

INNATE PREFERENCES FOR FLOWER FEATURES IN THE HAWKMOTH *MACROGLOSSUM STELLATARUM*

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Accepted 29 November 1996

Summary

The diurnal hawkmoth *Macroglossum stellatarum* is known to feed from a variety of flower species of almost all colours, forms and sizes. A newly eclosed imago, however, has to find its first flower by means of an innate flower template. This study investigates which visual flower features are represented in this template and their relative importance. Newly eclosed imagines were tested for their innate preferences, using artificial flowers made out of coloured paper or projected onto a screen through interference filters. The moths were found to have a strong preference for 440 nm and a weaker preference for 540 nm. The attractiveness of a colour increases with light intensity. The background colour, as well as the spectral composition of the ambient illumination, influences the choice behaviour. Blue paper disks against a yellowish

background are chosen much more often than the same disks against a bluish background. Similarly, under ultraviolet-rich illumination, the preference for 540 nm is much more pronounced than under yellowish illumination. Disks of approximately 32 mm in diameter are preferred to smaller and larger ones, and a sectored pattern is more attractive than a ring pattern. Pattern preferences are less pronounced with coloured than with black-and-white patterns. Tests using combinations of two parameters reveal that size is more important than colour and that colour is more important than pattern.

Key words: *Macroglossum stellatarum*, hawkmoth, Sphingidae, Lepidoptera, spontaneous choices, innate behaviour, colour vision, pattern vision.

Introduction

Approximately 1 day after eclosion, the imago of the hummingbird hawkmoth *Macroglossum stellatarum* starts searching for nectar sources. How does it find the first flower? Like other flower-visiting insects, it must be guided by means of an innate flower template (Darwin, 1877). The template may contain various features, such as odour (see Knoll, 1925, 1926; Ilse, 1928), colour (Lepidoptera: Ilse, 1928; Swihart, 1971; Scherer and Kolb, 1987*a,b*; Cutler *et al.* 1995; bumblebees: Lunau, 1990; Lunau *et al.* 1996; honeybees: Giurfa *et al.* 1995*b*; hoverflies: Ilse, 1949; Lunau and Wacht, 1994) and flower size and pattern (Lepidoptera: Ilse, 1933; honeybees: Lehrer *et al.* 1995).

Analysing spontaneous preferences is interesting for two main reasons. First, it is a very useful method for studying animals that cannot easily be trained to a particular stimulus, such as crickets (Campan and Lacoste, 1971) and beetles (Varjú, 1976; Dafni, 1997). In several lepidopterans that cannot easily be trained to flower colours, the feeding response has been described using this technique as a wavelength-specific behaviour, the sensitivity of which depends mostly on the blue, and to a lesser extent on the green, receptor (Scherer

and Kolb, 1987*a,b*; Cutler *et al.* 1995). In different contexts, the same animals might perform different wavelength-specific behaviours or even demonstrate true colour vision, as has been shown in the case of the honeybee (Menzel, 1979). Second, spontaneous choices might tell us which sensory cues are behaviourally important in the environment. Giurfa *et al.* (1995*b*) gave an ecological explanation for innate colour preferences: they have shown that honeybees are guided to flowers that promise a large amount of nectar. Lehrer *et al.* (1995) analysed pattern preferences in the same insect and concluded that flower-like patterns are preferred to others. They did not, however, work with flower-naïve bees.

The day-active hummingbird hawkmoth *Macroglossum stellatarum* is attracted by visual stimuli alone, although odours may also influence its motivational state (Brantjes, 1973). In contrast to other species (Knoll, 1926; Ilse, 1928; Brantjes, 1973; Scherer and Kolb, 1987*b*), this influence is not necessary to induce feeding behaviour in *M. stellatarum* (Knoll, 1922). Despite the fact that no electrophysiological recordings from its photoreceptors exist, it can be assumed that *M. stellatarum* has three spectral receptor types with

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sensitivities similar to those of other sphingids (*Manduca sexta*: 357 nm, 450 nm, 520 nm; Bennett and Brown, 1985; *Deilephila elpenor*: 350 nm, 440 nm, 525 nm; Höglund *et al.* 1973; Schwemer and Paulsen, 1973), bees (Peitsch *et al.* 1992) and other insects (Chittka, 1996). Electroretinograms recorded from *M. stellatarum* by Hasselmann (1962) show a fourth maximum at 620 nm, this could, however, not be reproduced by K. Bartsch (personal communication). Behavioural experiments (Knoll, 1922, A. Kelber, unpublished observations) suggest that a colour seen as red by human observers (e.g. HKS 23N, see Fig. 1B) is not discriminated from one having even a low reflectance over the complete spectrum (black for humans) by *M. stellatarum*. Therefore, in the absence of exact receptor data, one should be careful in interpreting such data. Thus, the spectral reflectance curves of the colours used in the experiments are given in Figs 1B,C, and human colour names are used.

Hummingbird hawkmoths are known to feed from a variety of flower species of almost all colours, sizes and forms (Müller, 1881; Knoll, 1922; Hasselmann, 1962; Herrera, 1990; Ebert, 1994; S. Vogel, personal communication; M. Pfaff, personal communication). The early colour discrimination experiments were performed by Knoll (1922). However, with the exception of only one experiment, Knoll (1922) did not work with newly eclosed moths and did not analyse their spontaneous preferences specifically.

In the present study, the choices of newly eclosed *M. stellatarum* for flower features are analysed in some detail. The cues tested include colour, flower diameter and pattern. Additionally, the influence of ambient light and background colours on the choice behaviour of the moths was tested. In natural environments, the spectral composition of the illumination varies greatly (Endler, 1993) and flowers might be seen against differently coloured backgrounds, such as the blue or cloudy sky, green leaves or a sandy ground. A flower-visiting insect should be able to identify food sources independently of these changing environmental conditions. Lunau *et al.* (1996) have demonstrated that chromaticity contrast has an influence on the colour choices of flower-naïve bumblebees. Colour constancy has been found in the honeybee (Neumeyer, 1981).

Experiments in which sizes or patterns are combined with colours reveal the relative importance of these three features. In the text, the stimuli (paper disks or lighted screens) presented to the moths are called artificial flowers, despite the fact that real flowers present much more complex stimuli than the artificial flowers used in these experiments.

Material and methods

Moths *Macroglossum stellatarum* L. were bred in the laboratory throughout the year. The day before eclosion, pupae were placed in dark cages, where the imagines spent their first day inactive and without food. The following day they were tested for their preferences in a 75 cm×75 cm×150 cm flight cage illuminated from above. In most experiments, the artificial

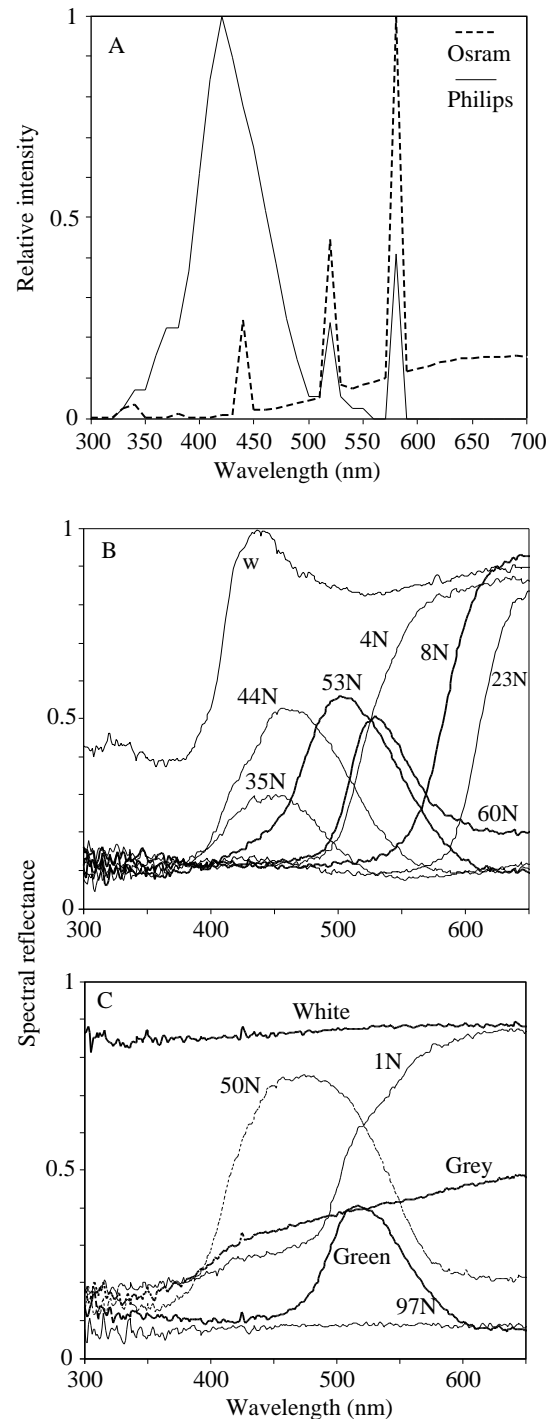


Fig. 1. Spectral curves of the lights and papers used in the experiments. (A) The spectral intensity curves of the two lamps used for cage illumination in the experiments using interference filters. (B,C) Spectral reflectance curves of the papers used in experiments on colour preferences. The colour numbers are the numbers of the HKS N paper series. These curves were measured and kindly provided by R. Menzel and M. Giurfa: 0, total absorbance; 1, total reflectance. (B) Colours used for artificial flowers: HKS 35N (violet), 44N (blue), 53N (blue-green), 60N (yellow-green), 4N (yellow), 8N (orange), 23N (red) and w (white). (C) Colours used as backgrounds for the artificial flowers: HKS 1N (light yellow), 50N (light blue), 97N (black) and grey, green and white.

flowers were paper disks 28 mm in diameter made out of standardized coloured paper (HKS N series produced by Hostmann-Steinberg in Celle, K+E Druckfarben in Stuttgart, H. Schmincke and Co. in Erkrath) and presented on vertical backgrounds, usually 20 cm×30 cm in size. The colour numbers in the text refer to the numbers of the HKS N paper series, and the spectral reflectance curves (Fig. 1B,C) were kindly provided by Professor R. Menzel, FU Berlin. To analyse preferences for spectral colours, the light from a halogen lamp was projected through interference filters (10 nm halfwidth) onto two frosted-glass disks (20 mm in diameter). A test flower (one of 12 wavelengths) and a reference flower (wavelength 470 nm) with equal quantal flux (0.5×10^{14} quanta $s^{-1} cm^{-2}$) were presented simultaneously in front of a white ultraviolet-reflecting background. During these experiments, the flight cage was illuminated with either an 18 W Osram Universal White tube or a Philips 40 W 09N tube (see Fig. 1A for the spectral intensity curves of these lights).

A test using a single moth lasted for as long as the animal was flying and touching artificial flowers. Like all sphingids, the hummingbird hawkmoth *M. stellatarum* sucks nectar whilst hovering in front of a flower; thus, touching the artificial flowers with its proboscis during flight is an unambiguous feeding reaction. This response was taken as a measure of the attractiveness of a feature in dual- or multiple-choice experiments (Knoll, 1922): each occasion when an animal approached and touched a flower with its proboscis was scored as a choice. Usually, a moth spent between 2 and 10 min touching the artificial flowers between 5 and 25 times. After a test, the moth was placed in a large flight cage, where it was fed and used for breeding. Each animal participated in only one test. In experiments using coloured paper flowers, eight different flowers were presented simultaneously in a horizontal row. In experiments using interference filters, only two colours were presented at a time, one of them being of a constant reference wavelength in all tests. In tests concerning flower size or pattern, 2–6 different flowers were presented. The spatial arrangement was changed randomly between individual animals to ensure that preferences for a particular position would not influence the results. The number of choices was summed for all animals used in equivalent tests. *G*-tests were applied to determine whether the choice distributions differed significantly from chance.

Results

Colour

In the first series of experiments, eight colours were presented on uniformly grey cardboard (Fig. 1C, grey). Given the choice between colours seen as red, orange, yellow, yellowish-green, bluish-green, blue, violet and white (not ultraviolet-reflecting) by humans (Fig. 1B), *M. stellatarum* only chose yellow, blue and violet (Fig. 2A). In this experiment, the very first choice was registered separately; out of 25 animals tested, 18 chose blue, four chose yellow and three chose violet first. All of them, however, subsequently

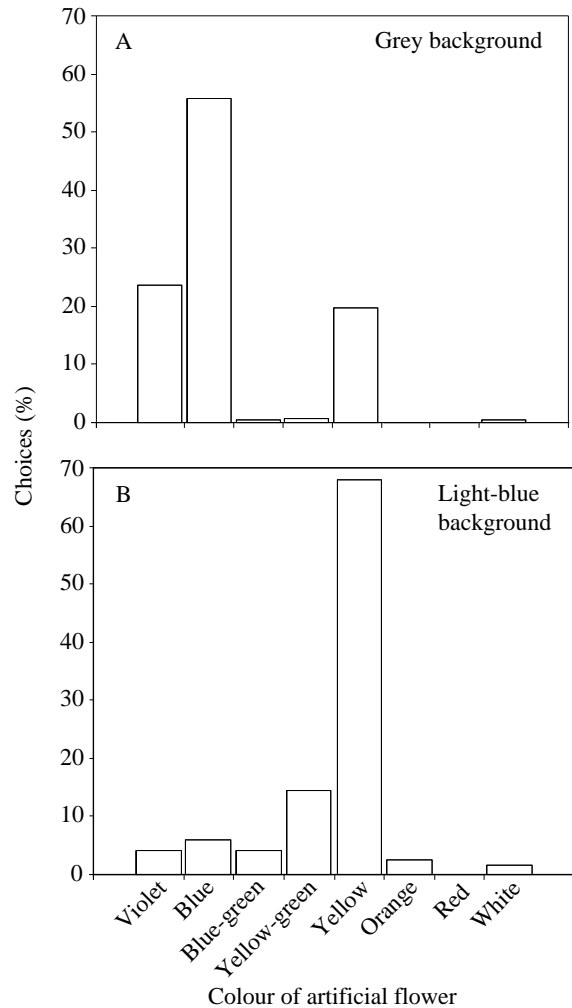


Fig. 2. Colour preference. Percentage of choices by *Macroglossum stellatarum* for the eight colours shown in Fig. 1B. Sixteen paper disks 28 mm in diameter were presented in two horizontal rows each containing all eight colours. The arrangement was changed between tests to avoid position effects. (A) Grey (see Fig. 1C) served as background; 371 choices by 19 moths. (B) Light blue (HKS 50N, see Fig. 1C) served as background; 202 choices by 35 moths.

visited the other two colours as well. The strong attractiveness of blue had been found previously in training experiments (Kelber, 1996).

When the moths ($N=22$) were tested with a set of eight colours including only green, yellow, orange and red (HKS 60, 2, 4, 5, 6, 7, 8 and 23; not all of these are shown in Fig. 1B), a result was found which cannot yet be explained: despite the fact that, in the first experiment, yellow had proved to be an attractive colour (Fig. 2A), only a small number of moths now visited the yellow flowers. A large number ($N=16$) of them did not respond to the testing arrangement at all. Thus, the presence of blue seems to have a facilitating effect in a similar way to that described for odours by Brantjes (1973).

The initial set of eight colours was also presented on backgrounds other than grey (for the spectral reflectances, see Fig. 1B). One example is shown in Fig. 2B (light-blue

background). The light-yellow (HKS 1N) and light-blue (HKS 50N) background colours were different from the blue and yellow flower colours (see Fig. 1B,C). Because, with all background colours, only blue (HKS 44N), violet (HKS 35N) and yellow (HKS 4N) flowers were chosen, visits to blue and yellow flowers were compared (Fig. 3). The moths hardly ever chose yellow on the light-yellow background and almost never visited blue on the light-blue background; the proportions of visits to these two colours were intermediate on green and grey backgrounds (Fig. 3A). On black and grey backgrounds, yellow is chosen somewhat more frequently than on a white background (Fig. 3B).

Using coloured papers, it is not easy to analyse the influences of hue and intensity independently. For this purpose, Perspex disks were illuminated through interference filters. The preference for monochromatic lights was tested at 14 wavelengths (380, 400, 410, 420, 440, 460, 480, 500, 520, 540, 560, 570, 600 and 620 nm), each tested against a reference light of wavelength 470 nm. All lights had a quantal flux of 0.5×10^{14} quanta $s^{-1} cm^{-2}$. The tests were conducted using two different illuminations of the flight cage (see Fig. 1A). The

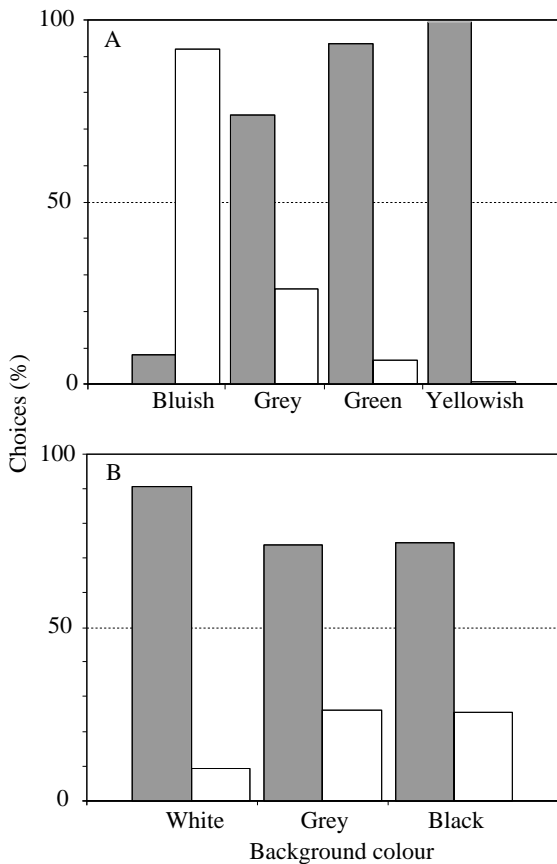


Fig. 3. Influence of the background colour on flower colour preference. Visits to HKS 44N (blue, shaded bars) and HKS 4N (yellow, open bars) artificial flowers presented on different backgrounds (abscissa, see Fig. 1C); 1196 choices by 154 animals. All choice distributions are significantly different from chance (G -test, $P < 0.001$); all distributions are different from one another (G -test, $P < 0.001$), except between those obtained with black and grey backgrounds (G -test, $P > 0.1$).

spontaneous choice curve obtained with the yellowish illumination (Osram Universal White) had a narrow major maximum at 440 nm and a minor peak at 540 nm (Fig. 4A). With the ultraviolet-bluish illumination (Philips 09N), two peaks were again found. However, the maximum at 540 nm was much more prominent and the entire curve was shifted to higher choice frequencies for the test wavelength (Fig. 4B).

Experiments investigating the intensity-dependence of the choices were carried out using 460 and 570 nm artificial flowers, again using 470 nm as reference wavelength. Both curves (Fig. 5) show similar sigmoid shapes. In the experiments using 570 and 470 nm, many animals headed for the brighter colour (570 nm) rather than the reference colour (470 nm) from far away (40–50 cm) and then changed their flight direction a few centimetres in front of the flowers. Thus, the influence of wavelength seems to be dominant over that of intensity.

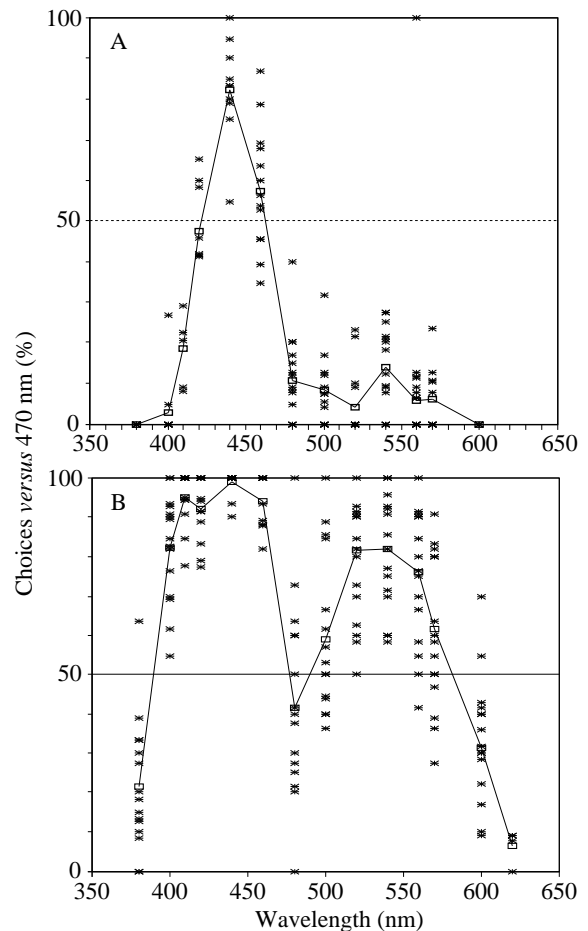


Fig. 4. Choices of flowers (projected onto screens through interference filters) of different wavelengths (abscissa) against blue (470 nm). All spectral colours had a quantum flux of 0.5×10^{14} quanta $s^{-1} cm^{-2}$. Each filled square represents the mean of at least 200 choices by 6–20 animals. Asterisks give the choice distributions of the individual animals. (A) Cage illumination using Osram Universal White (2965 choices by 208 animals). The choice distribution at 540 nm differs significantly from those at adjacent wavelengths (G -test, $P < 0.001$). (B) Cage illumination using Philips 09N (2798 choices by 474 animals).

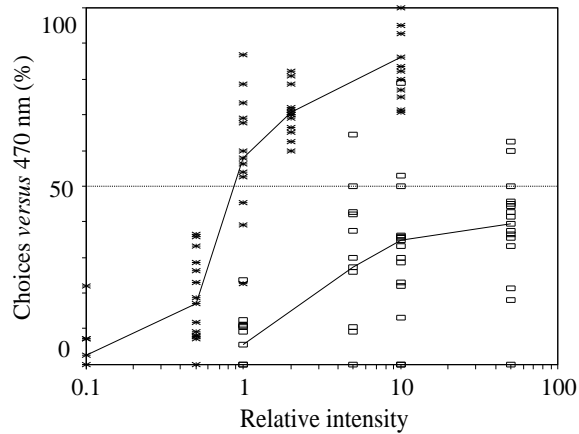


Fig. 5. Preference for higher light intensities. Values are percentage choices of each wavelength against the reference wavelength (470 nm). The intensities are normalized to the intensity (0.5×10^{14} quanta $s^{-1} cm^{-2}$) of the reference wavelength. Asterisks, 460 nm (1083 choices by 81 animals); open squares, 570 nm (904 choices by 60 animals). Cage illumination using Osram Universal White (see Fig. 1A). Lines indicate mean values for all animals.

Flower diameter

To test the role of flower size, three disks of different diameters were presented simultaneously in three different combinations. The animals preferred medium-sized disks (32 mm) to smaller and larger ones (Fig. 6A). The largest disks (128 mm) were hardly ever chosen. The reason for this behaviour might have been that they could no longer resolve the border when they approached a flower of this size (see Discussion). The preference was shifted to larger diameters when the disks carried a blue-and-white random-dot pattern (Fig. 6B).

Pattern choices

Initial experiments on pattern preferences turned out to be rather difficult. With several pairs of patterns, for example disks bearing horizontal *versus* vertical stripes, a pattern with long contours *versus* one with only short border lines (see Herrera, 1990) or symmetrical *versus* asymmetrical patterns, no distinct preferences could be found (data not shown).

Given a choice between a sectored and a ring pattern of equal size (36 mm diameter) and similar contour length (144 and 138 mm, respectively), *M. stellatarum* preferentially visited the sectored pattern. They behaved like the honeybees tested by Lehrer *et al.* (1995) using similar patterns. The preference was most pronounced with black-and-white patterns and less pronounced with violet-and-white and blue-and-white patterns (Fig. 7). That animals pay less attention to the pattern when the colour is attractive has also been observed in other Lepidoptera (Ilse, 1933) and honeybees (Giurfa *et al.* 1995a).

In the next experiment, small (8 mm diameter) yellow disks were attached to five of six larger blue disks (28 mm diameter) presented in a horizontal row. These spots enhanced the attractiveness of artificial flowers even when they had a less attractive colour than the large disk (Fig. 8). The position of the spot was less important, although a central spot was

somewhat more attractive than one positioned close to the periphery. This proved to be the case even with larger artificial flowers (64 mm diameter), despite the fact that, without a spot, these were preferentially touched close to the border (data not shown). With respect to the colour of a spot, results were similar to those for different colours on coloured backgrounds. On blue flowers, yellow spots were most attractive, and on yellow flowers, blue spots were preferentially chosen.

Hierarchy of flower features

Having tested the choices of *M. stellatarum* for three different features of artificial flowers (colour, size and pattern), the relative importance of these parameters in the moth's choice behaviour was then investigated. Pairs of artificial flowers were tested that differed from each other in two of the three parameters, e.g. either in colour and size, or in colour and pattern. Each pair of test flowers was chosen such that one

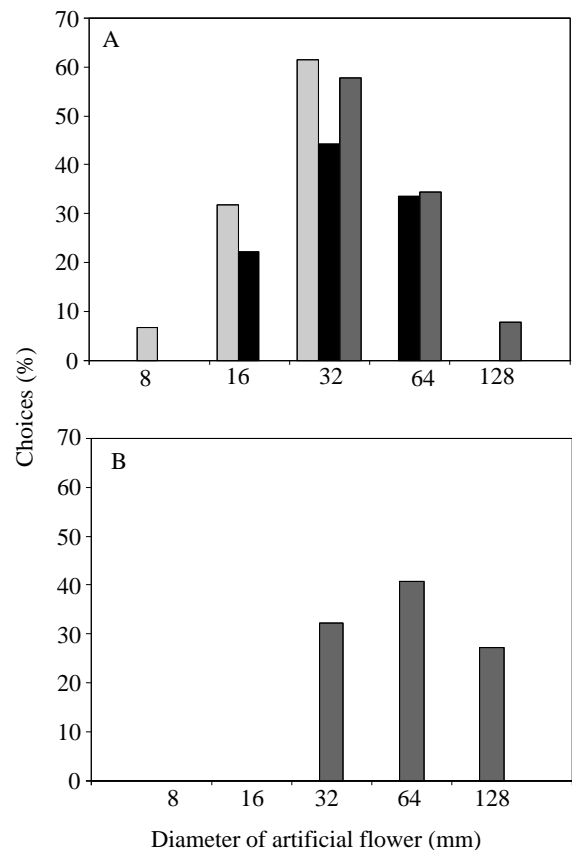


Fig. 6. Preference for disk diameter. (A) Three groups of animals were tested using sets of three blue disks of three different diameters. Light grey bars, choices between disks 8 mm, 16 mm and 32 mm in diameter, presented simultaneously. Filled bars, choices between disks 16 mm, 32 mm and 64 mm in diameter. Dark grey bars, choices between disks 32 mm, 64 mm and 128 mm in diameter; 512 choices by 48 animals. All choice distributions are significantly different from chance (*G*-test, $P < 0.001$, for the three intermediate sizes, $P < 0.01$). (B) Choices between disks with a blue-and-white random-dot pattern; 255 choices by 16 animals. This choice distribution differs from chance (*G*-test, $P < 0.05$) and from that among disks of the same diameters, but without a pattern (*G*-test, $P < 0.001$).

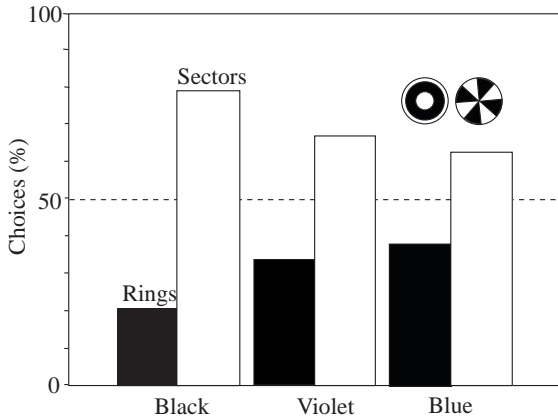


Fig. 7. Pattern preference. Choices between a sector and a ring pattern (inset) of different colours. The patterns had a diameter of 36 mm, the contour length was approximately the same and the amount of white and black was equal in both patterns. The background was grey in all experiments; 214 choices by 21 animals tested with black, 262 choices by 17 animals tested with violet, and 162 choices by eight animals tested with blue disks. All choice distributions are significantly different from chance (G -test, $P < 0.001$ for black and for violet, $P < 0.005$ for blue). The results using black patterns are significantly different from those using the other two colours (G -test, $P < 0.001$), whereas the results obtained using blue and violet are not significantly different from each other (G -test, $P > 0.1$).

flower had an attractive colour but an unattractive size (or pattern), whereas the other had a less attractive colour but a more attractive size (or pattern). The first step was to establish a pair of colours and a pair of sizes, as well as a pair of colours and a pair of patterns, in which choice frequencies were similar. The preference for violet when tested against yellow (Fig. 9, left pair of bars) was found to be similar to the preference for a sectored violet-and-white pattern when tested

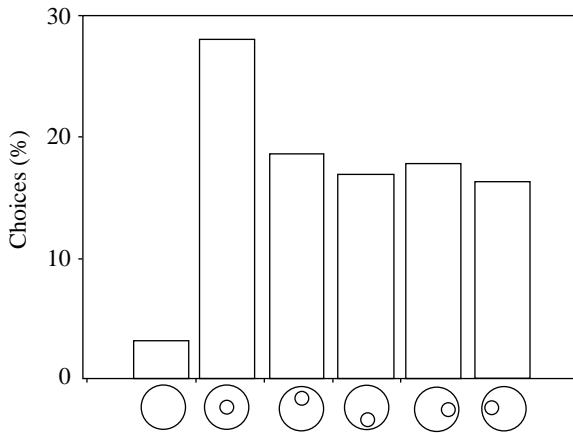


Fig. 8. Choices among blueflowers with small yellow disks ('nectar guides') placed in different positions; 587 choices by 29 animals. The choice distribution is significantly different from chance (G -test, $P < 0.001$). This also holds true for the choices among all five flowers with a 'nectar guide', but not among the four flowers with a 'nectar guide' close to the border (G -test, $P > 0.5$).

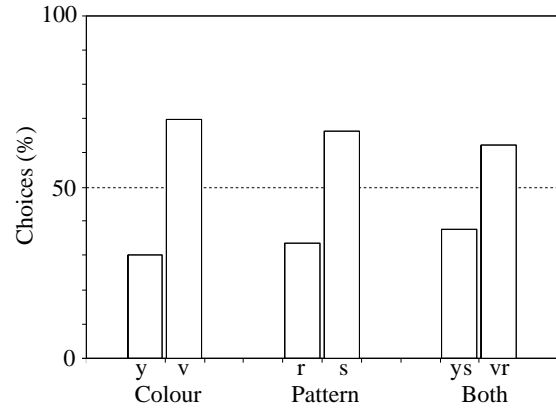


Fig. 9. Colour and pattern in competition. Different groups of moths were tested using yellow and violet flowers without patterns (y and v, left; 219 choices by 16 animals), violet flowers with ring and sectored patterns (r and s, middle; 262 choices by 17 animals) and yellow-and-white sectored and violet-and-white ring patterns (ys and vr, right; 236 choices by 13 animals). The colours and patterns were chosen such that the choice frequencies for the pair of colours and the pair of patterns were similar. Because the violet ring pattern is chosen much more frequently than the yellow sectored pattern (G -test, $P < 0.001$), colour must be the cue upon which *Macroglossum stellatarum* mainly relies in this test.

against a violet-and-white ring pattern (Fig. 9, middle pair of bars). Therefore, in the critical test, a yellow-and-white sectored flower was tested against a violet-and-white flower bearing a ring pattern. Similarly, because the preference for a blue flower when tested against that for a yellow flower (Fig. 10, left pair of bars) was similar to the preference for a

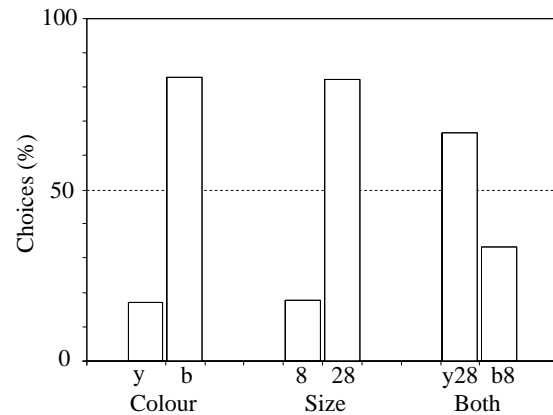


Fig. 10. Colour and size in competition. Different groups of animals were tested using yellow and blue disks 28 mm in diameter (y and b, left; 244 choices by 10 animals), blue disks 8 mm and 28 mm in diameter (8 and 28, middle; 525 choices by 34 animals) and blue disks 8 mm in diameter and yellow disks 28 mm in diameter (y28 and b8, right; 240 choices by 17 animals). The colours and diameters were chosen such that the choice frequencies for the pair of colours and the pair of sizes were similar. Because the large yellow disk was chosen much more frequently than the small blue disk (G -test, $P < 0.001$), size must be the cue upon which *Macroglossum stellatarum* mainly relies in this test.

blue flower 28 mm in diameter when tested against that for a blue flower 8 mm in diameter (Fig. 10, middle pair of bars), a yellow flower 28 mm in diameter was tested against a blue flower 8 mm in diameter in the critical test. The proportion of choices between the two flowers in each of the combinations ('colour-pattern' and 'colour-size') is expected to be 50% if the two parameters considered are equally important, but to be different from 50% if one of the two parameters is more important than the other. The results of these critical tests show that colour is more important than pattern (Fig. 9, right pair of bars) and that size is more important than colour (Fig. 10, right pair of bars). Thus, the hierarchy of the three parameters is size>colour>pattern.

Discussion

In the present study, using artificial flowers, the spontaneous choices of freshly eclosed hummingbird hawkmoths *Macroglossum stellatarum* for different visual flower features, such as colour, size and pattern, were examined in dual- and multiple-choice tests. Since the moths were tested prior to any visual experience and any uptake of food, and without presentation of other stimuli, this behaviour is likely to be innate to the adult moths. Although, in its natural habitat, this day-active moth feeds from flowers of various colours, shapes and sizes, the freshly eclosed animal first has to find a food source by means of an innate preference for particular colours, sizes and patterns. The expansion of the moth's preferences from the innate template is indirect evidence for the learning capability of this moth (Kelber, 1996). From the results of the present analysis it is concluded that the most attractive flower to a newly eclosed *Macroglossum stellatarum* would be a blue, radially sectored flower of approximately 32 mm in diameter and with a contrasting nectar guide situated at its centre. Size and colour are proved to be the more important than pattern.

Colour preferences

Hummingbird hawkmoths have a strong preference for wavelengths of 440 nm and a less pronounced preference for wavelengths of 540 nm (Fig. 4). The colours chosen in the experiments using coloured paper flowers reflect at these wavelengths (Fig. 1B; HKS 4N and HKS 44N). Additional influences are derived from light intensity (Fig. 5), background colour (Figs 2, 3) and the colour of the illuminating light (Fig. 4). The wavelengths attractive to *M. stellatarum* resemble those preferred by flower-visiting insects of other families (Nymphalidae, Pieridae, Satyridae: Scherer and Kolb, 1987a,b), other orders (honeybees: Giurfa *et al.* 1995b) and under other ecological conditions (e.g. nocturnal sphingids: *Manduca sexta*, Cutler *et al.* 1995; *Hyles gallii*, A. Kelber, unpublished data).

Despite these surprising similarities among species, there are also several differences. In contrast to the nocturnal sphingid *Manduca sexta* (White *et al.* 1994), newly eclosed adults of the day-active *Macroglossum stellatarum* do not visit white paper disks even when they absorb ultraviolet light. The records of

visits to white flowers (*Galium*, *Saponaria*, *Lonicera* spp.) by *Macroglossum stellatarum*, in the wild, may be due to colour learning rather than to innate preferences (Kelber, 1996). The spontaneous choice curves of butterflies (Scherer and Kolb, 1987a,b) are all similar with respect to the blue maximum, but differ in the yellow-red range. This may reflect the existence of a red-absorbing pigment in some of these species (Steiner *et al.* 1987).

From the present data, it cannot be determined whether spectral purity has an influence, as Lunau (1993b; Lunau and Maier, 1995) suggested for *M. stellatarum*, because this cue has not been analysed separately. A change in the spectral purity of a stimulus is always accompanied by a change in the amount of colour contrast between the stimulus and the background. Therefore, the effects of colour contrast and spectral purity are difficult to separate (see also Lunau *et al.* 1996). The single naive animal that was tested by Knoll (1922) using an arrangement of one yellow, one blue, one white, one black and 21 grey papers, chose yellow and blue. This observation is in accordance with the present results.

Wavelength-specific behaviour and colour vision

Since the spectral sensitivity functions of single photoreceptors have not been measured to date and since Peitsch *et al.* (1992) have shown that even small differences in spectral sensitivities strongly influence colour vision, it would be rather speculative to perform model calculations. If we assume the presence of three receptors, similar to those of the honeybee or *Manduca sexta*, an explanation similar to that proposed by Cutler *et al.* (1995) for *M. sexta* might be reasonable. The spontaneous choice curve of *M. stellatarum* could then be explained as wavelength-specific behaviour resulting from excitation of the blue and green receptors and inhibition of the ultraviolet receptor. The observed minimum at approximately 500 nm (Fig. 4) could be explained by inhibition between the blue and green receptor outputs either in the retina or at a later stage of visual information-processing.

Is colour-related behaviour in *M. stellatarum* restricted to wavelength-specific behaviour, as has been claimed for other Lepidoptera (Scherer and Kolb, 1987a,b; Cutler *et al.* 1995), or does this insect have true colour vision? Innate behaviour cannot be expected to be other than 'hard-wired' in the nervous system. It enables the newly eclosed adult to find its first nectar source. Lunau *et al.* (1996) assume that, in the bumblebee, spontaneous behaviour towards flower colours can be described in terms of true colour vision. In the hummingbird hawkmoth, there is no evidence for this at present. This does not mean, however, that the feeding reaction of a species remains restricted to the innate wavelength-specificity during its whole life. In learning experiments, *M. stellatarum* can be trained to visit colours different from those innately preferred (Kelber, 1996; Kelber and Pfaff, 1996). It seems, however, that such learning capacity differs among different species, possibly depending on the ecological needs of the animals (Kelber, 1996). Cutler *et al.* (1995) offered *Manduca sexta* a food reward during the tests, but reported no learning effect.

The influence of background and illumination colour

Lunau *et al.* (1996) found that a large chromaticity contrast between the colour of an artificial flower and its background leads to a high frequency of approaches by bumblebees. The results for hummingbird hawkmoths are similar. Thus, despite the fact that the sensitivities of the photoreceptors are not known, it can be assumed that chromaticity contrast between a flower and its background enhances its attractiveness.

The light reflected by the background could cause adaptation of the receptors such that they become more or less sensitive to the flower colour. Adaptation would have influenced the choice behaviour in exactly the direction observed: with ultraviolet-rich cage light, the ultraviolet receptor would be in a state of high adaptation and thus inhibit the preference less, resulting in a broadening of both maxima. With a yellowish background, and hence adaptation of the green receptor, the preference for blue would rise and, with the bluish background and hence adaptation of the blue receptor, the blue-green maximum would rise. The role of selective adaptation in the present task cannot be investigated further until the spectral sensitivity functions of the photoreceptors of *M. stellatarum* are known.

The role of flower size

Under natural conditions, *M. stellatarum* feeds from flowers of all possible sizes, including *Galium* spp. (3–4 mm in diameter) and *Lilium martagon* (40–50 mm in diameter). Thus, the initial size preferences reported here (Fig. 6) are abandoned later in life, on the basis of the animal's experience.

When Knoll (1922) tested the preferences of *M. stellatarum* between flowers of 10 mm and 40 mm in diameter, the moths preferred the smaller flowers. These animals, however, were not naive, and the flowers they had visited previously had a diameter of 8 mm. The preference was thus a clear result of learning. In experiments by Pfaff (1986), *M. stellatarum* spontaneously chose disks smaller than 55 mm in diameter. Flowers of 40 mm in diameter were touched mainly at the border, not in the centre, and they were often not touched at all. This is in agreement with the results presented here. Flowers with 'nectar guides', in contrast, were always touched close to, or even on, this contrasting spot, irrespective of the size of the flower and the position of the guide (Pfaff, 1986; see also Knoll, 1924).

In experiments with nymphalid butterflies, Ilse (1933) found the attractiveness of coloured paper flowers to increase with the size of the flowers. The butterflies landed on disks as large as 240 mm in diameter. Similar conclusions were drawn by Dafni (1997) investigating size preferences of the beetle *Amphicoma* sp: red model flowers were found to be the more attractive the larger they were. The preference for large flowers is probably closely related to colour preference: a large flower offers a large area of the preferred colour (see also Ilse, 1933). However, the results shown in Fig. 10 suggest that, at least in the hummingbird hawkmoth, size is more important than colour.

In the present study, when given a choice between flowers of

different sizes, *M. stellatarum* spontaneously preferred disks of 32 mm in diameter (Fig. 6). The discrepancy between the results obtained in the present study and those obtained by Ilse (1933) and Dafni (1997) might be due to the experimental procedure and the behaviour of the animals. Honeybees and beetles feed while sitting on the flowers; Ilse (1933) and Dafni (1997) therefore presented the model flowers on a horizontal plane and used, as the criterion for a choice, the animal landing on a flower. In the present study, the artificial flowers were presented on vertical planes, the choice criterion being touching a flower with the proboscis during free flight (see Materials and methods). It is possible that hovering in front of a pattern requires the presence of contours for stabilising the animal's position in space. *M. stellatarum* shows the strongest reaction to motion when it is seen with the frontal region of the eye (Kern, 1994). Farina *et al.* (1994) have shown that the reaction to expansion and contraction of projected artificial flowers depends on the number of contours, as long as these can be resolved. The contours are no longer seen with the frontal part of the eye when a large flower is approached closely enough to be touched (20–25 mm). This might explain why the moths avoided large disks (Fig. 6A). Patterned disks, however, offer contours not only at their boundaries but also within their area, which explains the shift of preference towards larger sizes when patterned flowers were used (Fig. 6B).

The role of nectar guides and flower pattern

Nectar guides are very common flower-pattern components in nature (Sprengel, 1793). These guides, which usually mark the entrance to the nectar reservoir, very often contrast in colour against the corolla and, according to Lunau (1993a), have a higher spectral purity.

Natural flowers differ in shape (geometry) as well as in pattern. Nectar guides are components of flower patterns, although the flower itself still possesses a particular shape. Knoll (1922) investigated the shape preferences of *M. stellatarum* by exploiting the fact that conspecific natural flowers of *Linaria vulgaris* differ in shape. He found that differences in flower shape did not influence the insect's choice behaviour as long as a nectar guide of the typical orange colour was present. The finding that colour is more important than shape is confirmed by the present study (Fig. 9). In a later study, using model flowers, Knoll (1924) concluded that nectar guides help the moth to find the entrance to the nectar reservoir of a flower with its proboscis. The present study again shows that contrasting spots representing nectar guides increase the attractiveness of flowers (Fig. 8). It is easy to understand how nectar guides act in attracting the insect, namely by presenting colour contrast against the corolla and by presenting additional contours, which makes it easier to stabilize the hovering position.

It is more difficult to understand the insect's spontaneous choice behaviour towards specific complex pattern features such as radially sectorised and ring patterns (Fig. 9). In order to recognise such patterns without previous learning processes, the insect's nervous system must be equipped with some type

of 'hard-wired' template tuned to global features such as radiating and tangential lines. A preference for radiating sectors and radiating bars over ring patterns and circular arrangements of bars has also been demonstrated in honeybees (Lehrer *et al.* 1995). On the basis of these and other results, Horridge (1994) postulated the existence of several types of 'pattern detectors' (filters) in bees, responding specifically to particular shape parameters, such as radiating patterns, tangential patterns and symmetry.

One problem of such a hypothetical mechanism, however, is whether the template functions in a space-invariant manner. In other words, whether a particular constant spatial relationship between the insect's eye and the pattern is required for the template to recognize the retinal image. Honeybees approaching a pattern presented in the frontal visual field were indeed shown to maintain a fairly constant spatial relationship between the eye and the pattern (Wehner and Flatt, 1977). In the case of the hummingbird hawkmoth, this question has not yet been investigated systematically. However, many casual observations of the approaches of these insects to flowers suggest that the moths view the flower with the lower frontal visual field. This particular eye region is equipped with several specializations that enhance spatial resolution (E. Warrant, K. Bartsch and C. Günther, in preparation) and it may therefore be the eye region most suitable for pattern analysis.

Thanks are due to D. Varjú for his support throughout the project, to K. Lunau for the loan of equipment and to R. Menzel for providing the spectral reflectance curves of the HKS papers. M. Pfaff, W. Pix and U. Henique carefully bred the moths, and M. Keller and H.-G. Hojak performed some of the tests. M. Pfaff, M. Giurfa and K. Bartsch are thanked for their readiness to discuss various aspects of the project. E. Warrant helped to correct the English, and the extensive and constructive comments made by two referees were very helpful.

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