Natur wissenschaften

Adrian G. Dyer · Lars Chittka

Fine colour discrimination requires differential conditioning in bumblebees

Abstract Accurate recognition requires that visual systems must be able to discriminate between target and distractor stimuli. Flowers are learned and recognised by bees using visual cues including colour and shape. We investigated whether bees were able to learn to discriminate between colours differently depending upon absolute or differential conditioning. For absolute conditioning bees were rewarded with sucrose solution for visits to target flowers. When distractor stimuli were subsequently presented, a high level of discrimination was observed if there was a perceptually large colour distance separating distractors and targets, but for a perceptually small colour distance the bees generalised and did not discriminate between stimuli. When provided with differential conditioning where both target and distractors were present, the bees learnt to discriminate stimuli separated by a perceptually small colour distance. This shows that for bees to learn fine colour discrimination tasks it is important to use differential conditioning. The findings are discussed within the context of the necessity for plants to produce distinctive flower colours.

A. G. Dyer (✉) · L. Chittka
Zoologie II, Biozentrum,
Universität Würzburg,
Am Hubland, 97074 Würzburg, Germany
e-mail: a.dyer@latrobe.edu.au
Fax: +61-3-94793692

A. G. Dyer
School of Orthotics, Faculty of Health Sciences,
La Trobe University,
3086 Bundoora, Victoria, Australia

L. Chittka
Biological Sciences,
Queen Mary, University of London,
Mile End Road, London, E1 4NS, UK

Introduction

Bees visit flowers to collect nutrition and provide an important service by transporting pollen between plants. Bees often exhibit flower constancy and temporally restrict visits to one species whilst its flowers are available (Chittka et al. 1999). Flower constancy may be of considerable reproductive benefit to plants and there is good evidence that flowers have evolved colour signals to suit the visual system of bees (Chittka and Menzel 1992; Chittka 1996). Currently however, it is not clear how well bees discriminate between colours depending upon their conditioning with the target colour. Backhaus et al. (1987) found that there was no statistical difference in honeybee colour discrimination when honeybees received absolute conditioning or differential conditioning, although they did note slightly better discrimination for differential conditioning when similar colours were tested. Absolute conditioning means that bees learn target stimuli in the absence of distractors, while differential conditioning means that bees learn target stimuli in the presence of distractors. Recently Giurfa et al. (1999) showed that honeybees used different visual strategies depending upon whether absolute or differential conditioning was used during training for pattern recognition tasks. Giurfa et al. (1999) found that differential conditioning resulted in an inhibitory conditioning of non-rewarding patterns, and increased the demands on the bees' selective attention to information from the whole of a pattern.

The ability of bees to make colour discriminations depending upon their conditioning potentially plays an important role in how plants evolved flower signals. Dyer and Chittka (2004) showed that the ability of bumblebees to correctly choose a target colour was dependent upon the colour distance separating target and distractor colours. Using differential conditioning, that study showed that discriminating small colour distances appeared to be a much more difficult task than discriminating large colour distances, and bees learnt large colour distances more rapidly. Here we test how well bumble-

bees learn to discriminate between different colours depending upon whether or not distractor stimuli are present during conditioning.

Methods

Experiments were conducted indoors with bumblebees (*Bombus terrestris*) housed in a two-chamber wooden nesting box (28×16×11 cm) connected via a Plexiglass tube to a flight arena (120×100×35 cm). Prior to experiments inexperienced bees were allowed to collect 2 M sucrose solution from a clear plexiglass feeder placed at random positions in the arena. A UV-transparent Plexiglas sheet covered the arena and illumination was provided by six Duro-Test 40 W True-Lite tubes and one Osram 36 W Blacklight tube to simulate bee daylight. Tube flicker was converted to 1,200 Hz using special ballasts (Osram Quicktronic QT-Eco 1×58/230-240).

Stimuli were plastic disks ($\emptyset$=26 mm, 4 mm thick) with a hole in the centre to hold fluid ($\emptyset$=4 mm; depth 2.5 mm) painted with matt blue paint [target with 60:40 (vol.) mixture of nos. 55 and 56 Revell (Germany) paint, similar distractor flower with a 40:60 mixture; and dissimilar distractor with no. 65 Humbrol (UK) paint]. Spectral reflectance was measured with a Varian DMS100 reflectance spectrophotometer calibrated against a Varian polytetrafluoroethylene standard (Fig. 1A.). Stimuli were presented on the arena floor which was painted green to match the spectral reflectance of foliage (Fig. 1A). The colour loci of stimuli were calculated in a hexagon colour space (Chittka 1992) considering the spectral sensitivity functions of bumblebee photoreceptors (Peitsch et al. 1992).

The relative amount of light absorbed by each photoreceptor class is given by P:

$$P = R \int_{300}^{650} S_i(\lambda) I(\lambda) D(\lambda) \mathrm{d}\lambda \tag{1}$$

where $S_i(\lambda)$ is the spectral sensitivity of the (UV, Blue, Green) receptor classes, $I(\lambda)$ is the spectral reflectance function of the stimulus and $D(\lambda)$ is the spectral distribution of the illuminant.

The variable R simulates adaptation to the painted green background (I_B),

$$R = 1 / \int_{300}^{650} S(\lambda) I_B(\lambda) D(\lambda) \mathrm{d}\lambda \tag{2}$$

The transduction of photoreceptor absorption (P) into receptor excitations (E) is given by:

$$E = P / (P + 1) \tag{3}$$

Coding is assumed to be performed by two unspecified opponent mechanisms and colour distance can be calculated as the Euclidean distance between colour loci (Chittka 1992). Figure 1B shows the loci of flower colours in colour space.

Five bees were individually trained with absolute conditioning using five target flowers presented in the arena where each flower was filled with 20 μl of 2 M sucrose solution. When a bee filled its stomach it returned to the nest. The flowers were then washed in 30% alcohol to remove any olfactory cues and the spatial arrangement was randomised to avoid position learning. Over 11–13 foraging bouts, each bee collected sucrose solution until 60 target flowers had been visited. At the conclusion of this conditioning, each bee's colour discrimination ability was tested in non-rewarded trials. In a non-rewarded trial five target and five distractor flowers were arranged at randomly determined positions in the arena. Each bee was first tested with target and distractor colours separated by a colour distance of 0.045 hexagon units, and then tested with target and distractor colours separated by 0.152 hexagon units. Immediately following these non-rewarded trials, each bee was provided with differential conditioning to the colour distance of 0.045 hexagon units with five target and five distractor flowers arranged in the arena. The distractor flowers contained a
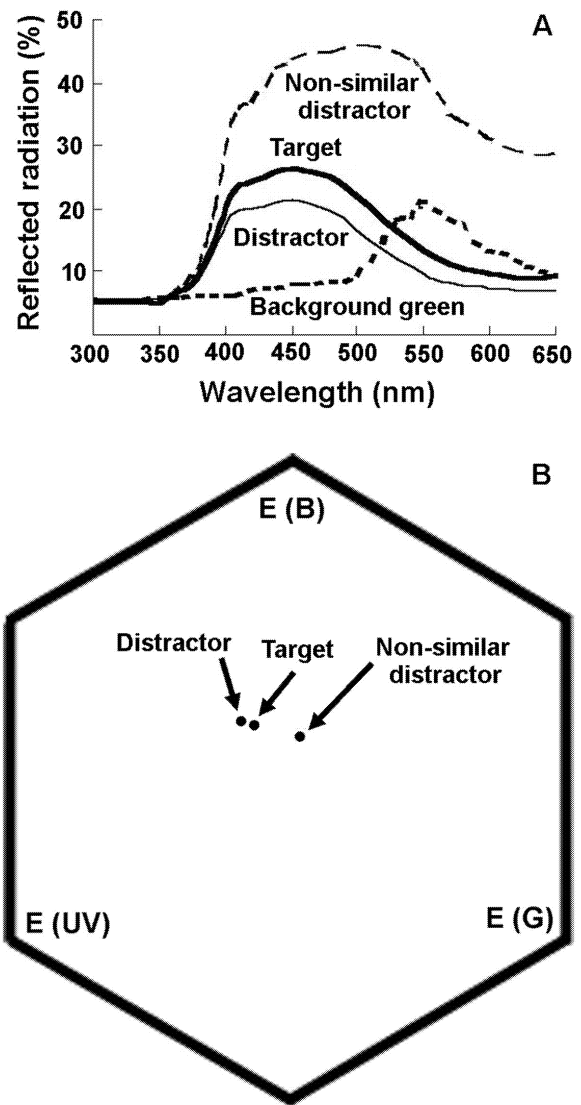

Fig.1 A The spectral reflectance of stimuli. **B** Loci of flower colours in a hexagon colour space

bitter punishment of 20 μl of 0.12% quinine hemisulphate salt in water to ensure motivation to avoid distractors (Chittka et al. 2003). After the bees had made 60 visits to flowers, their ability to discriminate between the flowers was retested in non-rewarded trials. Training and testing of each individual bee with absolute and then differential conditioning was completed in a single half-day session (approximately 4–5 h).

To test the possibility that improvement in discrimination might be a continued learning effect for the bees trained first with absolute and then with differential conditioning, a separate group of five bees was trained only with differential conditioning. Two of the bees from the second group were also tested over a number of days to see how the differential conditioning influenced the precision of long-term memory in bees.

Results

Bees initially provided with absolute conditioning to a target colour did not discriminate a perceptually small
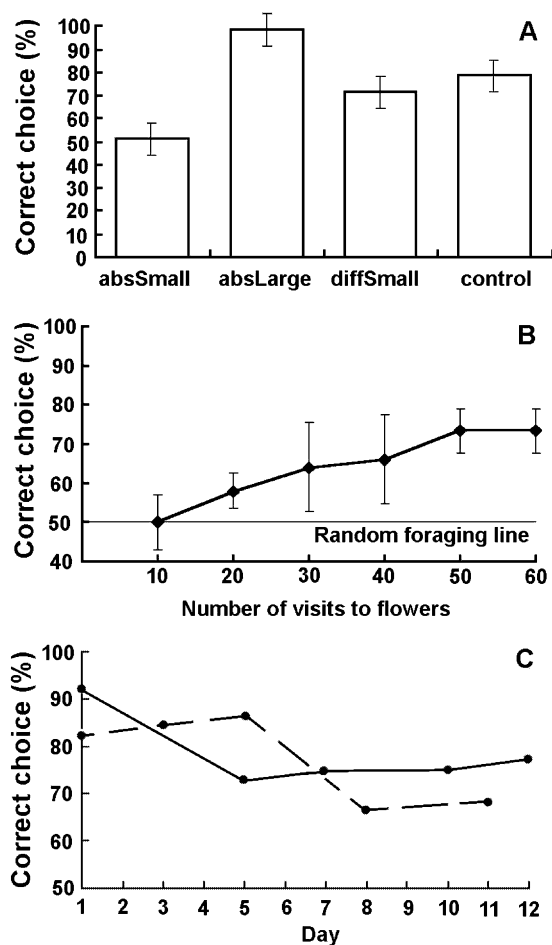
Fig. 2 A Choice frequencies of bumblebees discriminating between coloured stimuli in non-rewarded trials ($n=5$ bees ± SE). *Condition (absSmall)*: With absolute conditioning, bees do not discriminate between similar colours separated by 0.045 hexagon units. *Condition (absLarge)*: With absolute conditioning, bees do discriminate between colours separated by a large colour distance of 0.152 hexagon units. *Condition (diffSmall)*: With differential conditioning, bees are able to discriminate between similar colours. *Condition (control)*: A control group of bees given only differential conditioning perform at a similar level of discrimination to the initial test group. **B** With differential conditioning, bees gradually learn to discriminate between similar colours ($n=5$ bees ± SE). **C** Differential conditioning forms a long-term memory, as two bees tested were able to repeat the high level of discrimination for a number of days

colour distance ($\chi^2=0.031$, $df=1$, $P=0.578$; Fig. 2A, column absSmall), but showed a high level of discrimination for a perceptually large colour distance ($\chi^2=104.3$, $df=1$, $P<0.001$; Fig. 2A, column absLarge). One possibility for the failure to discriminate 0.045 hexagon units might have been that this colour distance was too small for the bees to discriminate. However, when these bees were provided with differential conditioning, they progressively learnt the task (Fig. 2B) and showed a significant improvement in discrimination ability (Fig. 2A, condition absSmall vs condition diffSmall; paired samples *t*-test, $t=-4.415$, $df=4$, $P=0.005$). Thus, with differential conditioning the bees

learnt to solve a colour discrimination task that they were not able to solve with absolute conditioning.

A separate group of five control bees trained only with differential conditioning showed a similar level of discrimination to the initial group after differential conditioning (independent samples *t*-test, $t=-0.746$, $df=8$, $P=0.477$; Fig. 2A, control column), indicating that the improved performance in the initial group was not just due to prolonged training. The differential conditioning formed a long-term memory, as two bees were able to repeat the high level of discrimination over a number of days (Fig. 2C).

Discussion

Increasing the level of training to a visual task improves bee choice performance (Giurfa et al. 2001); however, the expertise level that bees achieve in solving visual tasks depending upon the type of conditioning is not well understood (Giurfa et al. 1999). We show that bees provided with absolute conditioning did not discriminate between stimuli separated by a small colour distance, but were very capable of discriminating between stimuli separated by a large colour distance. However, when differential conditioning was applied, the bees learned to discriminate perceptually small colour distances, and differential conditioning formed a long-term memory that lasted for days. This is the first demonstration that differential conditioning is important for bee colour discrimination.

Most flowers offer a nutritional reward, with the exception being rare plants that use Batesian mimicry and receive pollinator visits by mimicking a rewarding species (Dafni 1984). Mimic species have low density compared with model species (Dafni 1984; Gumbert 2000), and consequently in nature bees are more likely to receive absolute rather than differential conditioning to flower colours. The prediction that bees often do not get differential conditioning in nature and do not learn to make fine colour discriminations agrees with data from field studies. For example, Chittka et al. (1997) showed that bees generalise on flowers separated by a colour distance of about 0.1 hexagon units, and very high levels of flower constancy are only observed when flowers have distinctly different coloration greater than 0.2 hexagon units (Chittka et al. 2001). Thus, there is considerable evolutionary pressure on plants to produce distinctive flower coloration so that flowers of different species are not perceptually similar. This is despite evidence that bees can make fine colour discriminations in a laboratory with differential conditioning. The finding that differential conditioning is important for colour discrimination tasks also has implications for studies that attempt to understand colour vision mechanisms in bees.

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