

## The concepts of 'sameness' and 'difference' in an insect

[Letters to Nature]

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Received 19 October 2000; accepted 29 January 2001.

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### Abstract

Insects process and learn information flexibly to adapt to their environment. The honeybee *Apis mellifera* constitutes a traditional model for studying learning and memory at behavioural, cellular and molecular levels [1](#). Earlier studies focused on elementary associative and non-associative forms of learning determined by either olfactory conditioning of the proboscis extension reflex [1](#) or the learning of visual stimuli [2](#) in an operant context. However, research has indicated that bees are capable of cognitive performances that were thought to occur only in some vertebrate species. For example, honeybees can interpolate visual information [3](#), exhibit associative recall [4,5](#), categorize visual information [6-8](#) and learn contextual information [9](#). Here we show that honeybees can form 'sameness' and 'difference' concepts. They learn to solve 'delayed matching-to-sample' tasks, in which they are required to respond to a matching stimulus, and 'delayed non-matching-to-sample' tasks, in which they are required to respond to a different stimulus; they can also transfer the learned rules to new stimuli of the same or a different sensory modality. Thus, not only can bees learn specific objects and their physical parameters, but they can also master abstract inter-relationships, such as sameness and difference.

An important cognitive capacity is the ability to learn relationships between stimuli. In vertebrates, the capacity to acquire sameness-difference concepts has been studied using two experimental procedures, the matching task and the oddity task. A variation of the former is the 'delayed matching-to-sample' task, in which an animal is presented with a 'sample' and subsequently with two or more secondary stimuli, one of which is identical to the sample. The animal is required to respond to the stimulus just encountered. The 'delayed non-matching-to-sample' task is a variation of the oddity task. The procedure is similar to the matching-to-sample task except that the animal is required to respond to the 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 completely new set of stimuli, which it had not experienced during training.

We trained honeybees, *A. mellifera*, in a delayed matching-to-sample paradigm to examine whether they could form a concept of sameness. Training was carried out using a Y-maze placed close to a laboratory window. Each bee entered the maze by flying through a hole in the middle of an entrance wall. At the entrance, the bee encountered the sample stimulus. The sample was one of two different stimuli, A or B, alternated in a pseudo-random sequence. The entrance led to a decision chamber, where the bee could choose one of two arms. Each arm carried either stimulus A or stimulus B as secondary stimulus. The bee was rewarded with sucrose solution only if it chose the stimulus that was identical to the sample. If the bees managed to learn the original discrimination, they were presented with new

sample and secondary stimuli in 'transfer tests': the bees had to choose between stimuli C and D, when the sample was either C or D.

Four experiments were carried out, each by training a fresh group of bees. In experiment 1, A and B were colours, whereas C and D were vertical and horizontal black and white gratings. In experiment 2, A and B were gratings, whereas C and D were colours. In experiment 3, A and B were radial and circular gratings, whereas C and D were oriented linear gratings. In experiment 4, A and B were odours, whereas C and D were colours. In all experiments the stimuli chosen were well distinguished by the bees, as indicated by preliminary investigations.

The results for experiments 1 and 2 are shown in Fig. 1. In experiment 1, the bees were trained to match a given colour (blue versus yellow). The acquisition curve showed a significant improvement during training (Fig. 1a:  $P < 0.0001$ ): bees preferred the colour that was identical to the sample, independently of the sample colour. In experiment 2, the bees were trained to match a given grating orientation (vertical versus horizontal). The acquisition curve also increased significantly along the training (Fig. 1a;  $P < 0.0005$ ): bees preferred the orientation of the grating that was identical to that of the sample, independently of the sample orientation. At the end of the learning phase, the choice frequency for the matching stimulus was above 70% in both experiments 1 and 2.

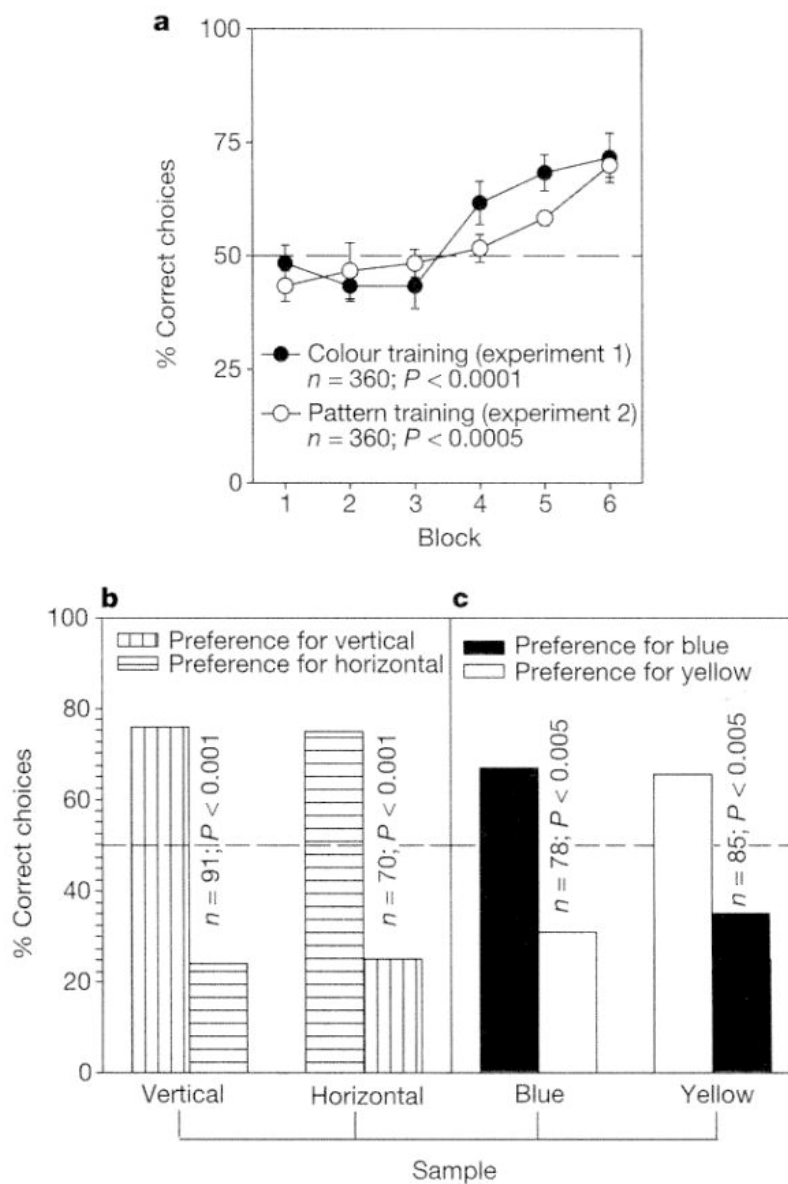


Figure 1 Experiments 1 and 2. **a**, Learning performance of bees during training on colours (experiment 1) and on vertical and horizontal gratings (experiment 2). Data show the results of blocks of ten consecutive training visits for each experiment. **b**, **c**, Results of transfer tests. **b**, Transfer tests with patterns (colour training). In experiment 1, bees trained on the colours were tested on the gratings. **c**, Transfer tests with colours (pattern training). In experiment 2, bees trained on the gratings were tested on the colours.  $n$ , number of choices evaluated.

These results, however, do not necessarily show a capacity to learn the concept of sameness between stimuli. Bees could solve the problem by simply learning the appropriate choice for each of the configurations used in training (for example, in experiment 1 'choose blue and not yellow if blue was seen at the entrance' and 'choose yellow and not blue if yellow was seen at the entrance'), without abstracting a general concept of sameness. The capacity to abstract a general concept of sameness is shown in the transfer tests, where the bees were confronted with new samples and secondary stimuli. In such tests, bees that had been trained to match the colours (experiment 1) could match the gratings (Fig. 1b); and bees that had been trained to match the gratings (experiment 2) could match the colours (Fig. 1c).

The performance in these transfer tests was similar to that attained in the training. In the transfer tests of experiment 1 (Fig. 1b), the matching orientation was chosen with a frequency of 76.3% ( $n = 91$ ,  $P < 0.001$ ) if the sample was a vertical grating, and 75.0% ( $n = 70$ ,  $P < 0.001$ ) if the sample was a horizontal grating. The difference between the preferences for vertical in the two cases was highly significant ( $P < 0.001$ ). In the transfer tests of experiment 2 (Fig. 1c), the matching colour was chosen with a frequency of 67.3% ( $n = 78$ ,  $P < 0.005$ ) if the sample was blue, and 65.3% ( $n = 85$ ,  $P < 0.005$ ) if the sample was yellow. The difference between the preferences for blue in the two cases was highly significant ( $P < 0.001$ ).

Similar results were obtained in experiment 3, in which the bees were trained with radial and circular gratings. After acquisition, the bees chose the matching stimulus with a frequency of 78.0% ( $n = 477$ ,  $P < 0.001$ ) if the sample was a radial grating, and of 77.7% ( $n = 491$ ,  $P < 0.001$ ) if it was a circular grating. In the transfer tests using linear gratings (oriented at  $+45^\circ$  and  $-45^\circ$ ), the trained bees chose the appropriate grating with a frequency of 59.8% ( $n = 92$ ,  $0.1 < P < 0.2$ ) if the sample was a  $45^\circ$  grating, and of 63.9% ( $n = 119$ ,  $P < 0.05$ ) if it was a  $-45^\circ$  grating. The difference between the preferences for the  $45^\circ$  grating in the two cases was highly significant ( $P < 0.001$ ). As in experiment 2, bees that had been trained on the radial and circular gratings could transfer their learned matching ability to colours (data not shown).

In experiment 4, we explored matching in a different stimulus modality, as well as transfer across stimulus modalities. The bees were trained to match odours (lemon and mango; Fig. 2). After acquisition, the trained bees showed a significant preference for the matching odour (Fig. 2c, left). When the trained bees were subjected to transfer tests using colours (blue and yellow), they showed a preference for the appropriate colour (Fig. 2c, right). Details and statistics are given in Fig. 2. The difference between the preferences for yellow in the two cases was highly significant ( $P < 0.001$ ). Thus, bees can learn to perform a delayed match-to-sample task even in the odour domain. Furthermore, they can transfer this capacity to the visual domain.

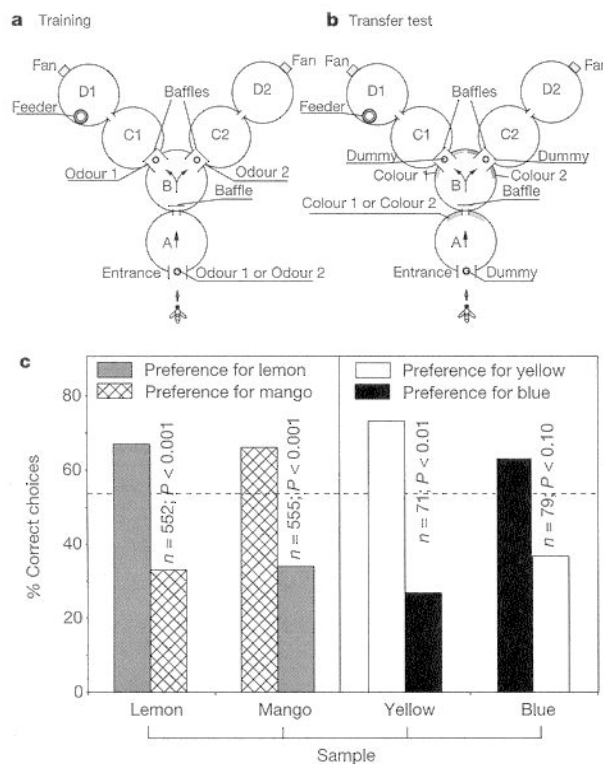


Figure 2 Experiment 4. **a**, Set-up in which bees were trained to match odours. **b**, Set-up used for transfer tests in which bees trained on odour matching were tested on colour matching. **c**, Results. Left, learning tests for odour matching; right, transfer tests for colour matching.

Finally, we trained bees in a delayed non-matching-to-sample task to examine whether they could form a concept of difference. Training was carried out as in experiments 1 and 2, except that in both cases bees were rewarded only if they chose the stimulus that was different from the sample. In experiment 5, bees were trained with the colours yellow and blue and tested with the vertical and horizontal black and white gratings. In experiment 6, bees were trained with the gratings and tested with the colours.

In experiment 5, the bees learned to choose the colour that was different from that of the sample. The acquisition curve showed a significant improvement during training (Fig. 3a;  $P < 0.02$ ). In experiment 6, the bees learned to choose the grating orientation that was different from that of the sample. The acquisition curve also increased significantly along the training (Fig. 3a;  $P < 0.03$ ). At the end of the training, the choice frequency for the matching stimulus was above 70% in both experiments.

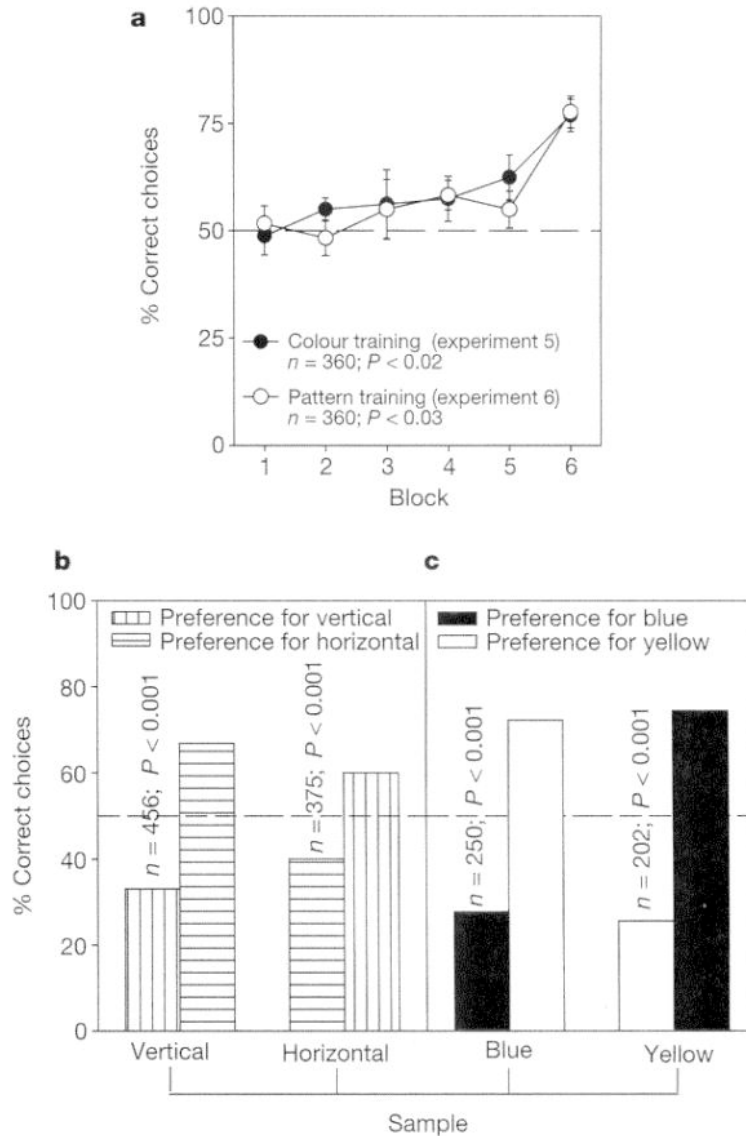


Figure 3 Experiments 5 and 6, investigating non-matching performance. **a**, Learning performance of bees during training on colours (experiment 5) and on vertical and horizontal gratings (experiment 6). Data show the results of blocks of ten consecutive training visits for each experiment. **b**, **c**, Results of transfer tests. **b**, Transfer tests with patterns (colour training). In experiment 5, bees trained on the colours were tested on the gratings. **c**, Transfer tests with colours (pattern training). In experiment 6, bees trained on the gratings were tested on the colours.  $n$ , number of choices evaluated.

In the transfer tests, the bees that had been trained to choose the different colour (experiment 5) transferred their choice to the different grating (Fig. 3b); and bees that had been trained to choose the different grating (experiment 6) transferred their choice to the different colour (Fig. 3c). In all cases, the bees showed a significant preference for the non-matching sample (for details and statistics, see Fig. 3).

Animals can learn matching or oddity tasks by using simple rules such as similarity judgements if they are trained and tested on the same stimuli. Our experiments reveal, however, that bees can do more than this: they can transfer the matching or non-matching ability to new stimuli for which the rules of choice are not specified in the training. Because the bees continue to choose the appropriate matching (or non-matching) stimulus even in new situations, we conclude that they can form and use a concept of sameness (experiments 1 to 4) and difference (experiments 5 and 6) in making their choices. The ability to judge sameness and difference has been shown in many vertebrates [10-20](#), but in some cases the results seem to depend critically on procedural details [20](#). Furthermore, transfer from one problem to another, when shown, is usually very weak [14](#).

Our findings with bees, however, are very consistent. They were repeatable in two different laboratories that used different experimental set-ups, test procedures and colonies. The concepts of sameness and difference, once learned, can be transferred to other stimuli that belong either to the same domain (as in the transfer from radial and circular gratings to oriented linear gratings) or to a different domain (as in the transfer from colours to oriented linear gratings and vice versa). Furthermore, the concept of sameness can be learned even in the olfactory modality, and can be transferred across stimulus modalities (from odours to colours). Thus, in the bee, the ability to form 'sameness-difference' concepts is broad and robust. Such concepts might contribute to improve foraging activities.

Our results question the view that vertebrates, and in particular primates, may be the only animals able to form 'sameness' or 'oddity' concepts [21](#). They also show that higher cognitive functions are not a privilege of vertebrates [22](#). Moreover, as such functions result from a relatively simple and accessible nervous system in which elementary associative learning pathways have been already characterized [23](#), there is a realistic chance of uncovering the neural mechanisms that underlie this capacity.

## Methods

### Bees and set ups

For each experiment, a group of 6-8 individually marked bees were trained to enter and collect 50% (weight/weight) sucrose solution in a Y-maze placed close to an open window in the laboratory. Experiments 1, 2, 5 and 6 were conducted in Berlin; experiments 3 and 4 were conducted in Canberra. All mazes were covered by an ultraviolet-transmitting Plexiglas ceiling. Bees entered the maze by flying through an aperture, 4 cm in diameter.

The set-up for training the olfactory stimuli is shown in [Fig. 2a](#). Bees received odour 1 as they entered chamber A. They then entered chamber B, where they were presented with two odours (1 and 2) and had to learn to choose the odour matching that at the entrance. The odours were presented by perforated vials containing tissue paper soaked in liquid odorant. To prevent mixing of odours in chamber B, and to remove any feeder odours in the reward chambers D1 and D2, suction fans were run for 5-10 s immediately after each time the stimulus positions in the decision chamber were interchanged; that is, every 10 min on average. Bees trained with odours in this way were subsequently tested for colour matching, using the apparatus shown in [Fig. 2b](#). Here the odours were replaced by colours, and the odour vials were replaced by dummy vials which carried no scent, but had the same visual appearance as the vials used in the training. Baffles prevented the bees from experiencing the stimuli in chamber B until they had entered it, and from viewing the feeder from chamber B.

### Visual stimuli

The visual stimuli used in the experiments were 11-cm diameter discs presented in the vertical plane. The colour stimuli were blue and yellow, cut from HKS-N 44 and 3 cardboards, respectively (K+E Stuttgart). All gratings were black and white and were printed using a high-resolution laser printer. The linear gratings had a period of 3 cm, the annular gratings had a period of 4 cm, and the radial gratings had an angular period of 60°.

### Training and experiments

During training in all experiments, the position (left or right) of the rewarded stimulus in the maze was changed after every two visits per bee (on average), and the sample was changed after every four visits per bee. We evaluated the learning of the training discrimination by determining significant changes in the acquisition curves (experiments 1, 2, 5 and 6) or by learning tests in which the performance was quantified by recording the choices of each bee after it entered the maze (experiments 3 and 4). In the latter case choices were registered as the first entry by the flying bee through an aperture that was associated with each stimulus.

Transfer tests were conducted after 60 training visits per bee (equivalent to one day of training) in experiments 1, 2, 3, 5 and 6 (which involved training on visual stimuli) and after 80 visits in experiment 4 (which involved training on olfactory stimuli). No reward was offered during these tests. Performance was recorded either as touches (flights towards a stimulus that ended with the antennae touching the stimulus surface) during 2 min (experiments 1, 2, 5 and 6) or as in the learning tests (experiments 3 and 4). In all cases, each test was divided into four parts: two with one stimulus as the sample and two with the alternative stimulus as the sample. Tests with a given sample were performed twice: once with the matching stimulus presented in the right arm of the maze, and once in the left arm. Between transfer tests, the bees were retrained (usually 5-10 visits to the apparatus) to maintain the level of learning at its plateau.

As the stimuli in the transfer tests were different from those used in the training, bees were sometimes reluctant to enter the apparatus and make a choice during the transfer tests. To eliminate this difficulty, in experiments 3 and 4 a 'familiarization procedure' was used from time to time during the training, in which the set-up presented the sample and secondary stimuli that would eventually be used in the transfer test. However, the feeder was placed at a 'neutral' position beyond the entrance, at the point where they would normally choose between the stimuli in the two arms. This procedure ensured that the bees became accustomed to the transfer stimuli, without being trained to choose between them.

## Statistical analysis

We used analysis of variance for repeated measurements to determine whether the bees' performance in experiments 1, 2, 5 and 6 significantly improved as a result of the training. In the learning tests in experiments 3 and 4 and in the transfer tests in all experiments, [chi]<sup>2</sup> tests were used to determine whether the experimentally measured choice frequencies were significantly different from random-choice. We used [chi]<sup>2</sup> × 2 tests to determine whether two choice frequencies were significantly different from each other.

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## Acknowledgements

We thank C. Bönisch and C. Wiley for help with experiments 5 and 6. M.G., A.J. and R.M. were supported by the Special Programme SFB 515 of the Deutsche Forschungsgemeinschaft. M.G. was also supported by a grant from the Deutsche Forschungsgemeinschaft. S.W.Z. and M.S. were supported partly by a grant from the Human Frontiers in Science Program and from the US Defence Advanced Research Projects Agency and the Office of Naval Research. The two laboratories contributed equally to the work.

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Accession Number: 00006056-200104190-00023

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