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Colour Vision in Diurnal and Nocturnal Hawkmoths[1]

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SYNOPSIS. Diurnal and nocturnal hawkmoths (Sphingidae, Lepidoptera) have three spectral types of receptor sensitive to ultraviolet, blue and green light. As avid flower visitors and pollinators, they use olfactory and visual cues to find and recognise flowers. Moths of the diurnal species *Macroglossum stellatarum* and the nocturnal species *Deilephila elpenor*, *Hyles lineata* and *Hyles gallii* use and learn the colour of flowers. Nocturnal species can discriminate flowers at starlight intensities when humans and honeybees are colour-blind. *M. stellatarum* can use achromatic, intensity-related cues if colour cues are absent, and this is probably also true for *D. elpenor*. Both species can recognise colours even under a changed illumination colour.

INTRODUCTION

Hawkmoths are an important group of flower visitors and pollinators. The majority of species are active in the late evening or in the darkness of the night, and sphingids were assumed to use olfaction rather than vision to find and recognise flowers (Brantjes, 1978). Flowers that are typically pollinated by hawkmoths tend to have a strong odour, and hawkmoths have been shown to be highly sensitive to flower odours (*e.g.*, Raguso *et al.*, 1996) and they learn flower odours rapidly (Daly and Smith, 2000). Raguso and Willis (2002, 2003) recently convincingly demonstrated that the crepuscular hawkmoth *Manduca sexta* depends on olfaction *and* vision to feed from flowers. In this report, we focus on the visual cues that diurnal and nocturnal hawkmoths use for flower detection and recognition.

Hawkmoths have highly light sensitive superposition eyes and excellent vision (Warrant *et al.*, 2003). Most hawkmoths feed while hovering in front of flowers and use visual cues to control hovering flight (Farina *et al.*, 1994). The diurnal hummingbird hawkmoth, *Macroglossum stellatarum*, has long been shown to use visual cues to find flowers (Knoll, 1922), and the nocturnal species *Deilephila elpenor* was among the first insects where visual pigments have been studied. They have three spectral types of receptor sensitive to ultraviolet, blue and green light (Fig. 1, Table 1; Schwemer and Paulsen, 1973; Höglund *et al.*, 1973). The receptors have long fused rhabdoms and are surrounded by a tracheal tapetum. Two distal receptors are sensitive to ultraviolet or blue light, seven proximal receptors and one basal receptor are sensitive to green light (Welsch, 1977; Warrant *et al.*, 2003). *Manduca sexta* has a very similar set of receptors, where the blue receptors are more abundant in the ventral part of the retina (Bennett and Brown, 1985; Bennett *et al.*, 1997; White *et al.*, 2003). For *M. stellatarum*, only electroretinograms (ERGs) are available. An early study (Hasselmann 1962) showed an ERG peak in the red region but this peak was not found in a later study (Klaus Bartsch, unpublished results). Behavioural tests imply that *M. stellatarum* does *not* use red receptors for colour vision (see below; Kelber and Pfaff, 1997; Kelber and Hénique, 1999) and the available ERG data suggest it most probably has three receptor types, similar to other hawkmoths (Table 1).

Diurnal hawkmoths are known to visit a large variety of differently coloured flowers of all sizes and shapes (own observations; Müller, 1881; Ebert, 1994). Flowers that are typically pollinated by nocturnal hawkmoths, in contrast, tend to be white, cream-coloured or bright yellow (*e.g.*, Wyatt, 1983), thus offering a strong intensity (or achromatic) contrast to the green vegetation or the dark night sky. Instead of colour, nocturnal hawkmoths might thus use achromatic intensity contrast for flower detection and recognition in dim light. Honeybees, forced to feed under dim light levels (0.01 cd m^{-2}) are colour-blind (Menzel, 1981) just as humans are under scotopic conditions (*i.e.*, less than 0.005 cd m^{-2}). Here we report and discuss evidence that (i) both diurnal and nocturnal hawkmoths use colour vision, that they (ii) possess colour constancy and (iii) that hawkmoths can use achromatic, intensity-related cues for flower detection.

METHODS

Colour vision tests

Animals are said to have colour vision if they can discriminate two stimuli of different spectral composition, regardless of their relative intensity (*e.g.*, Kelber *et al.*, 2003). Several experimental methods can be used to test colour vision and to exclude intensity-related or achromatic cues (Kelber *et al.*, 2003). Sphingids are nectar-feeders that feed while hovering in front of flowers. They express innate preferences for flower-like stimuli (Kelber, 1997) but some species can easily be trained to associate visual cues with a reward of sucrose. In training experiments, we have used two methods to study their colour vision.

First, the animal is required to discriminate two spectral lights of fixed intensity (in the experiments reported here, two lights emitting the same number of

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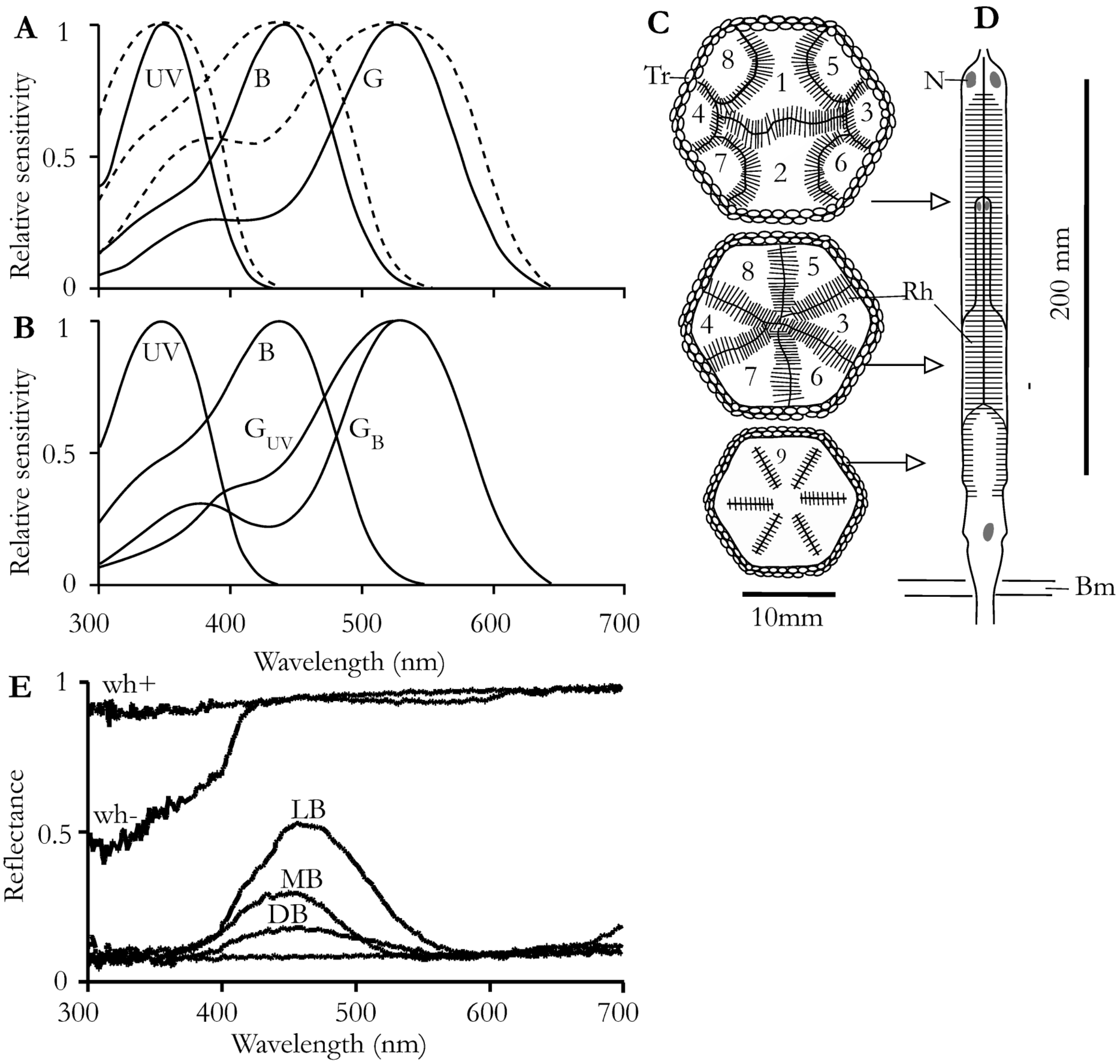


FIG. 1. Photoreceptors of *Deilephila elpenor*. **A.** Spectral sensitivity of the three receptor types of *D. elpenor*. *UV* ultraviolet receptor, *B* blue receptor, *G* green receptor, curves with *solid lines* calculated using data from Schwemer and Paulsen (1973) and the rhodopsin template of Stavenga, Smits and Hoenders (1993). *Dashed lines* are calculated from these curves for open rhabdoms of 414 μm length and with a typical absorption coefficient of 0.0067 μm^{-1} (Bruno *et al.*, 1977). **B.** Spectral sensitivity curves for the closed and tiered rhabdoms of *D. elpenor*, assuming that both distal receptors are either ultraviolet or blue receptors in one rhabdom. G_B and G_{UV} depict the green receptors in each of these two ommatidial types. Green receptors in ommatidia with one B and one UV receptor have intermediate sensitivity curves. **C** and **D.** Schematic drawings of the structure of the rhabdom of *Deilephila elpenor* (adapted from Schlecht *et al.*, 1978). *Bm* basement membrane, *N* nucleus, *Rh* rhabdom, *Tr* tracheal tapetum. *Numbers* refer to anatomical receptor types, where receptors 1 and 2 are blue or ultraviolet receptors and receptors 3–9 are green receptors. **E.** Spectral reflection of colours used in experiments. Two colours that look white to humans but differ in the reflection in the ultraviolet range (*wh*+ and *wh*−) were used in experiments on *Deilephila elpenor*. Three shades of blue (light blue, *LB*; medium blue, *MB* and dark blue, *DB*) and black (*unlabelled curve*) were used in experiments on *Macroglosssum stellatarum* (Fig. 7).

TABLE 1. *Sensitivities of the photoreceptors of three species of hawkmoths*.*

Species	UV	Blue	Green	Reference
Deilephila elpenor	345–350	440–450	520–525	Höglund *et al.*, 1973; Schwemer and Paulsen, 1973
Manduca sexta	357	450	520	Bennett and Brown, 1985
Macroglossum stellatarum	348–360	440–460	530–550	Hasselmann, 1962; K. Bartsch, unpublished results; E.W., unpublished results

* Sensitivity peaks were obtained using microspectrophotometry and electroretinograms. Data for *M. stellatarum* are preliminary.

photons). The animals are trained to associate one colour with a food reward and the other colour with no reward. In the critical tests, relative intensities of the lights are changed. An animal that uses colour will still choose the stimulus of the learned wavelength. An animal that uses an intensity-related cue will change its choice behaviour. Moths of *Macroglossum stellatarum* were tested as naive animals and trained using this method (Kelber, 1997; Kelber and Hénique, 1999). These diurnal hawkmoths approach and probe coloured stimuli with the proboscis indicating that they search for a nectar reward.

The second method was introduced by von Frisch (1914) to study colour vision in the honeybee. Animals are trained to associate a reward with a colour. Instead of one single unrewarding stimulus, many shades of grey are used. It is assumed that one of these many shades looks equally bright to the tested animals. If the animals are able to discriminate the colour from all shades of grey, it is assumed that they use colour vision. This method is especially convincing if animals do not use intensity-related cues, for instance two different shades of the same colour. We used it to study moths of *Deilephila elpenor*, *Hyles lineata* and *Hyles gallii*. Moths were trained to associate a colour (blue or yellow) with a reward of sucrose solution. They were then tested whether they discriminated the training colour from 8 different shades of grey (gr-1 to gr-8), from two additional colours and from a brighter and a darker shade of the training colour (Kelber *et al.*, 2002).

Experimental procedures

Larvae of *Macroglossum stellatarum*, *Deilephila elpenor*, *Hyles lineata* and *Hyles gallii* were fed their natural food plants to avoid visual pigment deficiencies, and pupae were kept under appropriate conditions, *i.e.*, 5°C for hibernating pupae (*D. elpenor*, *H. lineata* and *H. gallii*) and 20° for *M. stellatarum*. Adult nocturnal moths were kept under a 12:12 hr dark:light regime for the duration of the entire experiment, but diurnal moths were kept in dark moth containers between experiments. Training started one day after eclosion, with *M. stellatarum* and two days after eclosion with nocturnal hawkmoths. The experimental cage was 50 cm deep, 60 cm high, 70 cm wide and illuminated from above. For experiments in dim light, a broad spectrum UV-visible high-pressure mercury lamp (Leitz) could be dimmed by quartz neutral density filters (and a broad-spectrum yellow Schott FG-13 filter). Most experiments with *M. stellatarum* were performed in bright light provided by three fluorescent tubes. Spectral colours were produced using either a bright halogen lamp or a Xenon lamp together with narrow interference filters. Coloured paper stimuli were circular (28 or 30 mm in diameter) and presented vertically on the cage wall. Regular white printer paper served as UV-absorbing white (*wh*−), and laboratory filter paper as UV-reflecting white (*wh*+). Reflection curves for both colours are given in Fig. 1E. For some experiments on *M. stellatarum* papers of the HKS series were used, the reflection of these colours (HKS 50, *LB*; HKS 44, *MB*; HKS41, *DB*) are also given in Fig. 1E. Colours used in experiments on nocturnal hawkmoths were generated using an Epson colour printer and Epson ink jet paper. We give the quantum catches that these colours produce in the eye of *Deilephila elpenor*, under the lowest light intensity used in the experiments, in Fig. 4. A light grey background (*back*, in Fig. 4) was used in most experiments, but training to white stimuli required a black background otherwise the animals would not make an approach (for details see Kelber, 1997; Kelber and Hénique, 1999; Kelber *et al.*, 2002).

20% sugar solution was provided as a reward in the reversed tip of a syringe that could be accessed through a 3 mm wide hole in the centre of the training stimulus. One animal was released at a time. During training, it approached the stimulus, extended its long proboscis, and probed the stimulus until it found the hole leading to the sugar reservoir. During tests, no reward was present and the moth chose between different colour disks presented simultaneously, by touching them with the proboscis. The first colour disk probed during an approach was taken as the moth's choice. The positions of colours were changed regularly in a pseudo-random manner to avoid any influence from spatial cues. *G*-tests were used to decide whether the choice frequency for the training colour was different from random choice.

Light measurements and calculation of quantum catches

Light reflected from stimuli was measured using an International Light IL 1700 radiometer and a calibrated Ocean Optics S2000 spectrometer. We calculated photon catches for two ommatidial types found in the eyes of *Deilephila elpenor*, one containing two distal UV receptors and seven proximal green receptors, and one with two distal blue receptors and seven proximal green receptors. It is most probable that there is a third type of ommatidium with one ultraviolet receptor, one blue receptor and seven green receptors. Green receptor quantum catches of this ommatidial type are intermediate between those in the other two types, and quantum catches of blue and ultraviolet receptors must be smaller for this type. Evidence that *D. elpenor* has different ommatidial types similar to other lepidoptera comes from data on this (Fig. 1C, D; Welsch, 1977; Schlecht, 1979) and on the closely-related species *Manduca sexta* (White *et al.*, 2003). We calculated the number of photons N that are absorbed by each photoreceptor type in one ommatidium per integration time of the photoreceptor, using optical and electrophysiological data for *D. elpenor* and the following equation (Warrant, 1999; Kelber *et al.*, 2002):

$$N = 1.13\left(\frac{\pi}{4}\right)n\Delta\rho^2 D^2 \Delta t \int \kappa\tau[1 - e^{-kR_i(\lambda)l}]I(\lambda)\, d\lambda \quad (1)$$

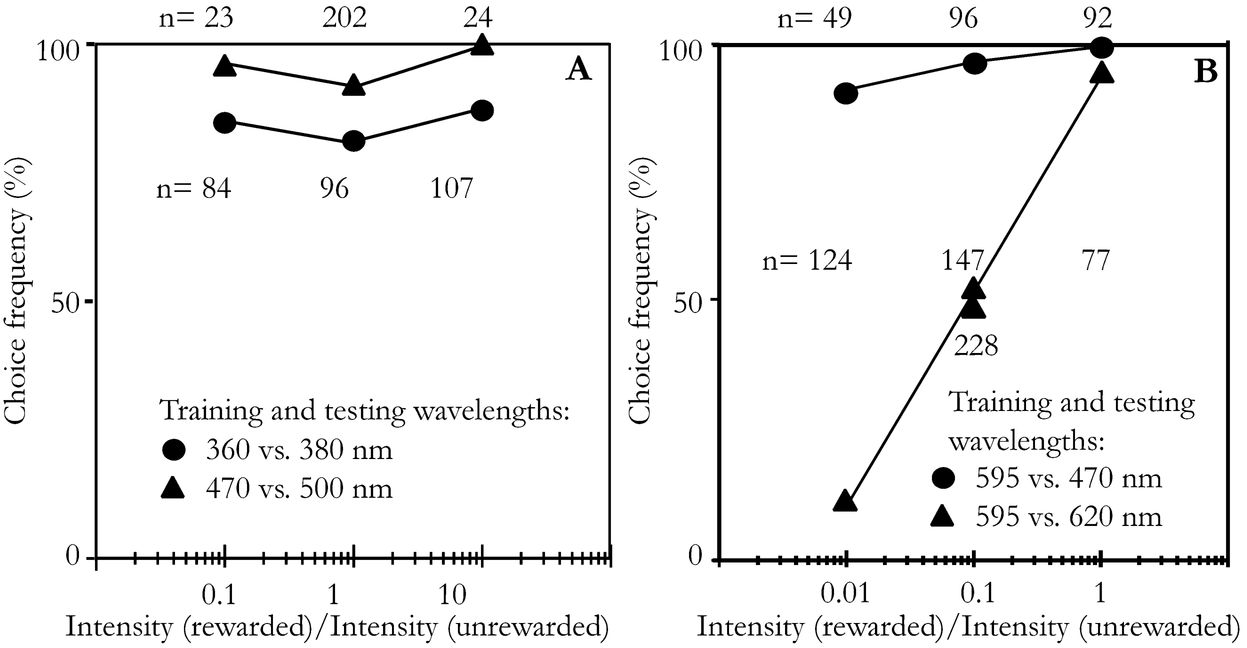


FIG. 2. Discrimination of chromatic and achromatic cues in *Macroglossum stellatarum*. Moths were trained to discriminate two lights of different wavelength (see *insets*) but equal photon flux (10^{13} photons sec^{-1} cm^{-2}). In unrewarded tests the intensity ratio of the lights could be changed by either reducing the intensity of one stimulus by a factor of 10, increasing the intensity of one stimulus by a factor of 10 or changing both intensities. **A.** Moths use colour vision to choose between lights of 360 and 380 nm (*filled circles*), and between 470 nm and 500 nm (*triangles*). All choice frequencies are significantly different from chance (*G*-tests, $P < 0.001$). **B.** Moths discriminate between 470 nm and 595 nm as colours (*G*-tests, $P < 0.001$) but use an achromatic cue to discriminate between 595 and 620 nm (at an intensity ratio of 0.1, choices are not different from chance, *G*-tests, $P > 0.1$; at intensity ratios of 1 and 0.01 they are different from chance, *G*-tests, $P < 0.001$). We deduce from this result that they use only one receptor type in this spectral range, and that this receptor type is more sensitive to light of 595 nm than to light of 620 nm. For details and additional data, see Kelber and Hénique (1999).

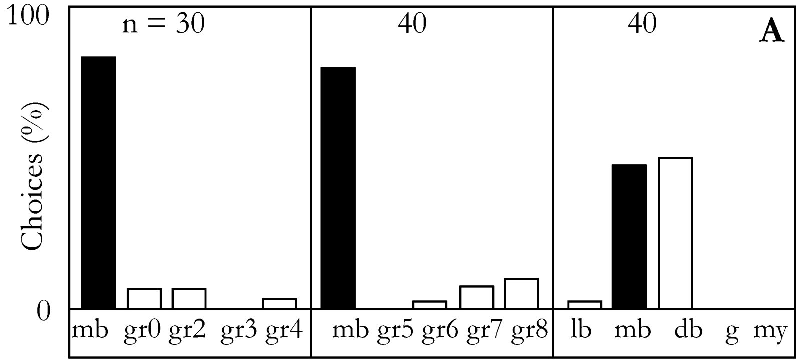
where $I(\lambda)$ is the stimulus light intensity in photons sec^{-1} nm^{-1} m^{-2} $steradian^{-1}$, the constant 1.13 accounts for the Gaussian shape of the receptive field of a receptor with acceptance angle $\Delta\rho$, $\Delta\rho$ is the photoreceptor acceptance angle = 3.0°(0.0524 rad, half-width of the receptive field; E.W., unpublished data), n is the number of effective facets in the superposition aperture = 568 (E.W., unpublished data), D is the diameter of a facet lens = 29 μm (E.W., unpublished data), κ is the quantum efficiency of transduction = 0.5, τ is the transmission of the ocular media = 0.8, Δt is the integration time of a photoreceptor = 0.036 sec (E.W., unpublished data), k is the absorption coefficient of the rhabdom = 0.0067 μm^{-1} (Bruno *et al.*, 1977), l is the rhabdom length, doubled by tapetal reflection = 414 μm, and $R_i(\lambda)$ is the spectral sensitivity of photoreceptor i (i = 1,2,3), calculated from the recorded sensitivity maxima (Höglund *et al.*, 1973; Schwemer and Paulsen, 1973) using the Stavenga-Smits-Hoenders rhodopsin template (Fig. 1A, *solid lines*; Stavenga *et al.*, 1993). We also calculated the quantum catch in a hypothetical ommatidium that contains only green receptors (Fig. 1A, *dashed line* for the green receptor).

COLOUR VISION

Flower-naive moths of *Manduca sexta* and *Macroglossum stellatarum* prefer blue and yellow as flower colours and avoid ultraviolet flowers (White *et al.*, 1994; Cutler *et al.*, 1995; Kelber, 1997). This is an indication that both species most probably use colour vision to choose flowers. Training experiments were performed to study colour vision abilities in more detail in *M. stellatarum* and *Deilephila elpenor*.

M. stellatarum were trained and tested with spectral lights of various wavelengths between 360 and 620 nm. After training to a rewarded and an unrewarded colour, in dual choice tests, they chose correctly even if one of the colours was made ten times brighter than the other colour (Kelber and Hénique, 1999). In the experiments shown in Figure 2A, moths discriminated colours that differed by only 20 or 30 nm in wavelength. Colours in the ultraviolet range were learned more slowly (Kelber and Hénique, 1999) and discriminated less precisely than colours in the human-visible range (Fig. 2A, *circles*). Animals trained to associate a light of 595 nm wavelength with a food reward and 470 nm with no reward, chose correctly even when the rewarded colour was 100 times dimmer than the unrewarded colour (Fig. 2B, *circles*). *M. stellatarum* moths could be trained to a new pair of colours every day, for several days in a row (Kelber and Hénique, 1999). Their learning capability enables them to find rewarding food sources during their long migration from the Mediterranean to central and northern Europe (Müller, 1881).

D. elpenor moths were able to discriminate each of the training colours (yellow, *my* in Fig. 3B, and blue, *mb* in Fig. 3A) from 8 shades of grey (*gr0* to *gr8*, in



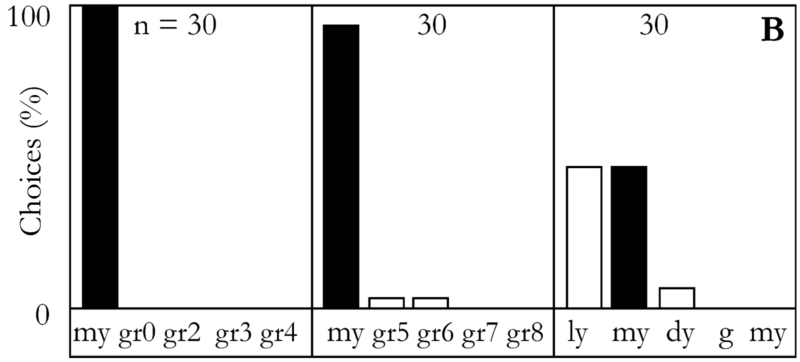


FIG. 3. Colour vision in *Deilephila elpenor*. **A.** Moths trained to blue artificial flowers discriminated the training colour, a medium shade of blue (*mb*; *black bars*) from four bright shades of grey, *gr0* and *gr2–gr4*, (*left*; *G*-test, $P < 0.001$), four dark shades of grey, *gr-5–gr-8*, (*centre*; *G*-test, $P < 0.001$) and from yellow (*my*), green (*g*) and light blue (*lb*), but not from a darker shade of blue (*db*; *right*). **B.** A different group of moths were trained to yellow and tested in the same way, using a lighter shade (*ly*) and a darker shade (*dy*) of yellow in addition to the colours mentioned above. Ambient light intensity during both tests was 0.0001 cd m^{-2}. For details and additional data, see Kelber *et al.* (2002).

Fig. 3) and from other colours (yellow or blue, and green, *g* in Fig. 3). They did not discriminate well between different shades of the training colour (*ly* and *dy*, and *lb* and *db*, respectively) indicating that they did *not* use achromatic (intensity-related) cues for discrimination in this experiment. This does not, however, prove that they are not able to use intensity-related cues for discrimination. The results shown in Figure 3 were obtained at a light intensity of only 0.0001 cd m^{-2}—a light intensity similar to light levels on a starlit night. All 16 animals used in both training experiments performed similarly well under brighter light levels (Kelber *et al.*, 2002). Of these 16 animals only seven were motivated to choose colours in tests at starlight intensities suggesting that this intensity is close to their threshold of colour vision. In a separate experiment, we trained three moths to discriminate between two colours that look white to the human eye, but differed in reflection in the ultraviolet range (*wh*+ and *wh*−, in Fig. 1E). The moths discriminated both colours extremely well (23 of 23 choices, *G*-test, $P < 0.001$) proving that the colour vision of *D. elpenor* also extends into the ultraviolet range.

We calculated the number of photons captured by the receptors in each ommatidium, for starlight intensities, and found that the numbers are between 1 and 70 photons, per receptor channel and integration time of the receptors (equation 1; Fig. 4). for the blue training colour (*mb*, in Fig. 4), the quantum catches are between 1 and 16 photons. With these numbers of photons, photon shot noise ($\sqrt{N}$; for $N = 16$, $\sqrt{N} = 4$) would make discrimination from similar shades of grey (*gr6* and *gr7*, in Fig. 4) impossible. We therefore propose that *D. elpenor* must use spatial and/or temporal summation (Warrant, 1999) to improve the signal-to-noise ratio and allow the colour discrimination we observed. For comparison, we also calculated the number of photons captured by a hypothetical ommatidium with only one receptor type, and it turned out that this ommatidium would capture more photons (Fig. 4, black bars). This difference is even more pronounced under the natural illumination of sunlight. By having three spectral types of receptor, the hawkmoths thus sacrifice absolute sensitivity, and this suggests that colour vision may be highly relevant for them.

We trained and tested moths of *M. stellatarum*, *H. gallii* and *H. lineata*, in a similar experiment to that we used for *D. elpenor*, to determine under which light levels these species chose the correct colour in tests. The results are summarised in Fig. 5. *M. stellatarum*, as a strictly diurnal species, stopped approaching the stimuli at 0.05 cd m^{-2} when only one of eight animals was still approaching stimuli. The remaining seven animals were still able to fly and land safely but instead of approaching stimuli they searched a spot on the cage wall where they settled down. They approached

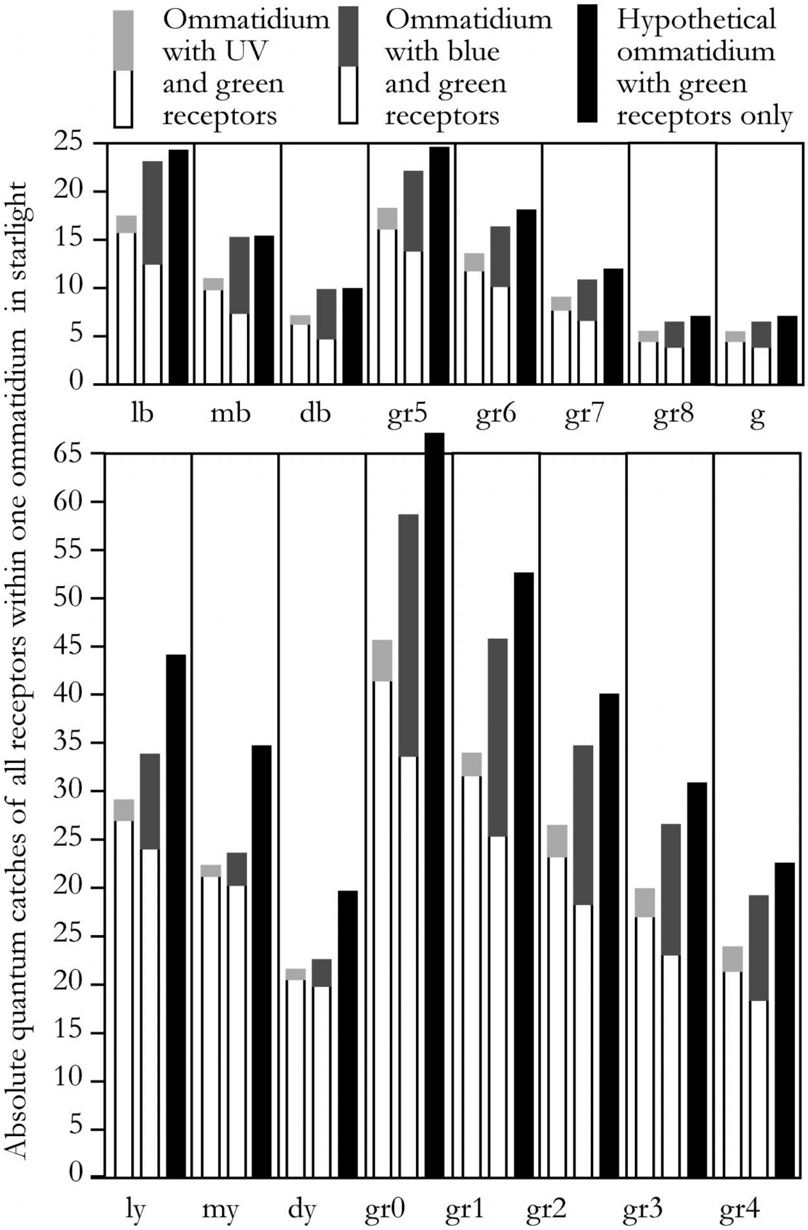


FIG. 4. Quantum catches of all *D. elpenor* photoreceptors in real (two ultraviolet and seven green receptors, two blue and seven green receptors) and hypothetical (nine green receptors) ommatidia viewing all test colours and the background colour at the dimmest light intensity used (0.0001 cd m^{-2}). Ommatidia with one ultraviolet, one blue and seven green receptors have lower quantum catches in the short wavelength receptors and intermediate quantum catches in the green receptors. Lights were measured using a calibrated S2000 Ocean Optics spectrometer and an International Light IL1700 photometer, in units of photons sec^{-1} nm^{-1} m^{-2} $steradian^{-1}$, and quantum catches were calculated using equation 1 (see Methods). Note that nine green receptors together capture more photons than two ultraviolet (or blue) receptors together with seven green receptors.

stimuli immediately when brighter light was again switched on.

Both species of *Hyles* are known to be active both during day and night (Knoll, 1926; Ebert, 1994; and own observations) and continued feeding even under similarly dim light levels as *D. elpenor*. How their eyes are adapted to see in such a large range of light intensities is still unknown. Colour vision in humans and honeybees is restricted to brighter light intensities than those accessible to nocturnal hawkmoths (Fig. 5).

COLOUR CONSTANCY

Many species of hawkmoth are active at dawn and dusk when the colour of light changes considerably. To find and recognise rewarding flowers, independent of the colour of the illuminating light, they require a colour-constant visual system. We tested colour constancy in *D. elpenor* and *M. stellatarum* by allowing them to discriminate between two stimuli, one green and one turquoise. Discrimination was tested in two different illumination colours, white and broad-spec-

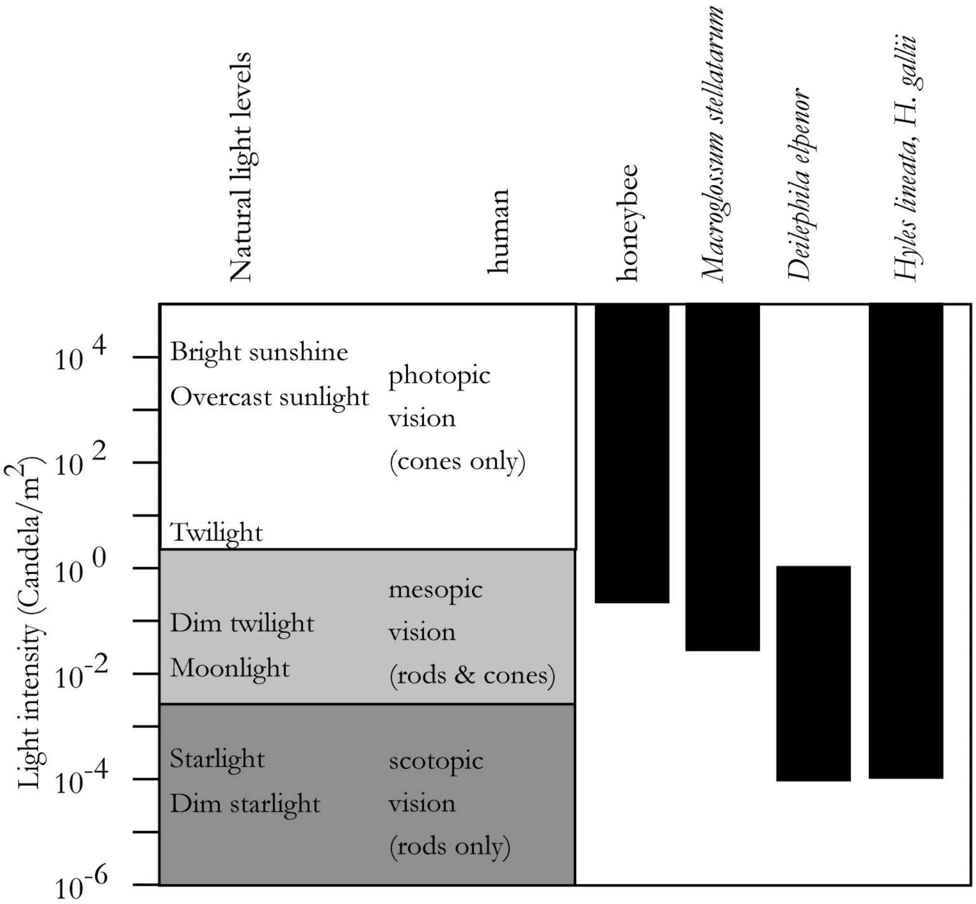


FIG. 5. Light intensities at which colour vision functions in humans, bees and hawkmoths.

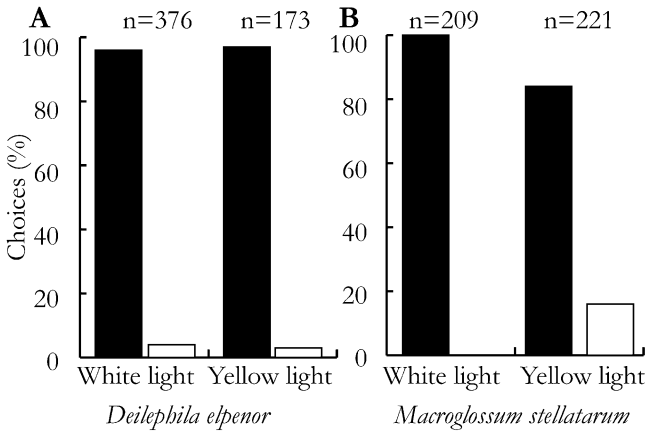


FIG. 6. Colour constancy in *D. elpenor* (**A**) and *M. stellatarum* (**B**). Ten animals of each species were trained to green (*black bars*) as the rewarded and turquoise (*white bars*) as the unrewarded colour and tested under the training illumination and a broad-band yellow illumination. Under yellow illumination, turquoise generates almost the same relative quantum catches in the three receptor types of *D. elpenor* as green does under white illumination. All results are statistically significant from chance (*G*-tests, $P < 0.001$).

trum yellow. In yellow light, the turquoise stimulus generates almost the same relative quantum catch in the UV, blue and green photoreceptor classes as the green stimulus does in white light (Kelber *et al.,* 2002; Balkenius and Kelber, unpublished results). Without colour constancy moths would confuse these two stimuli.

Moths trained in white light to associate a food reward with the green stimulus easily discriminated this stimulus from the turquoise one, irrespective of whether they were illuminated by white light or yellow light (Fig. 6). Hawkmoths of both species chose the rewarding colour with high precision in both illuminations (*G*-tests, $P < 0.0001$), proving they have colour constancy.

ACHROMATIC CUES

Besides a chromatic aspect, colour has an achromatic intensity (or brightness). Many animals use chromatic and achromatic cues for different tasks. Honeybees, as an example, use achromatic intensity (as seen by the green receptor only) for motion vision and pattern perception (Lehrer, 1993). Their colour vision system, in contrast, does not have an achromatic dimension (Brandt and Vorobycv, 1997) and bees have major difficulties to discriminate colours by achromatic cues only (von Frisch, 1914).

Nocturnal flowers tend to look white or yellow to the human eye, and even if they do not reflect ultraviolet light (White *et al.,* 1994; Raguso and Willis, 2003), intensity contrast between white or yellow flowers and the dark green background of leaves might be a strong cue for nocturnal insects. In the experiments described in the last two sections, we purposely made achromatic intensity unreliable, to prove that moths used the chromatic aspect of colour. Do hawkmoths use achromatic cues to find and recognise flowers?

The diurnal hawkmoth *M. stellatarum* is able to use achromatic cues. Animals were trained to associate a light of 595 nm with food, and a light of 620 nm with no food (Fig. 2B, *triangles*; Kelber and Hénique, 1999). They performed well under the training condition where both lights had equal intensities. When the intensity of the rewarding colour was reduced by one log unit, or when the intensity of the unrewarding colour was increased by the same amount, they chose randomly, and when the intensity difference between both colours was even larger, they chose the unrewarded colour exclusively. At 595 nm and 620 nm, only the moth's green receptor is sensitive, and colour vision is impossible. This green receptor is more sensitive to light of 595 nm than to light of 620 nm. We conclude that the moths learned to choose the stimulus that generated the higher quantum catch in the green receptors. After increasing the intensity of the 620 nm stimulus and decreasing the intensity of the 595 nm stimulus, the 620 nm light generated the higher quantum catch and was preferred (Fig. 2B; Kelber and Hénique, 1999).

Naive moths of *M. stellatarum* seem to have an innate preference for high achromatic contrast. Moths allowed to choose between two shades of blue, on a light grey background prefer the darker shade of blue (Fig. 7A; Kelber, 2002). We also allowed moths to choose between blue stimuli (28 mm diameter) with a central white or black spot (8 mm diameter). For three groups of moths, we used light blue, medium blue and dark blue as stimulus colour (see spectral curves *LB, MB* and *DB* in Fig. 1E). With a dark blue stimulus, moths preferred the white spot, with the light blue stimulus, they preferred the black centre, and with a medium blue, they showed some intermediate behaviour (Fig. 7B–D).

The fact that *D. elpenor* did not discriminate between different shades of blue or yellow (in the experiments discussed earlier, Fig. 3) does not mean that they are unable to learn achromatic cues (Land and Osorio, 2003). It is possible that moths learn achromatic cues only if chromatic cues are absent in the training situation. When we trained *D. elpenor* to discriminate between a white that absorbed ultraviolet light and one that reflected it, we made an interesting observation. Moths did not approach any of the stimuli when they were presented on a light grey background—a background that offered no chromatic and little achromatic contrast to the rewarded stimulus. On a black background—with a high achromatic contrast to the rewarding stimulus—they approached the stimuli and easily learned the task (Kelber *et al.,* 2002).

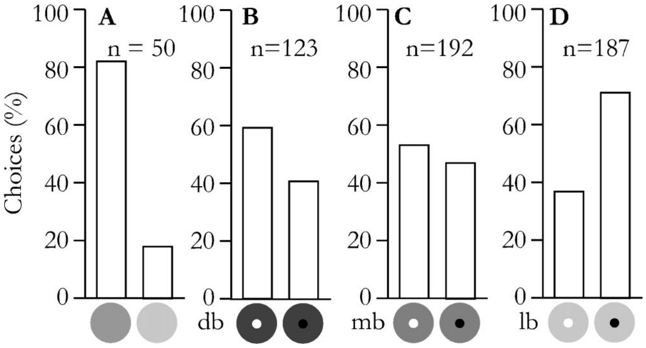


FIG. 7. Preferences for high achromatic contrast in naive moths of *Macroglossum stellatarum*. **A.** Ten moths chose the darker of two different shades of blue, on light grey background. *G*-test, $P < 0.001$. **B–D.** Moths chose between a blue artificial flower with a black spot and a blue pattern with a white spot. **B.** Flower colour dark blue (*db,* HKS paper, N41), five moths, *G*-test, n.s. **C.** Flower colour medium blue (*mb,* HKS paper, N44), six moths, *G*-test, n.s. **D.** Flower colour light blue (*lb,* HKS paper, N50), seven moths, *G*-test, $P < 0.001$. The choice frequency with light blue flowers differs significantly from those with dark blue and medium blue flowers (*G*-tests, $P < 0.001$). For reflection of the three shades of blue, see Figure 1E.

CONCLUSIONS

Diurnal and nocturnal hawkmoths use colour vision to find and recognise flowers. They recognise flower colours even under changed illumination colour indicating that they possess colour constancy. Diurnal hawkmoths do preferentially learn colour but are able to learn achromatic cues if no colour cues are present. It might be their preference for high contrasts that allows nocturnal moths to find flowers in very dim light. Observations in the field indicate that the hawkmoths *Agrius convolvuli* and *Hyles lineata* prefer blue flowers in bright light and white flowers in dim light (Michael Pfaff, personal communication; Martina Wicklein, personal communication). Even in dim starlight, however, nocturnal hawkmoths use chromatic cues rather than achromatic cues to recognize rewarding flowers.

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