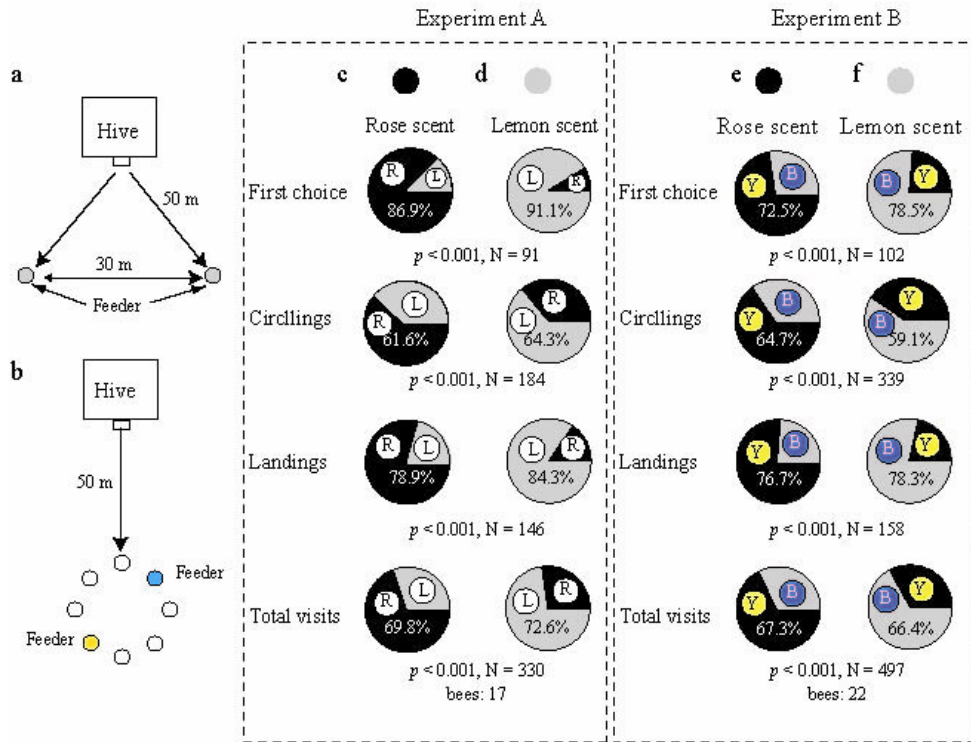


Figure 8. Olfactory cues triggering visual and navigational memories. Experimental configuration, showing feeder locations relative to the hive for experiment A (a), and for experiment B (b). Column c & d show results of Experiment A and e & f show results of Experiment B: Pie charts show the distribution of visits to the two test feeders in Experiments A and B, when the respective scents were blown into the hive (dark: former lemon feeder and light grey: former rose feeder). The experimental data are accumulated from 4 tests for each scent, and are shown separately for first visits, circlings, landings and total visits. The number of individually marked bees that visited both test feeders, the number of choices (N) and P -values for tests of statistical significance between the two feeders when the respective scents were blown (χ^2 test) are shown below the pie charts. Details are given in the text. (Modified from Reinhard et al., 2004b).

Categorization of Visual Objects by Honeybees

Categorization can be thought of as an information processing strategy in which objects or events are grouped together in order that a similar response or responses can be made to all members of the group or “class” (Keller & Schoenfeld, 1950; Troje, 1999). This involves both generalization within and discrimination between classes, and has been convincingly demonstrated in vertebrates. Categorization is the basis for any identification and classification task, and, accordingly, has enormous biological relevance. It would be especially interesting to know the extent to which invertebrates are able to employ this ability in spite of their miniature central nervous systems (see Prete, 2004, for some examples).

Honeybees can easily learn object orientation as an independent parameter. If trained to discriminate vertical from horizontal stripes with one set of patterns, they can transfer what they have learned to novel patterns differing in template and sharing the features vertical vs. horizontal (van Hateren, Srinivasan, & Wait, 1990;

Srinivasan, Zhang, & Witney, 1994; Zhang & Srinivasan, 1994). Apparently, the bees make the discrimination based on geometric cues intrinsic to the pattern and not on cues derived from the apparent motion of the pattern as it is perceived while the bees are flying. Honeybees can also learn to discriminate visual stimuli based on their radial or circular symmetry and they can transfer what they have learned to novel patterns (Horridge & Zhang, 1995). Similarly, they can learn to discriminate between stimuli that are bilaterally symmetrical versus asymmetrical and then transfer what they have learned to novel stimuli (Giurfa, Eichmann, & Menzel 1996; Horridge, 1996). Bumblebees, too, can learn to associate a color with a reward and can form simple categories based on floral colors which enhance their ability to discriminate between rewarding and non rewarding flowers (Dukas & Waser, 1994). These findings for bees mirror work demonstrating that monkeys and other primates are able to categorize complex visual images, such as photographs of human faces, trees and other animals, and that pigeons have the capacity to group objects into different categories, such as people, other pigeons, trees, water, landscapes, and so on (e.g., Freedman, Riesenhuber, Poggio, & Miller, 2000; Huber, Troje, Loidolt, Aust, & Grass, 2000; Martin-Malivel & Fagot, 2001; Roitblat, 1987; Vogels, 1999).

Zhang, Srinivasan, Zhu, and Wong (2004) investigated whether bees can categorize or group similar, natural, visual images together. Bees were trained to distinguish between different types of naturally occurring scenes, and to group them into four distinct categories: Category F (uppercase) consisted of images of flowers that were star-shaped and of different colors; Category f (lowercase) comprised images of flowers that were nearly circular in shape and different colors; Category P consisted of images of plant stems, of various shapes; and, Category L was composed of images of landscapes. Within each category, individual images differed in details of shape, texture, and, sometimes, color. The bees were trained in a multiple-choice maze in which they saw a picture as a sample stimulus in the entrance chamber (C1). To continue through the maze, they had to fly through a small opening in the middle of the picture to enter chamber two (C2). The back wall of C2 was transparent with a 3 cm (dia) hole in its centre. The small aperture restricted the bees' flight speed, and the transparent wall allowed them to see four additional pictures (or comparison stimuli) on the rear wall of chamber three (C3). During training, the sample stimuli and the four comparison stimuli were all from Group 1. (Groups 1-4 each consisted of one unique example of each of the four categories; Figure 9c). If the bee chose the correct test stimulus in C3, she would be able to receive a reward of sugar solution from a feeder that was placed in the reward box, R, behind that stimulus, by landing on and crawling through a tube in the centre of the stimulus (Figure 9c).

In transfer tests, Zhang et al. (2004) examined whether the trained bees could match a sample stimulus from one group with a stimulus of the same category from a different group. In these tests, the sample was always a stimulus from Group 1, but the comparison stimuli were from Group 2, 3 or 4 (Figure 9c). The bees performed very well in these transfer tests. In fact, in each case, the bees showed a clear and significant preference for the test stimulus that belonged to the same category as the sample (Figure 10a, b). Particularly noteworthy is the transfer test using Group 4, in which the comparison stimuli were entirely novel (Group 4,

Figure 10c). These stimuli had never been used in the training phase, or in the learning tests or transfer tests. Again, the bees performed very well at picking the test stimulus that was in the same category as the sample (Figure 10c).

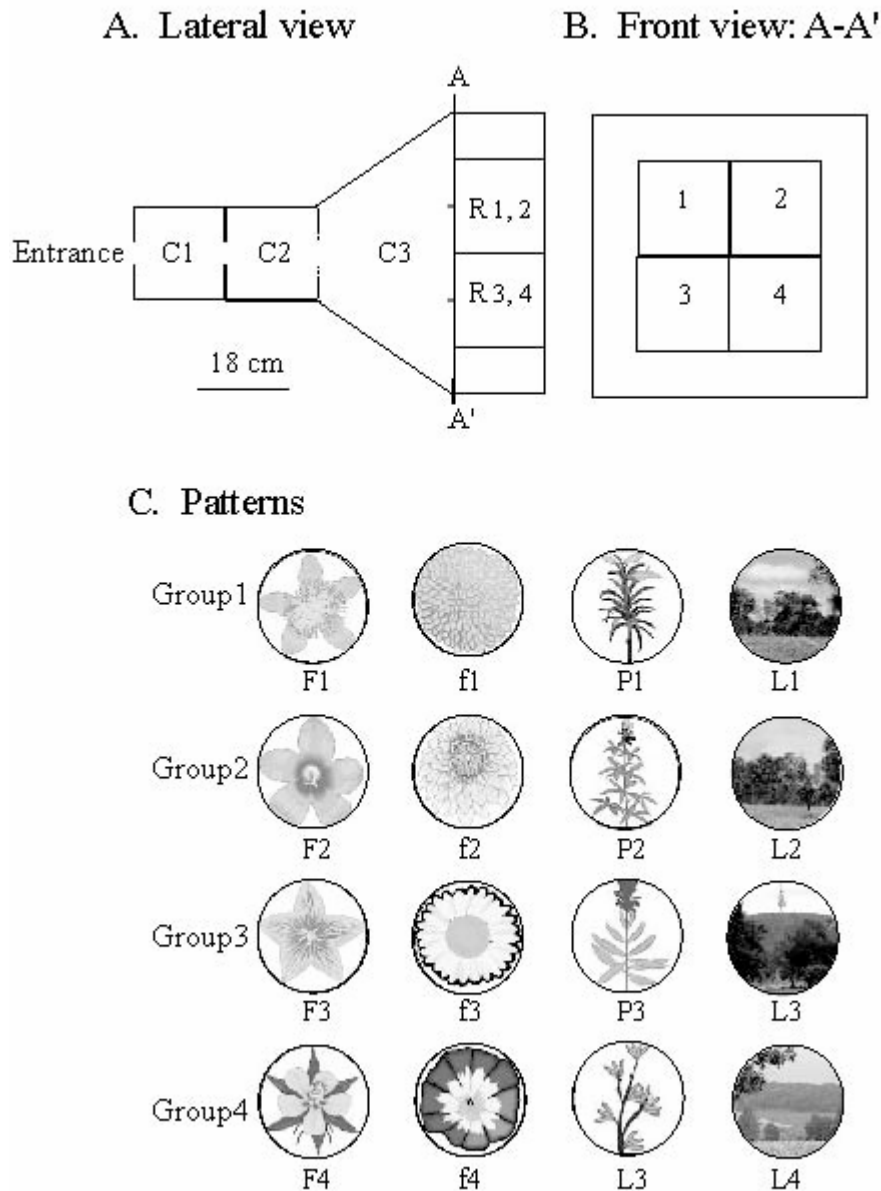


Figure 9. Illustration of experiments showing the multiple-choice maze apparatus (A, B) and the four groups of stimuli that were used. (C). Details are given in the text. (Adapted from Zhang et al., 2004).

The results of the transfer tests with novel stimuli (Figure 10c) show that the bees performed very well at picking the novel test stimulus that was in the same category as the sample. The honeybees exhibited the same response to novel stimuli that differed greatly in their individual, low-level features. That is, bees treat

these highly variable stimuli as equivalent. Our findings suggest that the honeybee possesses an ability to group similar visual stimuli into categories.

Learning the Abstract Concepts of “Sameness” and “Difference”

A natural extension of the previous experiments was to examine whether honeybees can group stimuli according to certain rules, or concepts, such as “sameness” or “difference.” In vertebrates, the capacity to acquire sameness-difference concepts has been studied using two experimental procedures, the delayed matching-to-sample task (DMTS) and the delayed non-matching-to-sample task (DNMTS) (Holmes, 1979; Wright, Cook, & Rivera, 1988; Zentall & Hogan, 1978). In the DMTS task, an animal is presented with a sample and then, after a brief delay, with two or more secondary stimuli, one of which is identical to the sample. The task is to choose the matching stimulus. In the delayed non-matching-to-sample variant, the animal is required to choose the second stimulus that is different from the sample. In both cases, broadly construed sameness and difference concepts are shown only if the animal exhibits positive transfer to a novel set of stimuli. Giurfa, Zhang, Jenett, Menzel, and Srinivasan (2001) tested bees in these types of experiments simultaneously at the ANU and the Free University in Berlin and obtained the same results.

The apparatus used in the experiments was similar to that used for the SDMTS tasks (Figure 7a, c). Bees were trained to choose a stimulus with a sector or a ring pattern in the decision cylinder based on which pattern they had seen in the first chamber of the maze (Figure 11a, left-hand panel). The trained bees were then given a transfer test using two blue and yellow stimuli. The bees successfully and immediately transferred their ability to do the task to the novel colored stimuli (Figure 11a, right-hand panel). In addition, they were able to transfer their matching ability to other novel stimuli, such as gratings oriented at plus or minus 45 deg.

Bees can also learn to match odors, as shown in the left-hand panel of Figure 11b. Furthermore, bees trained on odors can immediately transfer the learned matching ability to colors, as shown in the right-hand panel of Figure 11b. Thus, the concept of “matching,” once learned, can be transferred even across sensory modalities.

Finally, bees can also learn the concept of “difference.” That is, they can be trained to choose the non-matching stimulus, rather than the matching one. Figure 12a shows learning curves obtained in two experiments investigating this capability. In one experiment, the training stimuli were colors (blue and yellow). Here, bees had to learn to choose yellow in the decision cylinder when they encountered blue at the entrance, and vice versa. In another experiment, the training stimuli were linear gratings, oriented horizontally and vertically. In this case, bees had to learn to choose the vertical grating in the decision cylinder when they encountered a horizontal grating at the entrance, and vice versa. It is evident from Figure 12a that the bees learned both non-matching tasks well. Furthermore, in each case the trained bees were immediately able to transfer the learned, non-matching concept to novel stimuli; bees trained on the gratings were able to perform non-matching on the colors, and vice versa (Figure 12 b, c).

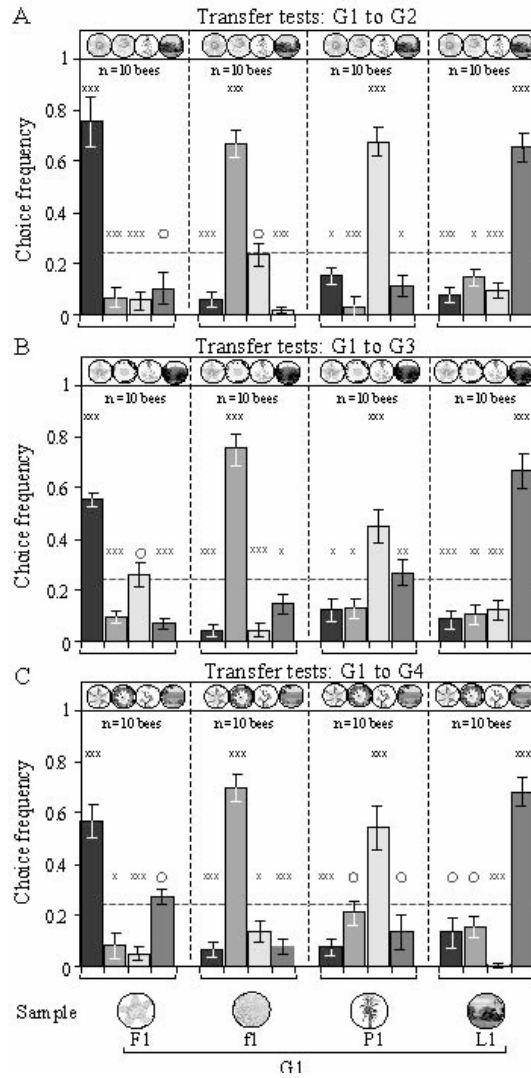


Figure 10. Results of transfer tests examining the ability of bees, which encounter a sample stimulus from Group 1, to choose a test stimulus of the same category in Group 2 (A), Group 3 (B), and a novel Group 4 (C). In each case, the bees are able to learn to choose the test stimulus that belongs to the same category as the sample. For each group, the bars show the relative preferences for the four comparison stimuli when the sample was a star-shaped flower, a circular flower, a plant stem or a landscape, as shown underneath the abscissa. In each panel, n denotes the number of bees tested in the experiments, as shown above the histograms. The results of tests for statistical significance from a random choice level of 25% are shown above each bar. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. Black stars mean significantly greater than the random choice level of 25%, but red stars mean significantly smaller than 25%. The circle symbol represents no significant difference from 25%. The error bars show the Standard Error of Means (S.E.) of the data. In each case, the bees are able to learn to choose the test stimulus that matches the sample. Details are given in the text. (Adapted from Zhang et al., 2004).

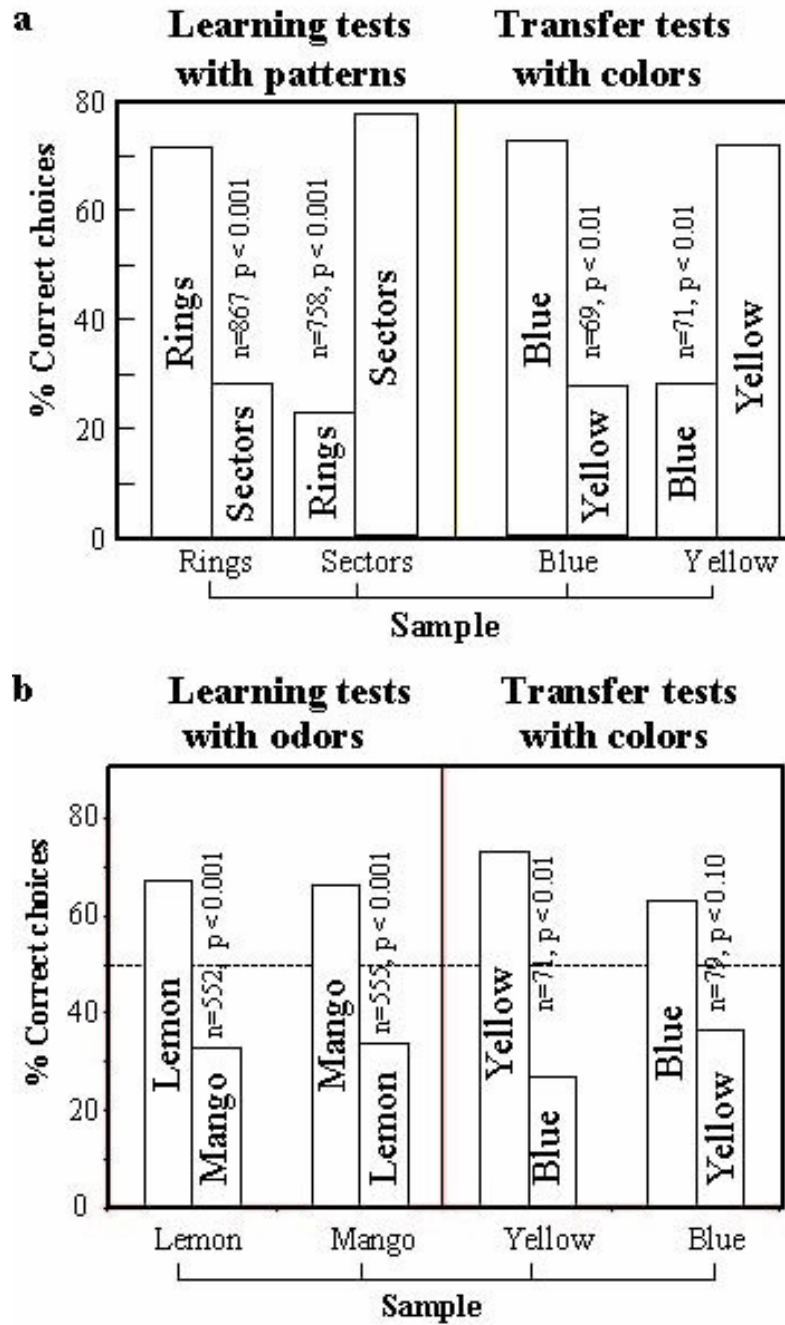


Figure 11. Learning the concept of “sameness,” and transferring it into novel stimuli in the same sensory modality and in different sensory modalities. (a) Results of learning tests with sectored and ring patterns (left panel) and transfer tests with colors (right panel). (b) Learning tests with odors (left panel) and transfer tests with colors (right panel). Details are given in the text. (Adapted from Zhang & Srinivasan, 2004b).

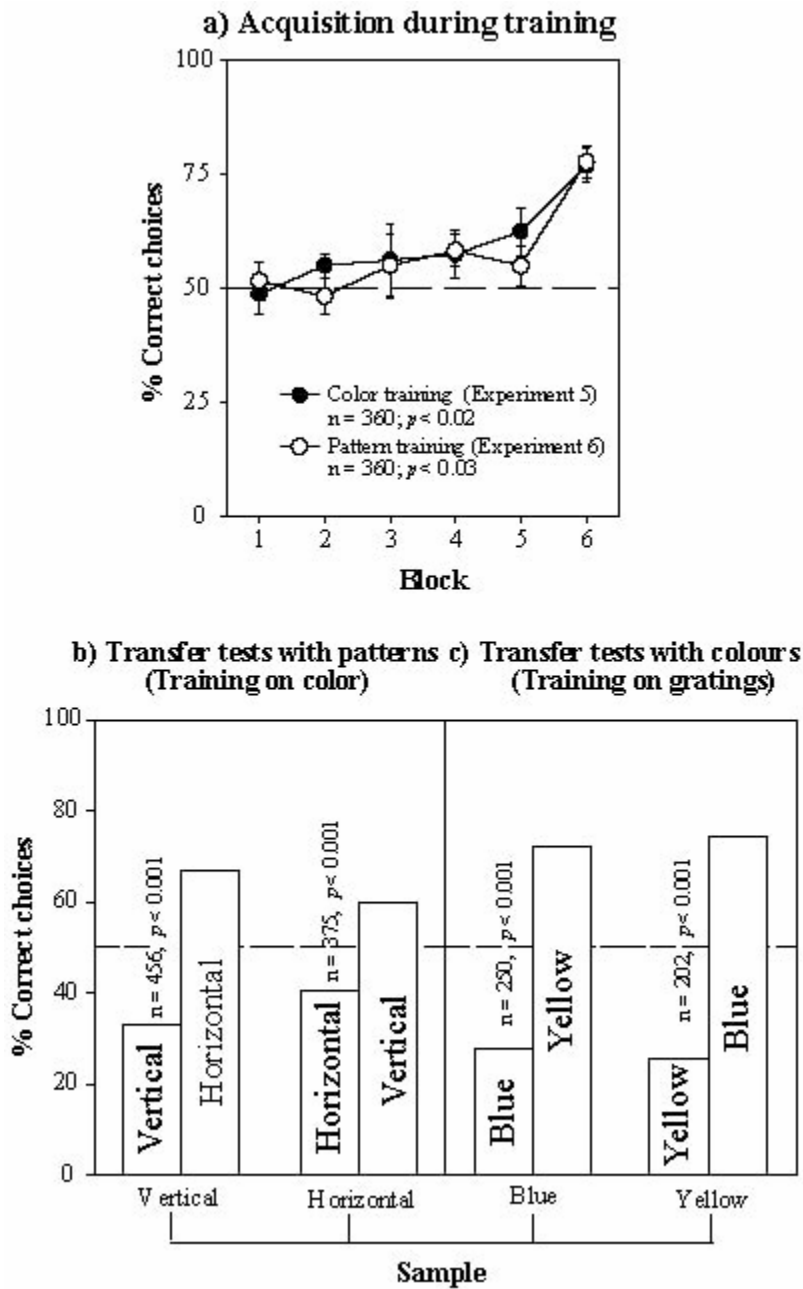


Figure 12. Learning the concept of “difference.” (a) Learning curves for bees trained on colors (filled circles) and on gratings (open circles). (b, c) Results of transfer tests. Details are given in the text. (Modified from Giurfa et al., 2001).

Learning the Abstract Concept of “Order”

Terrace and his colleagues discovered that rhesus monkeys can learn the correct order of arbitrary sets of images, and apply that knowledge to answer new questions about that order (e.g., Terrace, Son, & Brannon, 2003). Zhang, Bock, Si,

Tautz, and Srinivasan (2005) tested this ability in bees using a delayed matching-to-sample (DMTS) paradigm in a specially designed apparatus that consisted of a tunnel with a maze attached to the far end. In this set-up the sample pattern was presented in the middle of the tunnel (Figure 13a). The first of the three maze chambers had two exit holes, each of which was in the center of a large, square comparison stimulus. Blue and white gratings, with a period of 4 cm, oriented at 45 deg and 135 deg to horizontal, were used as stimuli and the bees were trained to match the sample pattern seen in the tunnel to one of the two patterns in the first (decision) maze chamber. During training in the order-learning experiments, bees saw two sample patterns in the tunnel placed 50 cm apart in varying positions within the tunnel. In one experiment the correct sample was encountered first; in another, it was second. In the first experiment (Figure 13b), the bees were able to learn to match the first (rather than the second) grating in the decision chamber with a correct choice frequency of 0.73 ± 0.03 , ($n = 23$, $p < 0.001$, Learning test in Figure 13b).

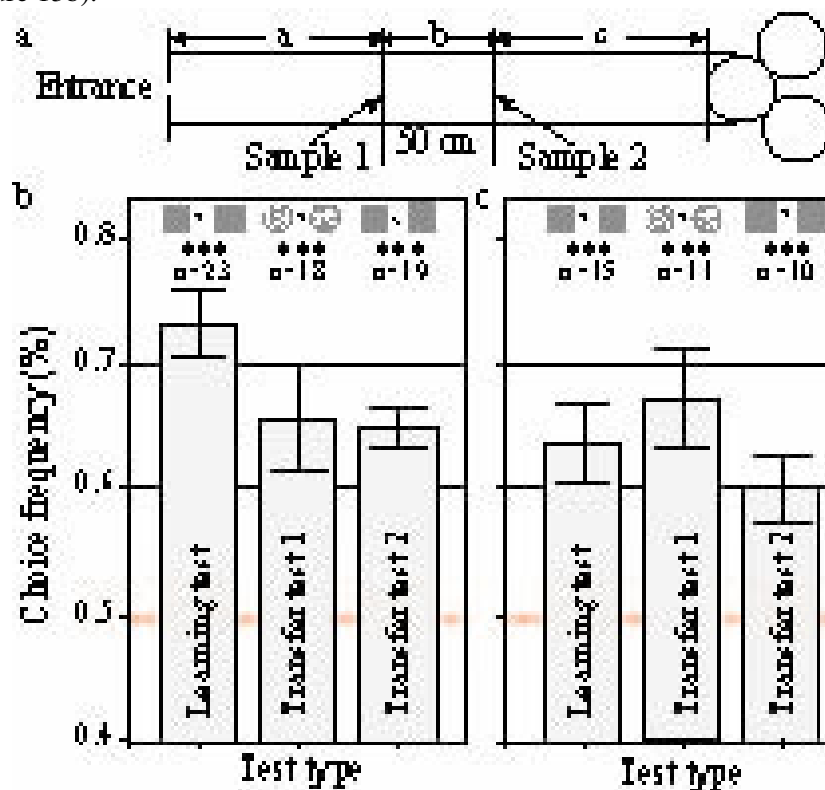


Figure 13. (a) Schematic illustration of apparatus used in the experiments to study the learning of order by honeybees. Bees were trained with two patterns (Sample 1 and Sample 2) in the tunnel, 50 cm apart. (b) The results indicate that bees can learn whether it is the first stimulus, or the second stimulus, that is the one to be matched in the decision chamber. Furthermore, they can apply this learnt rule to novel pairs of patterns, never previously encountered. Details are given in the text. (Adapted from Zhang et al., 2005).

Then, in transfer tests, Zhang et al. (2005) tested whether bees could use their knowledge regarding order to guide their choice in the decision chamber (i.e.,

match the first or second stimulus seen in the tunnel) if all of the stimuli were novel. The bees did well in these tests and made correct choices in more than two-thirds of the tests when the novel stimuli were rings and radial sectors (correct choice frequency = 0.66 ± 0.04 , $n = 18$, $p < 0.001$, Transfer test 1 in Figure 13b), and gratings of 90 deg and 0 deg (correct choice frequency = 0.65 ± 0.02 , $n = 19$, $p < 0.001$, Transfer test 2 in Figure 13b). Bees did equally as well when they had to learn that the second sample in the tunnel was the correct target stimulus (Figure 13c). Consequently, learning which of two sequentially encountered patterns is the one to be matched in a subsequent pattern-discrimination task is well within the honeybees' capabilities.

Conclusions and Discussion

Research over the past thirty or so years is beginning to suggest that learning and perception in arthropods is much more intricate and flexible than was originally assumed. Honeybees in particular are capable of a variety of visually-guided tasks that involve cognitive processes that operate at a surprisingly high level. They can learn to use symbolic rules for navigating through complex mazes, and to apply these rules in flexible ways. Honeybees are able to form concepts of sameness and difference and learn the order of visual objects in a sequence. They can also abstract the general properties of a stimulus, such as its orientation or symmetry (or even a combination of abstract features) and classify objects based on this information. Srinivasan and colleagues (e.g., Srinivasan, Zhang, & Rolfe, 1993; Srinivasan et al., 1994) have even suggested that the existence of feature-extracting mechanisms in the insect visual system might be comparable, functionally, to those known to exist in the mammalian cortex. While the processes of learning and perception may be more sophisticated in vertebrates than in invertebrates, there seems to be a continuum of capacities across these groups rather than a sharp distinction between them (e.g., Prete, 2004). The abilities that an animal acquires (evolutionarily) seems to be governed largely by what it needs in order to pursue its lifestyle, rather than on whether or not it possesses a backbone.

In colloquial terms, the honeybee's brain is small, but its mind is smart. Consequently, it is a promising model system with which to investigate the emergence of intelligence from a small assembly of neurons. Nowadays, it is not a difficult task to design and build a chip with a million components, about the number of neurons in a bee's brain. Understanding how bees use this comparatively small number of information processing units to meet sophisticated biological challenges will give engineers insights into the performance capabilities of such a chip. This may lead to learning machines that can match the complex behaviors of the honeybee.

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