

RESEARCH ARTICLE

Illumination preference, illumination constancy and colour discrimination by bumblebees in an environment with patchy light

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SUMMARY

Patchy illumination presents foraging animals with a challenge, as the targets being sought may appear to vary in colour depending on the illumination, compromising target identification. We sought to explore how the bumblebee *Bombus terrestris* copes with tasks involving flower colour discrimination under patchy illumination. Light patches varied between unobscured daylight and leaf-shade, as a bee might encounter in and around woodland. Using a flight arena and coloured filters, as well as one or two different colours of artificial flower, we quantified how bees chose to forage when presented with foraging tasks under patchy illumination. Bees were better at discriminating a pair of similar colours under simulated unobscured daylight illumination than when foraging under leaf-shade illumination. Accordingly, we found that bees with prior experience of simulated daylight but not leaf-shade illumination initially preferred to forage in simulated daylight when all artificial flowers contained rewards as well as when only one colour was rewarding, whereas bees with prior experience of both illuminants did not exhibit this preference. Bees also switched between illuminants less than expected by chance. This means that bees prefer illumination conditions with which they are familiar, and in which rewarding flower colours are easily distinguishable from unrewarding ones. Under patchy illumination, colour discrimination performance was substantially poorer than in homogenous light. The bees' abilities at coping with patchy light may therefore impact on foraging behaviour in the wild, particularly in woodlands, where illumination can change over short spatial scales.

Key words: bumblebee, colour constancy, colour vision, insect foraging, novelty aversion.

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INTRODUCTION

The light environment in terrestrial habitats is not consistent across space or time. Temperate woodland presents a complex foraging environment for insect pollinators, particularly during the spring when the canopy of trees and large shrubs is only partially closed. Indeed, many areas are shaded by leaves for much of the growing season, altering both the overall illuminance and the chromaticity of the light beneath (Endler, 1993). Other patches are open, but will only receive direct sunlight for a small part of the day when the sun is overhead, at other times being lit only by skylight. Furthermore, the spectral content of sunlight and light from the sky varies over the course of the day and between days (Hernández-Andrés et al., 2001; Johnsen et al., 2006).

An animal seeking rewarding flowers or another food source in such a habitat is therefore faced with a considerable visual challenge. Without some way to compensate for changing hues and intensities, i.e. mechanisms of colour constancy, colour and brightness information would be unreliable. Simple receptor adaptation enables a basic form of compensation for changing illumination – if a photoreceptor is highly stimulated, it downregulates its sensitivity to light. This permits some basic compensation for changes in illuminant (Dyer and Chittka, 2004a; Neumeyer, 1981). Some animals, including honeybees, appear to have more central nervous involvement in compensating for illumination, i.e. true colour constancy (Neumeyer, 1981; Werner, 1987; Werner et al., 1988).

Thus, even when the spectral content of illuminating light changes, some insects learn to distinguish coloured stimuli with a high degree of accuracy (Balkenius and Kelber, 2004).

However, there is evidence suggesting that this ability, as in humans, is approximate rather than perfect. Data from previous experiments showed that larger changes in the illuminant caused the bees to make more mistakes when recognising coloured stimuli (Dyer, 1999; Neumeyer, 1981). Furthermore, if bees adapted flawlessly to changed illumination, they might not detect changes in the spectral content of illuminant light and would therefore not be able to use illumination information to influence their behaviour. However, it has been demonstrated that bees directly perceive changes in illuminating light (Dyer and Chittka, 2004b; Dyer, 2006). Additionally, bees can use the illumination as a contextual cue in foraging tasks (Lotto and Chittka, 2005). Further studies (Dyer, 1998; Dyer, 1999) used data on bees' photoreceptor responses and the reflectance spectra of natural flowers to predict that some flowers would appear to change in colour to bees' eyes under altered lighting, indicating that bees' colour constancy might only be approximate. However, none of these experiments addressed the responses of bees to short-term changes in illumination that are associated with foraging in patchy light, as they might be encountered in nature, e.g. when moving rapidly into and out of illumination patches, the illumination surrounding the bee changes over seconds rather than minutes.

Possessing only approximate colour constancy could give rise to a difficult situation for bees foraging in such environments with patchy light, e.g. woodland edges, hedgerows and gardens. Whereas bees may be accurate at discriminating flowers and spotting concealed predators under sunlight, they may make mistakes in finding the correct flowers under leaf-shade or skylight, or fail to spot flowers with predators (e.g. crab spiders) on them (Ings and Chittka, 2008); the same applies when moving from leaf-shade into sunlit patches. Some illuminants could therefore be considered by the bees to be more risky, and it may affect their choice behaviour. This can be further complicated by metamerism, when two items with different spectral reflectances that are discriminable under one illuminant become indistinguishable under another (Wyszecki and Stiles, 1982). For a foraging bee, this could cause misidentification of flowers that in other illuminations would be discriminable.

In the following series of experiments, we sought to test whether an illuminant that simulated leaf-shade was associated with lower accuracy in a colour discrimination task compared with their performance under simulated daylight. We then investigated whether a difference in performance under the two illuminants could lead to an experimentally naive group of bees behaving differently under the two illuminants, for example, spending more time under one illuminant compared with the other, or making more mistakes under one lighting condition than under the other.

MATERIALS AND METHODS

Experiments were performed indoors between January 2007 and December 2009. For each experiment, we connected a colony of bumblebees [*Bombus terrestris* (Linnaeus 1758)] (colonies were supplied by Koppert UK Ltd, Haverhill, Suffolk, UK, and Syngenta Bioline Ltd, Little Clacton, Essex, UK) to a flight arena (1.2 × 1 m) with an ultraviolet (UV)-transparent Perspex lid *via* a plastic tunnel. Bees were always released into the arena individually during training and testing periods. Each bee was uniquely marked with a paint spot on its thorax and was used in only one of the different experiments detailed, and thus had no prior experience of colour or learning experiments. Between experiments, bees were allowed to forage from an uncoloured feeder containing sucrose solution placed on the centre-line of the flight arena (the line perpendicular to the wall through which the bees entered). At these times, the arena was illuminated by the simulated daylight lighting setup detailed below; all bees had foraged at least once under this illumination condition.

Lighting was provided by four fluorescent ‘daylight’ tubes (Duro-Test Lighting, Philadelphia, PA, USA) and one UV blacklight (Maplin Electronics Ltd, Rotherham, UK). Lights had a high flicker frequency (>1000 Hz) as 50 Hz mains flicker can be resolved by insect eyes. A UV-transmitting white diffuser (White Light Ltd, London, UK) was placed beneath the lights to provide homogeneous illumination from the five tubes. This was the default illumination for the arena, and also the illumination in the ‘daylight’ patches during patchy light experiments. The illumination spectrum is taken from Lotto and Chittka (Lotto and Chittka, 2005). We simulated leaf-shade in this setup with coloured filters placed above the arena to alter the intensity and spectral composition of the incoming light. These filters consisted of a combination of two layers of green translucent plastic (Acco UK Ltd, Aylesbury, UK) and one layer of tracing paper (Simply Stationery, Ackerman Group, London, UK) (see Fig. 1A for the transmittance spectra for the filter combination and reflectance of green leaves). For experiment 1, a large leaf-shade filter was placed above the flight arena to illuminate the whole area uniformly with leaf-shade light. For the other experiments, we

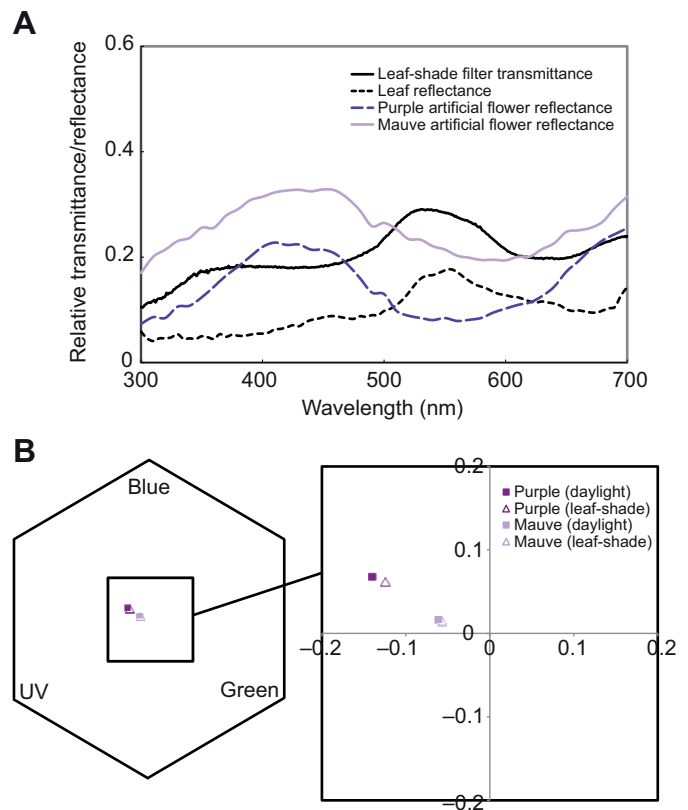


Fig. 1. Reflectance and transmittance spectra of materials used in the experiments, and colour hexagon coordinates of artificial flowers. (A) Spectral reflectance of leaves [averaged from multiple samples (Chittka, 1997)] and the transmittance of the leaf-shade filter used in our experiments, along with the spectral reflectances of the purple (rewarding) and mauve (unrewarding) artificial flowers. All spectra were measured with an Avantes AvaSpec 2048 spectrophotometer and a deuterium-halogen light source, relative to a BaSO_4 white standard. (B) Colour hexagon coordinates of the artificial flowers, under simulated daylight and leaf-shade illumination. In the colour hexagon, the angular position as defined from the centre defines hue, which is in turn determined by the relative receptor excitation signals – a position in the top part of the hexagon, for example, indicates a relative strong stimulation of the blue receptor. Distance from the centre defines spectral purity, so that colour loci further from the centre might be perceived as more saturated (Lunau et al., 1996). Colour loci are shown for the two types of artificial flowers, i.e. purple (containing sucrose) and mauve (where used, containing quinine) under the two illuminations as they appear to a bee (inset expanded for clarity). Colour distances in colour space correspond to subjective similarity of colours; therefore, the two colours are predicted to be somewhat harder to distinguish under green light than under unobscured daylight.

used leaf-shade ‘patches’, which were rectangular sheets one-quarter of the area of the top of the arena; two of these placed in diagonally opposite quadrants of the arena created a setup in which half the arena’s area was illuminated by simulated daylight and the other half was illuminated by leaf-shade light (Fig. 2). We refer to this as the ‘Battenberg design’ (referencing the sponge cake with a two-by-two coloured grid in cross section) for convenience.

Two types of artificial flower with different colours were used, representing two flower colour morphs. The rewarding (‘positive’) artificial flower consisted of a 2.4 × 2.4 cm UV-transmitting transparent plastic tile (thickness 4 mm) placed over a purple square of paper of the same size. The unrewarding (‘negative’) artificial flower was identical except that the paper was mauve in colour; reflectance spectra are shown in Fig. 1A.

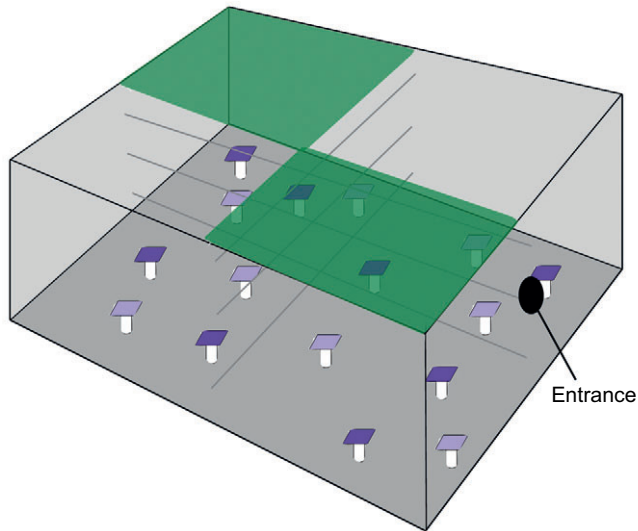


Fig. 2. Sketch of the flight arena. The figure shows a typical arrangement of artificial flowers, and the 'Battenberg' filter arrangement on the arena lid. Green patches indicate leaf-shade light filters, whereas the grey patches are illuminated by simulated daylight (produced by a combination of 'daylight' fluorescent tubes and a UV blacklight). Artificial flowers are shown in approximations of the two colours (mauve and purple), distributed equally across the four quadrants. The strings were strung across the arena at regular intervals below the boundaries of the colour filters to provide a clear delineation between illumination quadrants to improve accuracy of recording of bee behaviour.

Signals for these reflectance spectra in the bees' UV, blue and green receptors were calculated using the integral of the spectral sensitivity curves of *Bombus terrestris* photoreceptors (Skorupski et al., 2007), the illumination spectrum with or without a green filter (Lotto and Chittka, 2005) and the reflectance spectrum of the flower type in question, using methods described in Chittka (Chittka, 1992); their colour hexagon coordinates are displayed in Fig. 1B. The colour hexagon is a graphical representation of a bee's colour space where the discriminability of different colours correlates with the distance between them in colour space. Points in colour space are determined based on the object's reflectance, the photoreceptor sensitivities of the insect, and the illumination and background spectra. Under uniform simulated daylight, the colour distance between the two artificial flower stimuli is 0.095 hexagon units (hu), where the maximum distance between two opponent corners of the hexagon is 2. This indicates a challenging (but possible) discrimination task for bees; if bees are not penalised for errors, a distance of 0.1 hu will be distinguished with approximately 70% probability (Chittka et al., 1993). With differential conditioning, especially where bees are penalised for errors rather than unrewarded, smaller colour differences of the range used here can, however, be discriminated with some certainty by bumblebees (Dyer and Chittka, 2004c). Under uniform leaf-shade illumination, the colour distance between the two types of artificial flower is 0.083 hu, a smaller distance than under daylight, suggesting that discrimination should be more difficult under leaf-shade.

The tiles contained a small central indent (diameter 2 mm, depth 2 mm) in which a drop of sucrose reward could be placed. The tiles were placed on glass vials 4.2 cm in height, with equal numbers (either four or two, depending on the experiment) in each quadrant of the arena. After each bout we cleaned all the tiles and the arena floor, and pseudo-randomised the positions of the artificial flowers

within the arena so that the bees could not learn to associate any location in the arena with the predictable presence of either a rewarding or an unrewarding stimulus.

Experiment 1: colour discrimination in two separate illuminants

Each bee was trained and tested under one of two experimental treatments, both of which consisted of uniform illumination (not patchy). Treatment 1 was uniform simulated daylight; treatment 2 was uniform simulated leaf-shade, produced by placing the leaf-shade filters beneath the lighting array so that the entire arena was lit by green light. We tested 15 bees in each treatment.

In both treatments, a single bee was allowed to forage in the arena containing eight rewarding and eight unrewarding artificial flowers. The rewarding flowers contained 15 μ l of 40% sucrose solution, whereas the unrewarding flowers contained 15 μ l of 0.013% quinine hemisulphate solution, a known aversive substance to bees commonly used to penalise incorrect choices (Dyer and Chittka, 2004d). Although the crop capacity of very large foragers can be up to 180 μ l (Lihoreau et al., 2010), we found that most bees tested in this experiment were able to satiate on the 120 μ l of sucrose solution provided within a foraging bout, inducing them to return to the nest. Occasionally for very large or small individuals, sucrose solution volumes were adjusted slightly (microlitres) up or down consistently across all artificial flowers to ensure adequate satiation.

We recorded the bee's landings for 100 training visits. If any part of the bee made physical contact with the artificial flower, this was counted as a choice. After those 100 visits, we gave each bee an unrewarded test, in which clean artificial flowers were placed in the arena without either sucrose or quinine, and the bee's first 10 landings were recorded. The bee was not allowed to return to the nest until she had completed at least 10 landings. This experiment was designed to confirm that the bees had learned to discriminate the artificial flower types based on colour, without being able to rely on scent cues deposited during training.

Experiment 2: illumination preference and colour discrimination in patchy light

In this experiment we used the previously described 'Battenberg design' (Fig. 2). The filters were swapped in a pseudo-random fashion between the two diagonally opposite possible Battenberg arrangements between bouts. As the bees were able to pass freely between the two illuminants, they could choose whether to visit both illuminants equally or favour one over the other.

Parallel horizontal strings (beige colour, 2 mm diameter) placed 7.5 cm apart marked out the boundaries of the quadrants in three dimensions (Fig. 2). Foraging bees did not collide with the strings or attempt to land on them, but if the bee crossed one of the strings it could be easily and unambiguously identified as a change of quadrant and therefore recorded as a switch between illuminant patches. This reduced error in human observation, based on oblique viewing angles, of when the bee switched quadrants.

A 40% sucrose reward was provided to bees on the purple artificial flowers. Two types of experiments were performed. The first one had only a single flower type (reward of 10 μ l volume on all 16 flowers, so 160 μ l in total) to explore the bees' illumination preferences without the added challenge of target colour discrimination (absolute conditioning). In the second type of test with patchy light, the same two colours had to be discriminated as in experiment 1 (purple and mauve; differential conditioning). Eight flowers were of the purple rewarding type, and therefore the reward volume was increased to 20 μ l so that the bee could satiate within

a foraging bout and return home, and an equal volume of quinine solution was used on the eight unrewarding mauve flowers.

Bees did not always visit all the flowers, but the volumes available within only one type of illuminant (80 µl) did not suffice for the bees to fill their honey stomach, so they always had to visit flowers in both types of illuminant in order to fill up. The artificial flowers were arranged so that there were equal numbers of each colour in each quadrant (i.e. four rewarding flowers per quadrant for the absolute conditioning treatment, and two rewarding and two unrewarding flowers per quadrant for the differential conditioning treatment).

Each bee's behaviour was recorded for five foraging bouts. Using the program ETHOM (Shih and Mok, 2000) on a laptop computer, we recorded each time the bee switched between illuminants (both leaf-shade to simulated daylight and *vice versa*), when the bee landed on stimuli under the leaf-shade and daylight patches, and whether these choices were correct or incorrect in the differential conditioning treatment. The ETHOM program records each event with a time stamp, so that it is possible to calculate how much time the bee spent in different illuminants.

To compare search times under both illumination conditions, we divided the total time spent under each illuminant by the number of artificial flowers visited during this time under the illuminant for each bee. This can be used as an estimate for the time taken to find and handle flowers, i.e. 'search time'; there is no *a priori* reason to expect flower handling time to vary significantly between illuminants. This will indicate whether, for example, the bee appears to be spending longer flying in the arena per artificial flower visit under the simulated leaf-shade illumination, indicating that it is taking longer to locate the targets under these illumination conditions.

In addition to quantifying illumination preference, it is also important to explore whether bees minimise the frequency of transitions between illuminants when they have a choice. To this end we employed a constancy index originally derived for flower constancy (a pollinator's tendency to remain faithful to familiar flower species) (Raine and Chittka, 2007), here used to measure 'illumination constancy' (fidelity to a particular illuminant). The index (Q) is calculated as:

$$Q = 0.5 [(A - B) / (A + B) + (C - D) / (C + D)], \quad (1)$$

where A represents the number of constant flights from X to X, B the flights from X to Y, C the flights from Y to Y and D the flights from Y to X. Q ranges from -1 to 1 , with 0 representing random choices (e.g. randomly choosing flowers in leaf-shade or daylight illuminant), -1 representing complete inconstancy and 1 representing full constancy to an illuminant (Chittka et al., 2001). If the bees chose flowers entirely at random, the illumination constancy index would be expected to be close to zero. Conversely, if bees minimise the number of illumination switches they have to endure during foraging, they will tend to stay in a quadrant as long as possible before leaving it and the constancy index will be positive. Such behaviour could be indicative of an attempt to minimise error opportunities as the bee might have to adapt with each illumination switch to the new light conditions before being able to identify flowers correctly.

Control 1: effect of pretraining

For this experiment, the bees were trained with a paradigm identical to that of the previous absolute conditioning scenario (i.e. all artificial flowers were the same colour and all were rewarding, so no choice was considered an 'error'), but each bee had a minimum of one full bout of foraging under uniform leaf-shade light before the start of

the experiment. Therefore, the green light was no longer unfamiliar to the bees at the start of the experiments. We then proceeded as before, recording the bee's choices and flight times under the two illuminants for five foraging bouts.

Control 2: effect of light intensity

In this experiment, we balanced the light intensity in daylight and leaf-shade patches by placing a neutral density filter over the arena lid in the daylight patches. Light transmitted through the neutral density filter had an intensity (25.8% of white light) similar to that transmitted through the leaf-shade filters (18.0% of white light) across the 300–700 nm range, but the filter did not change the spectral composition of the light. The experiment otherwise proceeded as for the absolute conditioning scenario as described above (all artificial flowers were the same colour and all were rewarding, so no 'incorrect' choices were possible).

RESULTS

Experiment 1: colour discrimination in two separate illuminants

In this experiment, the bees were presented with a colour discrimination task under either uniform simulated daylight illumination or uniform leaf-shade, and their choices during the rewarded training period and the unrewarded final test were recorded. If bee colour constancy is perfect, one would expect bees to learn to discriminate two coloured stimuli as quickly and accurately under one lighting condition as under another. Conversely, imperfect colour constancy may result in a poorer colour learning performance under some illuminants. In this experiment, we used a colour learning task under two different illuminants to test whether bees performed equally well under both illuminants, or whether there was a difference in learning speed or accuracy for one of the illuminants.

The bees initially chose between the two colours of artificial flower at chance level under both the daylight and leaf-shade illuminants (Fig. 3); this would be expected based on previous work showing that discrimination between very similar colours requires extensive conditioning (Dyer and Chittka, 2004d). Under both illuminants, the bees gradually learned to prefer the purple (rewarded) artificial flowers [general linear model (GLM), $F_{9,20}=3.579$, $P=0.008$]. During the training phase, learning appeared to occur faster under the daylight condition than under the leaf-shade condition, though this effect was not significant (GLM, $F_{9,20}=1.763$, $P=0.139$). However, in the unrewarded test at the end of the training period, the bees trained and tested under the daylight condition exhibited a better performance than those tested under the leaf-shade condition, selecting the correct colour more often (t -test, $t=1.78$, d.f.=28, $P=0.043$; Fig. 3).

Experiment 2: illumination preference and colour discrimination in patchy light

Because colours are more difficult to distinguish under the green illumination condition than in unobscured daylight, one might ask how this affects the foraging behaviour of bees when they have an opportunity to choose the illuminant in which to forage. It seems likely that they would exhibit a preference for the 'easier' illuminant, in which they perform more accurately, in particular when mistakes are punished. In this experiment, bees were presented with either one or two colours of artificial flower and foraged on them under patchy light conditions; in the absolute conditioning setup, all artificial flowers contained a reward, whereas in the differential conditioning setup only one colour was associated with a reward

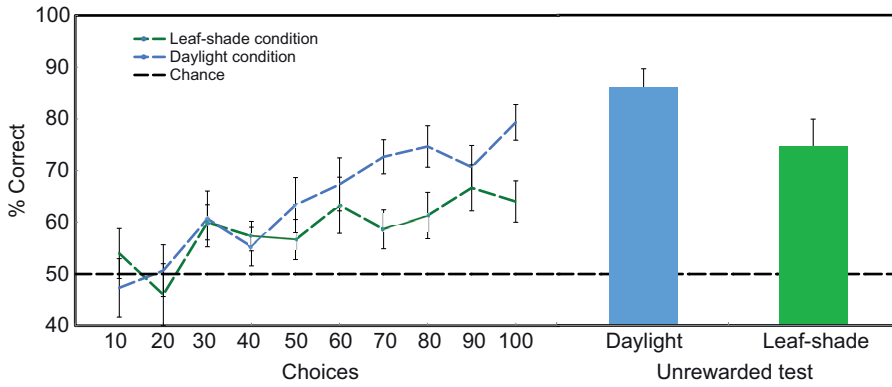


Fig. 3. Learning behaviour of bees under the two lighting conditions. The percentage of correct choices per block of 10 flower visits (\pm s.e.m.) and final unrewarded test results are shown. The unrewarded test results are the percentage of correct landings under the two illumination conditions, shown as means \pm s.e.m. for each treatment group. The results show that bees are more accurate at discriminating the two coloured stimuli under simulated daylight than under leaf-shade. $N=15$ bees under leaf-shade treatment, 15 bees under daylight. Bees were only used in one treatment.

and artificial flowers of the other colour contained a quinine penalty. If both colours of artificial flowers are equally rewarding, or if the unrewarding flower colour contains only water rather than quinine, then there is relatively little incentive for the bees to forage accurately (as the energetic cost of moving on to the next flower in a small flight arena is relatively negligible) (Chittka et al., 2003; Chittka and Spaethe, 2007). This means that one would expect the aversion to an illuminant under which it is more difficult to forage accurately to be more pronounced in a differential conditioning paradigm with quinine as a penalty.

We found that bees in both absolute and differential conditioning setups exhibited an initial preference for the simulated daylight illuminant (Fig. 4A). This preference was highly significant in the first bout (paired t -test, absolute conditioning: $t=-4.95$, d.f.=26, $P<0.0001$; differential conditioning: $t=-3.36$, d.f.=24, $P=0.0026$). However, this preference decreased with time, and by the end of the five bouts the bees spent equal time in both illuminants (paired t -test, absolute conditioning: $t=-0.63$, d.f.=26, $P=0.267$; differential conditioning: $t=-0.78$, d.f.=24, $P=0.446$). Because this decrease in preference occurred in both the absolute and differential conditioning treatments, it appears to be independent of the possibility of costly mistakes. In terms of artificial flower visits, the overall trends are similar (Fig. 4B): bees initially avoid flowers in the green leaf-shade illuminant, preferring to make visits under simulated daylight, but over time become more indifferent to the illuminant and visit artificial flowers in both types of patches equally often (paired t -test comparing initial and final preferences, absolute conditioning: $t=-5.00$, d.f.=25, $P<0.0001$; differential conditioning: $t=-4.67$, d.f.=23, $P=0.0001$).

Illumination constancy decreased in parallel with illumination preference (Fig. 5). In the early phase of the differential conditioning experiment, bees significantly minimised switches between illuminants, but this tendency vanished at the end of the observation period, when bees became indifferent to switching. A similar trend was observed in the absolute conditioning experiment in which only a single flower type was used, confirming that illumination preference and constancy were not directly linked to feedback on the difficulty of flower discrimination (Fig. 5).

One might expect that this progressive indifference to illumination conditions was the result of an increasing ability to discriminate colours in both conditions. This was not the case, however. The number of errors made under the two illuminants (in the differential conditioning paradigm in which choices of mauve artificial flowers were incorrect) was substantially higher in patchy light than in both illuminants in experiment 1, in which the illuminant was uniform and the bees could only see the artificial flowers under one illumination condition during a foraging bout. Although bees improved somewhat under the daylight illuminant, eventually

reaching a marginally improved performance of $57.4\pm 4.5\%$ correct choices in visits 41 to 50, they did not improve at all under the leaf-shade illuminant, finishing at a mere $53.3\pm 5.6\%$ correct choices under this lighting condition (Fig. 6). The final discrimination performance was not significant under either illumination condition

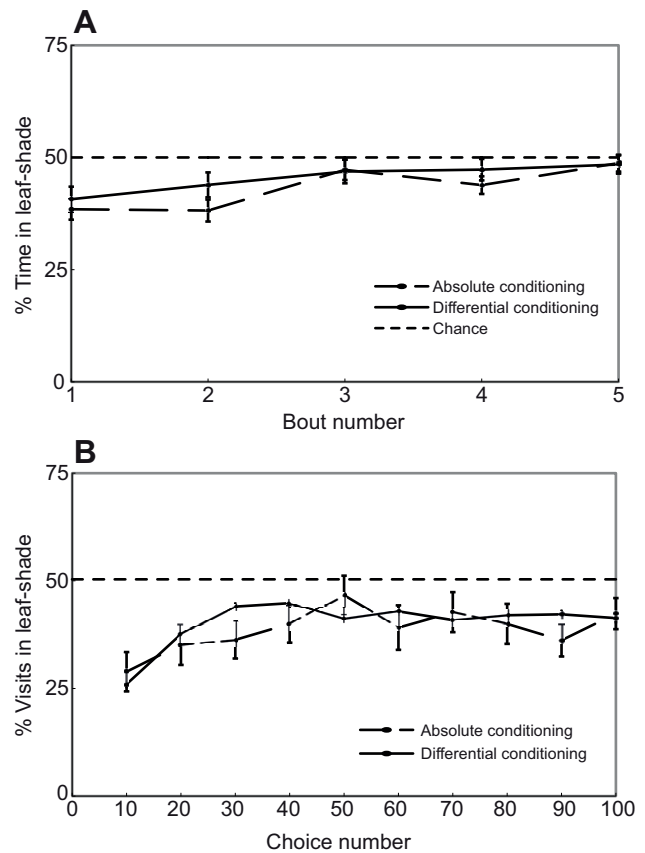


Fig. 4. Preference for the leaf-shade illuminant in the absolute and differential conditioning treatments. (A) Amount of time spent by bees over five foraging bouts (averaging 100 choices) under the two types of illumination, for two different training paradigms. In both cases, the bees initially showed a level of aversion to the leaf-shade, spending less than 50% of their time there, but by the end of the training showed no preference. Absolute conditioning: $N=27$ bees; differential conditioning: $N=25$ bees. (B) Flower visit data for the absolute and differential conditioning treatments. Over the course of 100 flower choices, initially the bees made fewer than 30% of their flower visits in the leaf-shade illuminant, but after the 100 visits they lost this aversion and the number of flower visits under the leaf-shade illuminant was not significantly different from chance. Absolute conditioning: $N=27$ bees; differential conditioning: $N=25$ bees. Data are means \pm s.e.m.

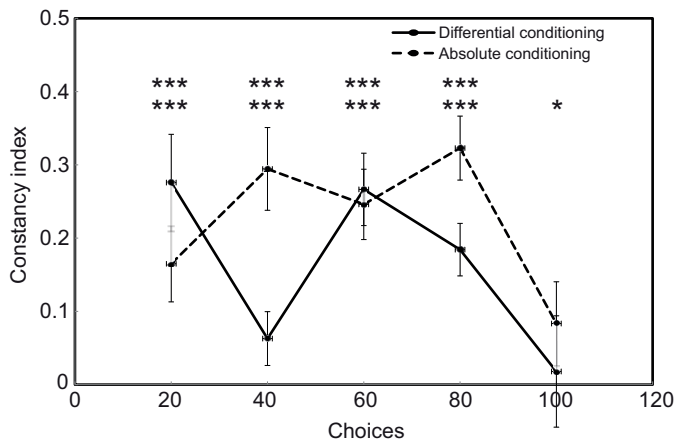


Fig. 5. Illumination constancy indices in patchy light conditions. Illumination constancy indices calculated according to Eqn 1 fluctuated somewhat with experience in both the differential and absolute conditioning paradigms. The significance of constant *versus* inconstant transitions between flowers was determined with a chi-squared 2×2 test (d.f.=1 in all cases). As 10 tests were performed, we used Bonferroni-adjusted α -levels of $0.05/10=0.005$ (*), $0.01/10=0.001$ (**), and $0.001/10=0.0001$ (***). The upper row of asterisks refers to differential conditioning and the lower row to absolute conditioning. Data are means \pm s.e.m.; $N=27$ bees at the start of the experiment, but only 12 of those bees completed 50 visits under both illumination conditions and were included as far as the final data point.

(daylight patches, $\chi^2=2.94$, d.f.=1, $P=0.08$; leaf-shade light, $\chi^2=2.33$, d.f.=1, $P=0.13$). It thus appears that under patchy light conditions, discrimination of similar colours is an extraordinary challenge for bees, even though the same colours are distinguishable under homogeneous lighting conditions. There was a slight increase in discrimination performance under unobscured daylight in the final phase of the testing (Fig. 6), which might explain why bees slightly avoided leaf-shade in this phase of the experiment (Fig. 7); however, neither effect was significant.

The average search times in both illuminants decreased between bouts 1 and 5 from 25.5 s per visit overall in bout 1 to 14.2 s per visit overall in bout 5 for the absolute conditioning treatment and from 20.7 s per visit in bout 1 to 18.6 s per visit in bout 5 for the differential conditioning treatment. However, we also found that the initial search times for foraging under the leaf-shade illuminant were consistently higher than the search times for the daylight illuminant. In the absolute conditioning treatment in the first bout, the bees averaged 58.0 s per flower visit under the leaf-shade illuminant, and just 24.1 s per visit under simulated daylight (paired t -test on log-transformed data, $t=2.118$, $P=0.046$). By bout 5, bees took only 15.0 s per flower visit in the leaf-shade and 15.2 s per visit in the simulated daylight (paired t -test on log-transformed data, $t=1.558$, $P=0.131$). Likewise, for the differential conditioning treatment, the initial time per visit was 30.5 s for the leaf-shade illuminant and 19.0 s per visit for the simulated daylight (paired t -test on log-transformed data, $t=3.135$, $P=0.005$), reducing to 21.8 and 18.3 s per visit, respectively, by bout 5 (paired t -test on log-transformed data, $t=-0.064$, $P=0.949$). The results indicate that the bees had more difficulty finding targets under the green leaf-shade illuminant when they had no previous experience with it, but showed a comparable performance at locating artificial flowers once they had experience.

Control 1: effect of pretraining

Bees in this treatment experienced absolute conditioning during the experiment in the same way as those in the absolute conditioning

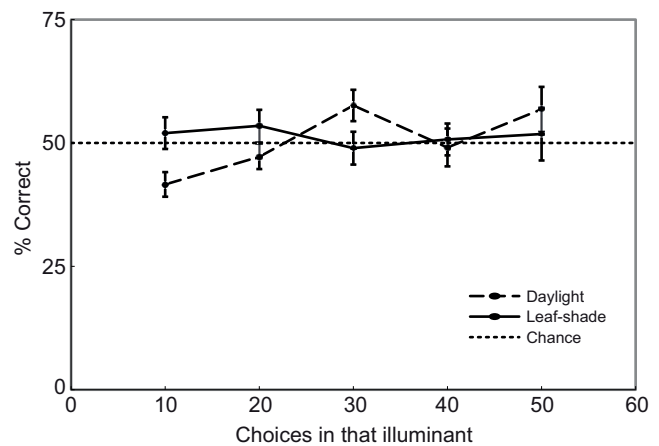


Fig. 6. Learning performance of bees foraging under patchy light. The percentage of correct choices made by bees in the patchy light setup of experiment 2, under leaf-shade and daylight illumination conditions. Note that not all bees completed 50 choices under leaf-shade illumination, and therefore only those who did are included in the later points. Also note that bees accumulated choices more quickly in the daylight patches, i.e. a bee that has made 20 choices under leaf-shade will likely have made considerably more than that by that time point under daylight, and therefore the leaf-shade choices represent a comparatively later learning stage. Data are means \pm s.e.m.; $N=25$ bees.

treatment of the previous patchy light experiments, but had received prior experience with green light so that green light was no longer an unfamiliar condition. Although the bees still showed a slight initial aversion to the green light (spending initially 44% of their time in the green light patches on average), this preference was not significant (paired t -test, $t=-1.37$, d.f.=14, $P=0.096$). This is confirmed by the flower visitation data shown in Fig. 7; the proportion of visits the bees with previous exposure to green light made under leaf-shade was initially significantly higher than the proportion of visits made by bees with no prior experience of leaf-shade (t -test, 44.2% of visits in leaf-shade *versus* 28.9%, $t=-1.75$, d.f.=36, $P=0.045$).

In the previous experiments in which the leaf-shade illuminant was unfamiliar to the bees whereas the simulated daylight was familiar, they initially took much longer finding flowers under leaf-shade than under simulated daylight. With pretraining, however, this difference was eliminated. In bout 1, the pretraining control bees visited flowers in leaf-shade at a speed of 15.2 s per visit, and in the simulated daylight at 16.4 s per visit, which is not a significant difference (paired t -test, $t=-0.425$, $P=0.339$).

As in Experiment 2, significant illumination constancy was observed, i.e. bees tended to make transitions between flowers presented under the same illumination. As above, a decrease in illumination constancy was observed between the first 20 visits and the final 20 visits made in the experiment (mean \pm s.e.m. constancy index $Q=0.23\pm 0.06$ during the first 20 visits, and $Q=0.16\pm 0.06$ during visits 81 to 100). Constancy was significant during visits 1 to 20 ($\chi^2=22.5$, d.f.=1, $P<0.0001$) and remained so, albeit at a slightly lower level, during the final visits 81 to 100 ($\chi^2=15.2$, d.f.=1, $P<0.0001$).

Control 2: effect of light intensity

In terms of flight time, the behaviour of the bees in this scenario was similar to that in the patchy light experiment with leaf shade *versus* unattenuated daylight, i.e. the bees initially avoided flying

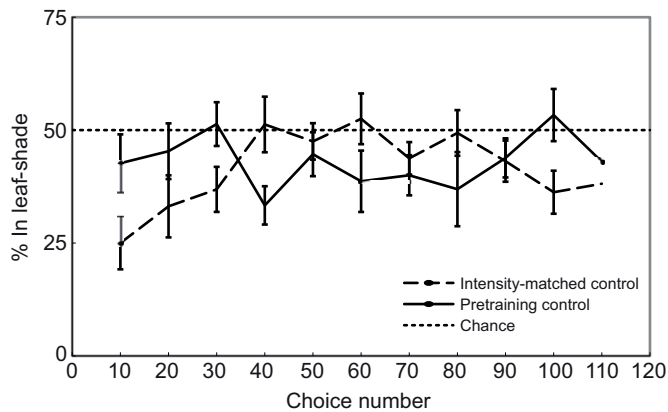


Fig. 7. Bee visit preference for artificial flowers in the two control experiments. In one control ('pretraining'), bees had been provided with prior experience of foraging in green leaf-shade light. In the other ('intensity-matched') control, the simulated daylight patches were reduced in intensity by application of neutral density filters so the daylight and leaf-shade patches had similar light intensity. The preference for the familiar simulated daylight illumination persists even when the intensity of the patches is similar. However, the preference for simulated daylight that was observed initially in bees with no prior experience with the illumination is not present in bees with leaf-shade foraging experience. Data are means \pm s.e.m.; $N=16$ bees for the intensity-matched control and 15 bees for the pretraining control.

in the green light patches. This preference is smaller than that for the initial absolute conditioning experiment (41.9% of time spent under leaf-shade *versus* only 38.6% in the original absolute conditioning experiment), but remains significant (paired *t*-test, $t=-3.80$, d.f.=15, $P=0.0009$), and the flower-visiting behaviour, shown in Fig. 7, is not significantly different from the original patchy light experiment (*t*-test, $t=-0.70$, d.f.=37, $P=0.245$). This indicates that although there may be a small tendency for bees to avoid areas with lower light intensity whilst foraging, this does not explain their preference for daylight over leaf-shade entirely.

The data on the visit speeds show that, similarly to experiment 2, bees searched more slowly for the artificial flowers under the leaf-shade illuminant in bout 1 (12.3 s *versus* 9.2 s per visit; paired *t*-test, $t=2.83$, d.f.=15, $P=0.006$), but this difference is largely eliminated as the bees gain experience with the leaf-shade illuminant (11.0 s *versus* 10.0 s; paired *t*-test, $t=1.12$, d.f.=15, $P=0.140$). This suggests that it is not merely the low light intensity in the leaf-shade patches that causes the bees to take longer to locate targets initially.

As in the other experiments with patchy light conditions, bees tended to remain constant to illumination conditions. As above, a decrease in illumination constancy was observed between the first 20 visits and the final 20 visits made in the experiment ($Q=0.3\pm 0.04$ during the first 20 visits and $Q=0.27\pm 0.06$ during visits 81 to 100), although this decrease was less pronounced than in experiment 2, so that constancy was still significant in the last 20 visits ($\chi^2=8.7$, d.f.=1, $P=0.0032$), but less so than during visits 1 to 20 ($\chi^2=27.5$, d.f.=1, $P<0.0001$).

DISCUSSION

When foraging in nature, bees often move in and out of patches of different illuminants, such as daylight and leaf-shade (Dyer and Chittka, 2004a; Lotto and Wicklein, 2005; Lythgoe, 1979). Unless they can compensate for such changes in illuminant, this could affect their perception of colours and possibly their ability to select rewarding flowers, especially if transitions between illumination

conditions occur frequently. To explore how bees cope with such challenges, we first selected a pair of flower colours that was difficult to discriminate and quantified bees' performance under two different illumination conditions, but where each bee only ever encountered one illuminant during the tests (experiment 1). We found that bees selected the correct flower colour less frequently under a light condition resembling leaf-shade than under conditions resembling natural daylight. Search times for flowers were also increased under green light relative to unobscured daylight.

We thus predicted that bees would, when given the choice between the two lighting conditions in a patchy environment, prefer to forage in the open rather than in leaf-shade. This was indeed the case, at least at the start of the bees' exposure to patchy light conditions, but over several foraging bouts this preference vanished and finally bees spent equal times in both illuminants. However, there are several lines of evidence suggesting that this preference for daylight is entirely independent of challenges related to flower identification. If bees have prior experience with homogenous leaf-shade illumination (control 1), the initial aversion to green light is not observed once bees have a choice between both illumination conditions in patchy environment. Our second control (where daylight and leaf-shade patches are intensity-matched) confirms that the initial avoidance of leaf shade in experiment 2 is related to the unfamiliarity of the spectral content of the illuminant, not its lower intensity. There is thus a clear tendency of bees to remain in familiar illumination conditions when possible.

We also predicted that bees might minimise transitions between lighting conditions, because each transition might necessitate adaptation to the new lighting and carry a risk of compromising colour identification before adaptation is complete. Indeed, bees initially avoided switching between illumination conditions in patchy light, but gradually became indifferent to such transitions. The initial avoidance of switching between patches of different lighting clearly indicates that bees perceive changes in overhead light (Dyer, 2006; Lotto and Chittka, 2005), thus not 'discounting' the illumination as one might expect from a perfect colour constancy algorithm (von Helmholtz, 1896).

However, this gradual change in behaviour was again independent of challenges of identifying the correct flowers. This is evidenced by the fact that bees' early avoidance of leaf-shade, as well as its eventual acceptance, occurred not only when bees were faced with a difficult discrimination task, but also when there was only a single type of flower (with all individual flowers containing a reward), so there could be no costs to making errors. Moreover, the decline in preference for one illumination, and constancy to either illuminant, was entirely independent of discrimination performance in the differential conditioning paradigm: bees never learnt to discriminate the colours in either pair of illumination quadrants under patchy light conditions. If the illumination preference of bees had been influenced by their own performance and colour discrimination, one might have expected them to confine their foraging to one type of illuminant until discrimination had improved, but this was clearly not the case. We conclude that although bees have a clearly documented preference for continuing to forage in familiar lighting environments, this is independent of any feedback they might use from foraging performance. The preference gradually declines as bees become familiar with a new lighting environment, but again independently of challenges related to colour identification.

Although at the end of training in patchy light conditions bees were marginally superior at discriminating colours under simulated daylight, performance was not significant at the 5% level for either illuminant. This near-complete failure of bees to discriminate

mauve and purple flowers under patchy light conditions is surprising, given that bees were able to discriminate these colours well in homogenous illuminations of both types in experiment 1.

One possible explanation is that the boundaries between illumination patches are not sharply defined, but that some light mixing occurs at the edges, plus there is of course scattering from all surfaces in the arena; these conditions also occur in naturally patchy lighting conditions. Such light mixing should, if anything, make the illumination less patchy and more homogeneous, i.e. closer to the conditions in experiment 1 – but under such homogenous conditions, bees displayed typical learning curves of progressively improved discrimination and even eventually learned to discriminate correctly under homogeneous leaf-shade (see experiment 1, Fig. 3). It is also clear from bees' lighting preferences and constancy in the patchy light that the patchy conditions were indeed perceived and the crossing of light patch boundaries was avoided. This indicates that subtle light mixing across patches is unlikely to provide a straightforward explanation for the poor colour discrimination performance in patchy light. Instead it is more likely that bees' eyes were never fully able to adapt to one or the other illuminant, because the small spatial scale meant that some eye regions might have typically viewed some surfaces in areas of the arena illuminated by the opposite lighting type, and because the bees regularly (although initially reluctantly) flitted in and out of lighting patches. These difficulties are, however, also expected to be of relevance in natural foraging under patchy light conditions.

It thus appears that bees are reluctant to switch illumination conditions, unless they already have extensive experience with more than one condition. Even though this reluctance is not a result of feedback from misidentification of flowers, it is nonetheless adaptive. Given the apparent difficulties of discriminating colours under patchy light conditions, foraging bees would do well under natural conditions to steer clear of such conditions and remain in homogeneous lighting conditions under which they have experience with identifying rewarding flowers. Our data on search times also indicate that flower detection in unfamiliar lighting conditions is challenging: initially, bees took longer locating artificial flowers under the leaf-shade illumination than under the daylight illumination, but only when the leaf-shade illuminant was unfamiliar. This included conditions when the daylight patches were of similarly low intensity. However, over time the search times under both types of illumination in all our tests became statistically indistinguishable. This means that location of the flowers, if not their correct identification, is possible for bees even under more than one illuminant, at least after extended exposure to both.

Our findings may have wide-ranging implications for foraging from natural flowers, as well as for colour signalling strategies in flowers. Bees in nature may adjust foraging routes to minimise switching between lighting conditions, which in turn would affect pollen flow between plants. However, boundaries between differently lit local patches will not remain stable during the course of the day, depending, for example, on the position of the sun and clouds, meaning that travel circuits might have to be adjusted, to some extent, from one foraging bout to the next. At larger spatial scales, some illumination patches are likely to remain relatively stable – lighting conditions within a forest or in an open field will be subject to change over small spatial scales only near the edges, not within. When given the choice, bees may confine their foraging activity entirely to such more homogeneously lit environments to avoid the added foraging cost imposed by increased search times and misidentifications of flowers in light climates that are either unfamiliar or to which the visual system will take some time to adapt.

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