

## Colour constancy in diurnal and nocturnal hawkmoths

Anna Balkenius\* and Almut Kelber

Department of Cell and Organism Biology, Lund University, Helgonavägen 3, S-223 62 Lund, Sweden

\*Author for correspondence (e-mail: anna.balkenius@cob.lu.se)

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### Summary

Diurnal and nocturnal hawkmoths have been shown to use colour vision for flower discrimination. Here, we present evidence that the nocturnal hawkmoth *Deilephila elpenor* and the diurnal hawkmoth *Macroglossum stellatarum* also have colour constancy. Colour constancy was shown in *D. elpenor* in two multiple-choice experiments with five different bluish colour patches under white and blue illumination and with five yellowish colour patches under white, blue and yellow illumination. The mechanism underlying colour constancy in both species was investigated in two dual-choice experiments. The choice behaviour is consistent with the use of the von

Kries coefficient law. Although the moths have colour constancy, they react to the colour of the illumination. They make fewer choices when tested under the changed illumination, where they never receive a reward, compared with the training illumination. Even if colour constancy can be explained by a von Kries adaptation mechanism, the fact that the animals discriminate between different illuminations indicates that some additional process must be involved.

Key words: colour vision, colour constancy, insect, hawkmoth, *Macroglossum stellatarum*, *Deilephila elpenor*.

### Introduction

When we recognise the colour of an object, we do not realize that different illuminations alter the spectral distribution that reaches our eyes. A yellow lemon looks yellow under bright sunlight as well as under the blue sky or a light bulb, and we are not aware that the spectra reaching our eyes are strikingly different in the three cases. This is the result of visual processes in our retina and brain that produce colour constancy. Although these processes may be useful for humans, they must be absolutely essential to animals such as bees and moths that rely on colour vision to identify their food sources. This is especially true for hawkmoths that are active during dawn and dusk, when the spectrum of natural illumination changes the most.

Colour constancy greatly contributes to the usefulness of colour vision, which is thought to be more reliable for object recognition than intensity vision since colour is less affected by changes of illumination (Kelber et al., 2003a,b).

Receptor adaptation contributes a large part to constancy (Komatsu, 1998). The sensitivity of the receptors decreases as a result of adaptation of the photoreceptor cells being stimulated by the background spectrum (Neumeyer, 1980, 1981; Komatsu, 1998). For chromatic adaptation, it is assumed that the different receptor types adapt separately depending on the background spectrum (Neumeyer, 1981, 1998).

Chromatic adaptation can be described by the von Kries coefficient law, which scales the signals from the photoreceptors to the background illumination to keep the colour constant despite changing spectra (Kries, 1905;

Vorobyev et al., 1999). The model adjusts the sensitivity of the three photoreceptors independently in proportion to their response to the background illumination (Backhaus et al., 1998). However, the von Kries coefficient law does not lead to perfect colour constancy since the photoreceptors are not completely independent as the model assumes. Theoretical analyses have shown that the broad spectral sensitivity and large overlap in the sensitivity curves of the photoreceptors limit colour constancy (Worthey and Brill, 1986; Dyer, 1999).

The second mechanism to achieve colour constancy is lateral interaction. This process can occur on an opponent stage in the retina or higher up in the visual pathway (Neumeyer, 1981; Komatsu, 1998; Rinner and Gegenfurtner, 2000). Finally, cognition also contributes to colour constancy in many ways. The awareness of colours and prior experience influence the perception of colours (Craven and Foster, 1992; Hurlbert, 1999). However, the role of cognition in colour perception can only be tested in humans.

Besides humans, colour constancy has been studied in honeybees (Neumeyer, 1981; Werner et al., 1988), goldfishes (Dörr and Neumeyer, 2000; Neumeyer et al., 2002) and butterflies (Kinoshita and Arikawa, 2000). In an experiment with goldfish, the backgrounds were black, grey or white and this influenced how well colour constancy functioned (Neumeyer et al., 2002). In tests with white or grey backgrounds, goldfish showed perfect colour constancy but, when a black background was used, they failed colour

constancy under saturated illumination. With the grey and white backgrounds, the colour of the illumination was reflected and caused adaptation of the receptors. The black background reflected very little light, causing the adaptation cue from the illumination to be much weaker (Neumeyer et al., 2002). Neumeyer (1981) found that the choice behaviour of honeybees was almost the same under training illumination as under coloured illuminations. The bees were tested with increasingly saturated yellow and blue illuminations and, under the most saturated illuminations, colour constancy started to fail (Neumeyer, 1981). Bees were also tested with Mondrian patterns (multi-colour patterns inspired by the painter Mondrian), showing good colour constancy (Werner et al., 1988).

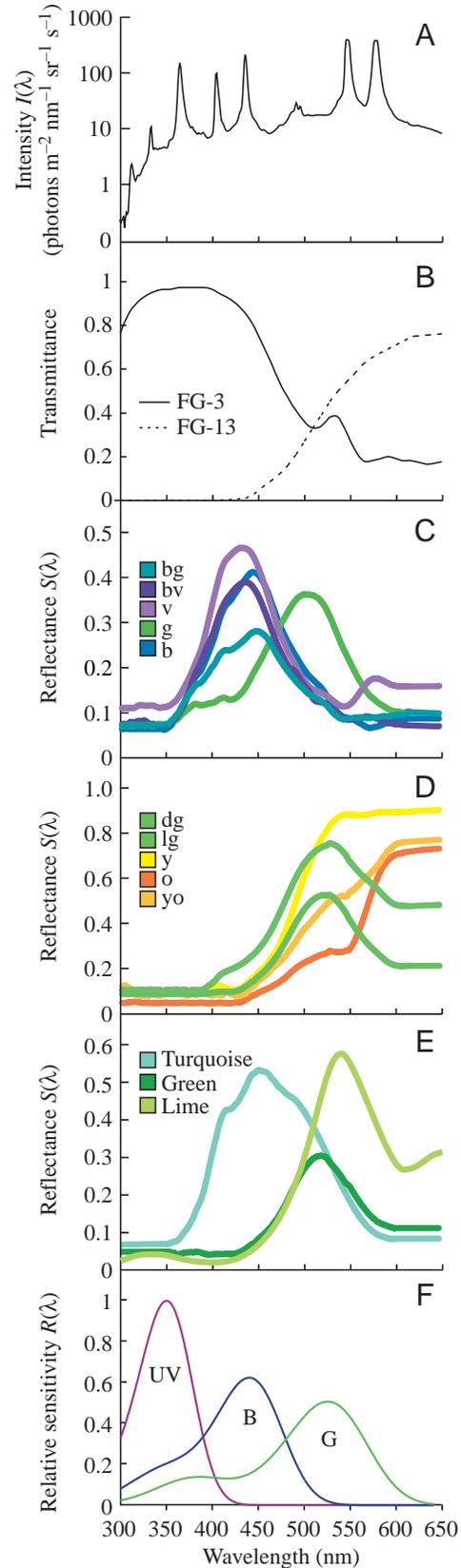
The butterfly *Papilio xuthus* was trained to recognise a colour in a Mondrian under differently coloured illuminations (Kinoshita and Arikawa, 2000). In a critical test, the butterflies were trained to discriminate a red rewarded stimulus from orange under white illumination. Under yellow illumination, orange reflected the same spectrum as red had done under white illumination, but the butterflies still chose red, thus showing colour constancy. However, the same behaviour would result if the butterflies had chosen the 'reddest' colour and thus showed relative colour learning.

Colour vision is assumed to be a general ability of hawkmoths (Kelber et al., 2003a,b). They have trichromatic colour vision with an ultraviolet-, blue- and green-sensitive receptor type (Höglund et al., 1973). *Macroglossum stellatarum* is a diurnal hawkmoth (Kelber and Hénique, 1999), and *Deilephila elpenor* is the first nocturnal animal proven to use colour vision (Kelber et al., 2002). Hawkmoths use their colour vision system to find and forage from suitable nectar flowers. Most hawkmoths are active at dawn and dusk, when light spectra change most. It would therefore be advantageous for them to have colour constancy (Kelber et al., 2002; Land and Osorio, 2003). We also tested whether a mechanism operating according to the von Kries coefficient law can account for colour constancy in both the nocturnal *D. elpenor* and the diurnal *M. stellatarum*.

## Materials and methods

### Animals and experimental procedures

The pupae of *Deilephila elpenor* L. hibernated in a refrigerator at 5°C. For each experiment, pupae were taken out and kept under 20°C and a 12 h:12 h L:D regime until they eclosed approximately 2 weeks later. *Macroglossum stellatarum* L. were bred in the laboratory throughout the year. After eclosion, the naïve moths were placed in a cage for one day without access to food. The following day, the training started with one moth at a time and a single rewarded paper flower. The experimental cage measured 50×60×70 cm and was illuminated from above by a high-pressure mercury lamp (Leitz, Germany). The spectrum of the illumination (Fig. 1A) could be changed by a yellow



(Schott FG-13; Mainz, Germany) or a blue (Schott FG-3) filter (Fig. 1B). The light intensity during experiments was 0.01 cd m<sup>-2</sup> with *D. elpenor* and 100 cd m<sup>-2</sup> with *M.*

Fig. 1. (A) The spectrum of the white cage illumination without filters. (B) The transmission of Schott filters FG-3 (blue) and FG-13 (yellow). (C) Reflectance of colours used in the first multiple-choice experiment with *D. elpenor* (experiment 1). Blue (b), blue-green (bg), green (g), violet (v) and blue-violet (bv). (D) Reflectance of colours used in the second multiple-choice experiment (experiment 2). Yellow (y), yellow-orange (yo), orange (o), dark green (dg) and light green (lg). (E) Reflectance of green, turquoise and lime used in the dual-choice experiments with *D. elpenor* and *M. stellatarum* (experiments 3–5). (F) The sensitivity curves of the ultra-violet (UV), blue (B) and green (G) receptors of *Deilephila elpenor*. The curves were calculated from the recorded sensitivity maxima (Höglund et al., 1973) using the Stavenga–Smits–Hoenders rhodopsin template (Stavenga et al., 1993) and normalised in such a way that the integrals equal 1.

*stellatarum*. The filtered illuminations are less than one logarithmic unit darker and thus well within the range where *D. elpenor* and *M. stellatarum* fly and feed. Animals were always rewarded under white illumination.

The moths learned to retrieve a small amount of 20% sugar solution through a 3 mm-wide hole in the centre of an artificial flower. Testing started on the second day. No reward was present during tests, and testing was followed by a feeding session. Testing continued over 10 days but not all animals continued to cooperate during this period. A visit was defined as each time the moth touched the colour patch with the proboscis. Each test lasted for as long as an animal continued to make choices, which was ~10–20 min. The positions of the colour patches were changed randomly to avoid place learning (Balkenius et al., 2004). All tests were performed on individual animals.

As stimuli, we used different coloured disks of 30 mm diameter on a light grey background. Stimuli and backgrounds were printed with an Epson colour printer (Model P952A) on Ink Jet paper. Experiment 1 was a multiple choice test in the short-wavelength range where eight *D. elpenor* were trained to discriminate five different bluish colours: blue (b), blue-green (bg), green (g), violet (v) and blue-violet (bv) (Figs 1C, 2A). Experiment 2 was a multiple-choice test in both the short- and the long-wavelength range. Ten *D. elpenor* and five different yellowish colours were used: yellow (y), yellow-orange (yo), orange (o), dark green (dg) and light green (lg) (Figs 1D, 2A). The moths were tested under white, blue (Schott filter, FG-3) and yellow (Schott filter, FG-13) illuminations. Experiments 3 and 4 were dual-choice tests on *D. elpenor* and *M. stellatarum*. Ten *D. elpenor* and 10 *M. stellatarum* were trained to turquoise as the rewarded colour, and another 10 specimens of each species were trained to green (Fig. 1E). They were tested under white and yellow illumination (Schott filter, FG-13; Fig. 1A,B).

To exclude the possibility of relative colour learning, we performed a fifth experiment. Six *M. stellatarum* were trained to green as the rewarded colour and turquoise as the unrewarded colour. After one week of training, the moths were tested with green and a ‘yellowish’ colour (lime; Fig. 1E). Using relative colour learning, moths trained to choose green and not turquoise should prefer lime to green. Absolute colour

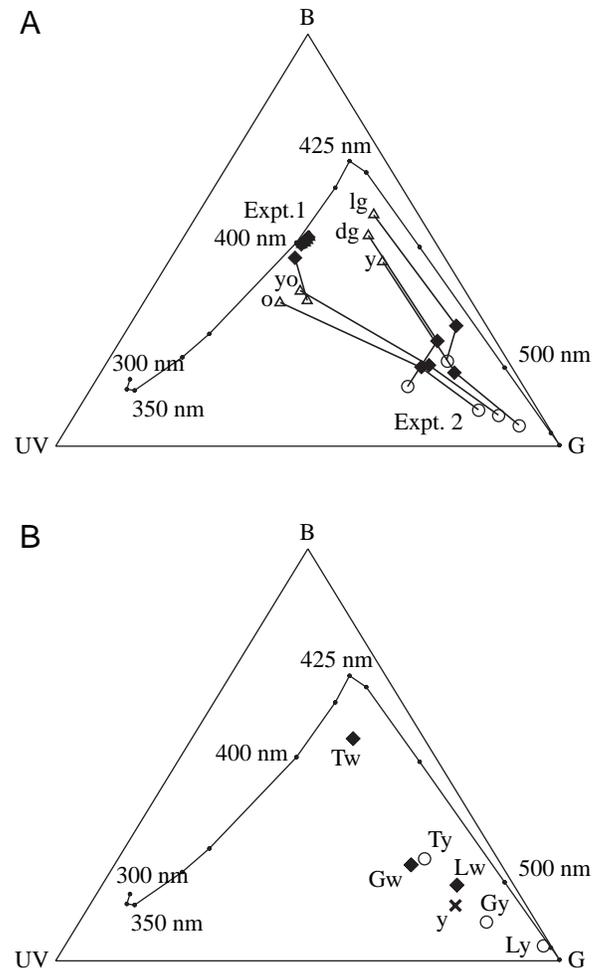


Fig. 2. Maxwell's triangle of *D. elpenor*. The corners of the triangle represent colours that excite only one of the three receptor types (UV, B, G). Loci within the triangle represent colours exciting all three receptor types. The line with wavelengths represents monochromatic colours in the colour space of the hawkmoths. For calculation of colour loci, see text. Diamonds, colour loci under white illumination; open circles, loci under yellow illumination; triangles, colour loci under blue illumination. (A) The loci of the colours used in experiments 1 and 2. The lines connect the same colour loci under the different illuminations. In experiment 1, the loci are very close and do not change much between the white and blue illumination. (B) The turquoise (T), green (G) and lime (L) colours were used in dual-choice tests under white (Tw, Gw, Lw; filled diamonds) and yellow (Ty, Gy, Ly; open circles) illuminations. y marks the locus of the yellow illumination.

learning should result in a high percentage of choices for green in all tests. Another group of six *M. stellatarum* were trained with green as the rewarded colour and lime as the unrewarded colour and tested on green and turquoise. In the Maxwell colour triangle, green lies between turquoise and lime (Fig. 2B).

#### Calculation of quantum catches and colour loci

The spectral composition of the light reflected from

the paper flowers was measured with a calibrated spectrophotometer (S2000; Ocean Optics). The quantum catch ( $Q_i$ ) of each photoreceptor is the number of photons absorbed by the receptor and is calculated as:

$$Q_i = \int_{300}^{700} I(\lambda)S(\lambda)R_i(\lambda)d\lambda, \quad (1)$$

where  $I(\lambda)$  is the spectrum of the illumination,  $S(\lambda)$  is the reflectance of a surface and  $R_i(\lambda)$  is the fraction of the incident light absorbed by a specific type of photoreceptor  $i$  for each wavelength ( $\lambda$ ) for the three receptor types sensitive to ultraviolet, blue and green light (Fig. 1F). The spectral sensitivities of the photoreceptors were calculated from the recorded sensitivity maxima (Höglund et al., 1973) using the Stavenga–Smits–Hoenders rhodopsin template (Stavenga et al., 1993).

The von Kries coefficient law assumes that the signals of the photoreceptors adapt to the background. The scaling factor,  $k_i$ , depends on the illumination spectrum (Vorobyev et al., 1999), and the quantum catch after adaptation,  $\phi_i$ , is calculated according to equations 2, 3:

$$k_i = \left[ \int_{300}^{700} I(\lambda)S_B(\lambda)R_i(\lambda)d\lambda \right]^{-1}, \quad (2)$$

$$\phi_i = k_i Q_i, \quad (3)$$

where  $I(\lambda)$  is the spectrum of the light reflected from the background,  $S_B(\lambda)$  is the reflectance of the background and  $R_i(\lambda)$  is the fraction of the light absorbed by a specific type of photoreceptor for each wavelength.

For an animal with trichromatic colour vision, colours can be represented as different loci in a Maxwell colour triangle, which is a projection of the three-dimensional colour space on a plane of equal intensity (Fig. 2; Kelber et al., 2003b). This is possible for animals that disregard intensity and predominantly use the chromatic aspect of colour as has been shown for both *D. elpenor* and *M. stellatarum* (Kelber and Hénique, 1999;

Kelber et al., 2002). In the Maxwell colour triangle, the colour loci are calculated as:

$$q_i = \frac{Q_i}{Q_{UV} + Q_B + Q_G}. \quad (4)$$

Here,  $Q_{UV}$ ,  $Q_B$  and  $Q_G$  are the quantum catches of the three photoreceptor types of the moths, and  $q_i$  represents the projection on the Maxwell triangle (Kelber et al., 2003b). Colour loci can also be calculated with von Kries coefficient law using  $\phi_i$  instead of  $Q_i$ .

For calculation of the quantum catches, the sensitivity curves were normalised in such a way that the integrals equal 1 (Fig. 1F).

The Euclidean distance  $d(x, y)$  between the colour coordinates in the Maxwell triangle was used as a measure of similarity:

$$d(x,y) = \left( \sum_{i=1}^3 (x_i - y_i)^2 \right)^{1/2}. \quad (5)$$

These colour distances were calculated for different colours and illuminations with and without a von Kries coefficient law (Tables 1, 2).

For the dual-choice experiments (experiments 3 and 4), we selected two colours that require a colour constancy mechanism to be distinguished under the changed illumination (Fig. 1E; Table 2). In yellow light, the turquoise colour generated almost the same quantum catch in the different photoreceptor types and occupied almost the identical colour locus as the green colour did in white light (Figs 2A, 3).

## Results

### Experiment 1: multiple-choice test in the short-wavelength range

Under white illumination, *D. elpenor* learned to discriminate the rewarded blue (b) colour from four other colours. The

Table 1. Euclidean distances between colour loci in colour constancy in experiments 1, 2 and 5

Experiment	Training colour	Test illumination	Distance to training colour in white	Without von Kries	
				Closest colour	Distance
1	Blue	White	0	b	0
		Blue	0.024	<b>bg</b>	<b>0.019</b>
2	Yellow	White	0	y	0
		Blue	0.304	y	0.304
		Yellow	0.154	<b>lg</b>	<b>0.029</b>
5	Green	White	0	G	0
		Yellow	0.183	G	0.183

In experiment 1, blue (b) was the rewarded training colour under white illumination ( $b_w$ ). The distance in the colour triangle from  $b_w$  to the closest colour under the blue illumination is blue-green ( $bgb$ ). The distance between  $b_w$  and  $b_b$  is larger than between  $b_w$  and  $bgb$ . In experiment 2, the training colour was yellow,  $y_w$ , and under blue illumination  $lgb$  is closer to  $y_w$  than  $y_b$ . Under the yellow illumination,  $y_w$  is closest to  $y$  and the second closest is yellow-orange ( $yo_y$ ). In experiment 5, the training colour green was closest to itself under yellow illumination. The closest distance is highlighted in bold.

Table 2. Euclidean distances between colour loci of turquoise and green in experiments 3 and 4, assuming presence or absence of colour constancy

Training	Test illumination	Without von Kries		With von Kries	
		T	G	T	G
T	White	<b>0</b>	0.339	<b>0</b>	0.214
	Yellow	<b>0.338</b>	0.517	<b>0.166</b>	0.243
G	White	0.339	<b>0</b>	0.214	<b>0</b>
	Yellow	<b>0.019</b>	0.183	0.398	<b>0.206</b>

The closest distance to the training colour under white illumination is highlighted in bold. In the absence of a von Kries mechanism, turquoise (T), under yellow illumination, has the shortest distance to green (G) under white illumination. With a von Kries mechanism, this is not the case.

colours were very similar (Table 1; Fig. 2A) so it was no surprise that the moths chose the training colour in no more than 34% of their visits (Fig. 4). Still, the choice distribution differed from chance ( $\chi^2$ -test,  $P < 0.001$ ). The choice behaviour under blue illumination did not differ from that under white illumination ( $\chi^2$ -test,  $P > 0.05$ ; Fig. 4). Under blue illumination, the blue-green (bg) resulted in almost the same quantum catch as the rewarded blue (b) colour did in white illumination (Table 1), but the moths chose this colour less frequently than in white illumination.

Experiment 2: multiple-choice test in both the short- and the long-wavelength range

In experiment 2, *D. elpenor* were rewarded at the yellow (y) colour under white illumination during the training sessions. Under the white, yellow and blue illuminations, they selected yellow most frequently (Fig. 5). The choice distribution for the colours did not differ under the white and yellow illumination ( $\chi^2$ -test,  $P > 0.05$ ; Fig. 5) and it differed from chance ( $\chi^2$ -test,  $P < 0.001$ ). Under the blue illumination, the moths chose orange less frequently compared with the white and yellow illumination, and the choice distribution differed from chance ( $\chi^2$ -test,  $P < 0.001$ ). Under the yellow illumination, light green (lg) resulted in almost the same quantum catch as the rewarded yellow under white illumination (Fig. 5; Table 1). Orange (o) was selected more frequently than yellow-orange (yo) under white illumination even though the yellow-orange was more similar to the rewarded colour than the orange (not shown). The behaviour changed under blue illumination, when the yellow-orange was chosen more frequently than orange (o).

Experiment 3: dual-choice tests on *D. elpenor*

In experiment 3, we tested whether the von Kries coefficient law can explain colour constancy in *D. elpenor*. If the moths did not have colour constancy, turquoise would occupy almost the same colour locus as the green did under white illumination (Fig. 3A). Still, the moths chose green. With turquoise as the rewarded colour, *D. elpenor* selected turquoise in 100% of the

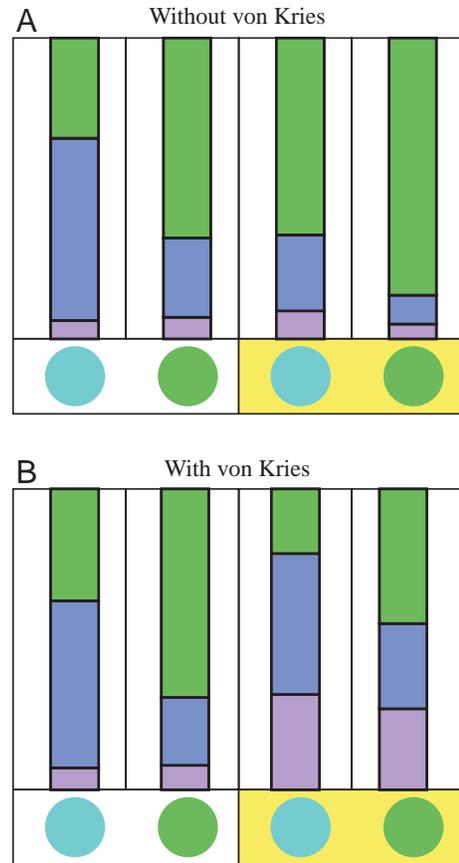


Fig. 3. Relative quantum catches for the three receptor types for the turquoise and green colour under white and yellow illumination, (A) without von Kries coefficient law and (B) with von Kries coefficient law (the colours indicate the corresponding receptor types).

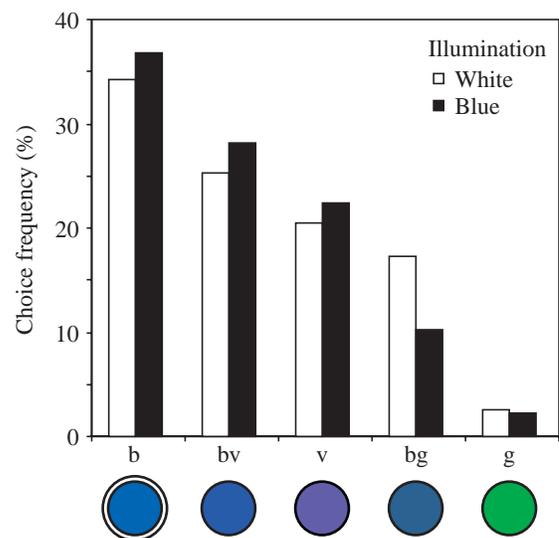


Fig. 4. Choices made by eight *D. elpenor* in experiment 1, under white illumination ( $N=201$ ) and blue illumination ( $N=138$ ). The colour surrounded by a white circle is the training colour. b, blue; bv, blue-violet; v, violet; bg, blue-green; g, green.

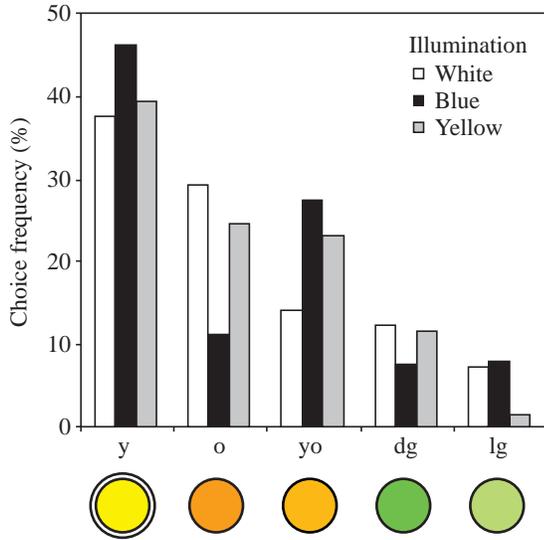


Fig. 5. Choices made by 10 *D. elpenor* in experiment 2, under white ( $N=263$ ), blue ( $N=189$ ) and yellow ( $N=69$ ) illumination. The colour surrounded with a white circle is the training colour. y, yellow; o, orange; yo, yellow-orange; dg, dark green; lg, light green.

trials under white illumination and in 98% under yellow illumination (Fig. 6A). With green as the rewarded colour, the moths selected green in 96% of the cases under white illumination and in 98% under the yellow illumination (Fig. 6B). The choices of the moths can be explained by the use of von Kries coefficient law.

#### Experiment 4: dual-choice tests on the diurnal hawkmoth *M. stellatarum*

We performed the same experiment on the diurnal species *M. stellatarum*. The experimental design was identical to that of the previous experiment, with the exception that the light intensity was  $10^4$  times higher. With turquoise as the rewarded colour, the moths selected the turquoise in 87% of the trials under white illumination and in 99% under the yellow illumination (Fig. 7A). When green was rewarded, the moths chose correctly in 100% of the cases under white illumination and in 84% under the yellow (Fig. 7B). This indicates that *M. stellatarum* also has colour constancy.

#### Experiment 5: test of relative colour learning

The dual-choice experiments might have allowed the animals to perform relative choices. They might have chosen the 'bluer' or 'yellower' colour rather than the learned colour. Therefore, a group of six moths were trained on a rewarded green colour and an unrewarded 'yellower' colour (lime) and tested with the green and a 'bluer' colour (turquoise; Fig. 2). They selected green in 91% of the cases (Fig. 8A) even if it was the 'yellower' of both test colours. The second group of six moths were trained to green as the rewarded and turquoise as the unrewarded colour and tested with green and lime. They selected the green in 85% of the cases (Fig. 8B, white bars). These moths were also tested with green and lime colours, for

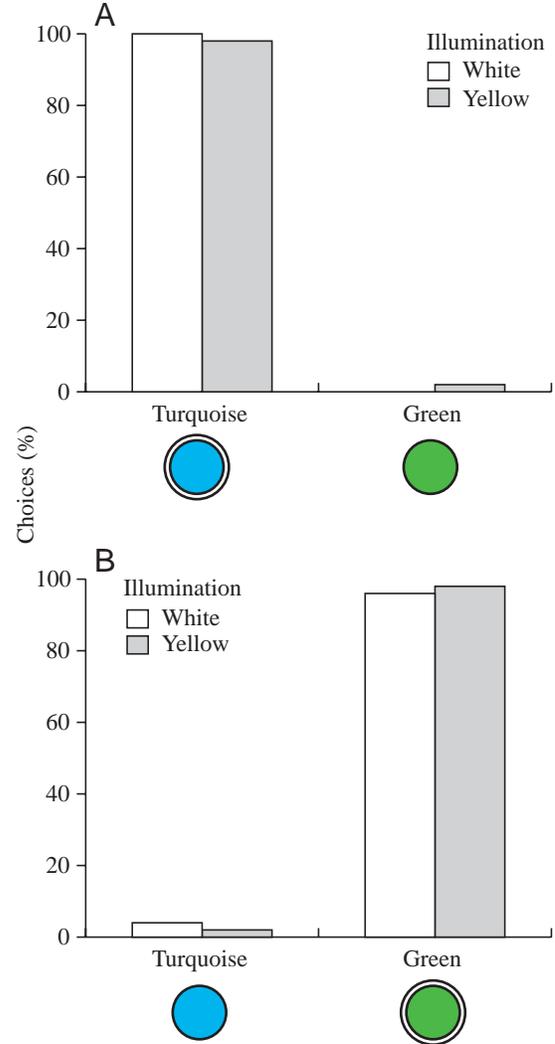


Fig. 6. Dual-choice experiment on *D. elpenor* (experiment 3). (A) Turquoise used as rewarded colour patch (surrounded by a white circle). Choices made by 10 *D. elpenor* for the different colour patches under white ( $N=376$ ) and yellow ( $N=173$ ) illumination. (B) Green used as rewarded colour (surrounded by a white circle). Choices made by 10 *D. elpenor*, under white ( $N=137$ ) and yellow ( $N=55$ ) illumination.

colour constancy, under white and yellow illumination. The moths selected the green in 85% of the cases under white illumination and 91% under the yellow illumination (Fig. 8B, grey bars;  $\chi^2$ -test,  $P<0.001$ ). These results exclude relative colour learning as a possible explanation for the results of experiments 3 and 4.

#### Discrimination of illumination

The moths did not change their preferred colour when the spectra of the illumination changed and thus showed colour constancy. However, the total numbers of choices differed under different illuminations (Fig. 9). The difference could be observed in all experiments, but only in one experiment did a sufficiently large number of animals ( $N=4$ ) make choices on

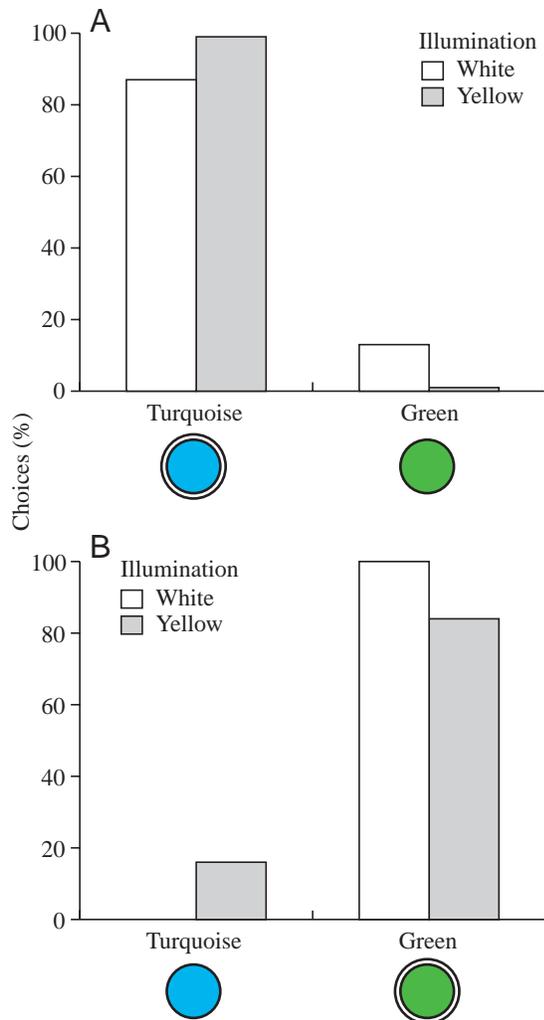


Fig. 7. Dual-choice experiments on *M. stellatarum* (experiment 4). (A) Turquoise was used as rewarded colour (surrounded by a white circle). Choices made by *M. stellatarum* under white ( $N=274$ ) and yellow ( $N=183$ ) illumination. (B) Green used as rewarded colour (surrounded by a white circle). Choices made by 10 *M. stellatarum* for the different colour patches under white ( $N=221$ ) and yellow ( $N=209$ ) illumination.

eight subsequent days. In this experiment, *M. stellatarum* were trained with turquoise as the rewarded and green as the unrewarded colour. Moths made fewer choices the first day when they saw the changed illumination. This implies that they noticed the change in illumination even if they compensated for it (Fig. 9). There was a significant difference in the total number of choices made under white and yellow illumination (day 1, one-tailed  $t$ -test,  $P<0.04$ ). On days 2–5, they made similar numbers of choices under the yellow and the white illumination (day 5, two-tailed  $t$ -test,  $P>0.82$ ). After five days, the number of choices under yellow illumination decreased again and differed significantly from the number of choices under white illumination (day 8, one-tailed  $t$ -test,  $P<0.03$ ).

## Discussion

Our results demonstrate that two species of hawkmoth, *Deilephila elpenor* and *Macroglossum stellatarum*, have colour constancy. The colour choices of the moths are independent of the spectrum of the illumination, and the mechanism can be described by the von Kries coefficient law. In addition, both species can learn to associate colours that are not typical flower colours with food. *M. stellatarum* have an innate preference for blue and yellow (Kelber, 1997). Nevertheless, the moths from both species could quickly learn to accept green as a rewarded colour. They continued to select the green colour even when a ‘bluer’ colour, i.e. turquoise, or a ‘yellower’ colour, i.e. lime, was present.

### Colour constancy

In the dual-choice tests, green under white illumination and turquoise under yellow illumination resulted in almost identical quantum catches in the photoreceptors and it would have been impossible to recognise the green colour under yellow illumination without colour constancy (Fig. 3A,B; Table 2).

The moths did not show perfect colour constancy under the blue illumination in the second multiple-choice experiment (Fig. 5). They selected the orange and dark green colours less frequently under the blue illumination than under the white illumination. The blue illumination was highly saturated and the moth’s visual system is perhaps not adapted to this spectrum.

If chromatic adaptation resulted in perfect colour constancy, we would expect all colours in the Maxwell colour triangle to show a zero shift when the illumination changes. In our dual-choice experiments, the von Kries coefficient law does not completely eliminate the shift. This limitation depends on the spectral width and the overlap of the different photoreceptor sensitivities (Worthey and Brill, 1986). The coloured oil droplets in birds are thought to improve colour constancy since they narrow the spectral sensitivities, which makes the von Kries coefficient law more effective (Vorobyev et al., 1998). Narrowing the width of the different photoreceptor sensitivities has the disadvantage of reducing sensitivity, which seems useless in a nocturnal hawkmoth. In the butterfly *Papilio xuthus* and in the goldfish, it has previously been shown that colour constancy is not perfect under strongly saturated illumination or with a black background (Dörr and Neumeyer, 2000; Kinoshita and Arikawa, 2000; Neumeyer et al., 2002). Humans do not display perfect colour constancy either. We fail colour constancy under saturated illuminations and on solitary colour surfaces surrounded by black (Bäumli, 1999; Hurlbert, 2002). It is known that the colour constancy in humans is based on several mechanisms. Thus, not only the adaptation mechanism fails under these extreme conditions.

### Possible alternative explanations

Could the moths have used achromatic cues instead of a colour constancy mechanism? In previous experiments, *D. elpenor* did not use achromatic cues to recognise colour

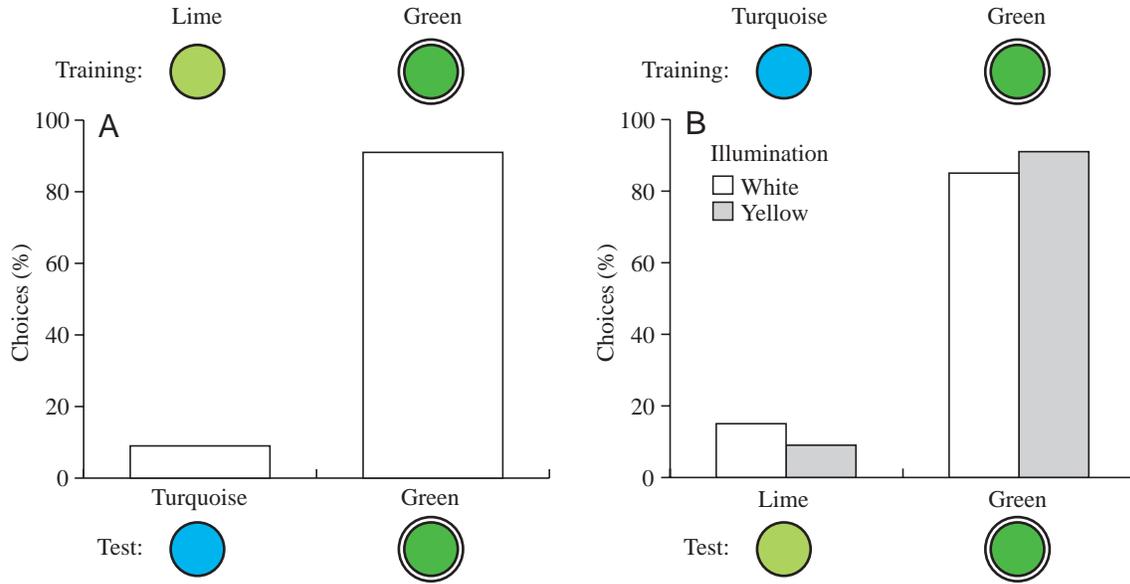


Fig. 8. ‘Relative colour learning’ with *M. stellatarum* (experiment 5). (A) Six moths were trained to green as the rewarded colour (surrounded by a white circle) and lime and tested with green and turquoise ( $N=268$ ). (B) Six moths were trained to green and turquoise using green as the rewarded colour, and tested with green and lime, under white illuminations ( $N=243$ ). Colour constancy was tested under yellow illumination ( $N=35$ ).

(Kelber et al., 2002) but this does not guarantee that they cannot use achromatic cues to select the correct flower. In the second multiple-choice experiment, two colours (orange and dark green; Fig. 5) reflected less light under the blue illumination and the moths did not select them as frequently as under the white and yellow illuminations. This might indicate that the moths use achromatic cues when they fail in colour constancy. However, in the other experiments, no strong intensity cues were available.

In three of the experiments, one of the unrewarded colours occupied a colour locus closer to that of the rewarded colour when a coloured illumination was used (Tables 1, 2). In all three cases, animals still chose the training colour (Figs 4–7).

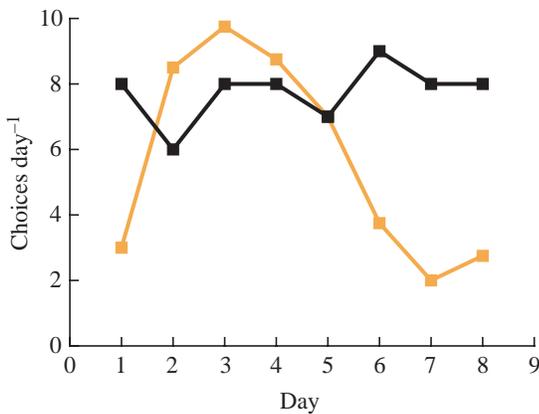


Fig. 9. Total numbers of choices made by four *M. stellatarum* during eight subsequent days. Turquoise was the rewarded colour. Black line: white training illumination. Yellow line: yellow illumination. See text for explanations.

Did the moths learn relative rather than absolute colour? To our knowledge, the only animal that has been shown to learn relative colour is a dichromatic marsupial, the tamar wallaby (*Macropus eugenii*). Trained to choose a 450 nm stimulus and to avoid a 500 nm stimulus, the wallabies preferred the stimulus with the shorter wavelength in any pair they were tested with, thus they preferred 400 nm to 450 nm, the training colour (Hemmi, 1999).

In our fifth experiment, two groups of moths were tested for relative colour learning. The first group of moths were trained on green and turquoise and tested with green and lime. The lime colour is ‘yellow’ for the moths than the rewarded green colour (Fig. 2), but the moths still selected the original green patch. The second group of moths were trained on green and lime and, when tested with green and turquoise, they still selected the green colour. If the moth had learned to select the ‘yellowest’ or ‘bluest’ colour, they would have switched to the other colour when tested. Instead, they stayed with their original choice, which rules out their use of relative colours. Their choices are consistent with a comparison between the test colour and the recollected rewarded colour assuming they have colour constancy.

It is highly probable that the tetrachromatic *Papilio* butterflies do not use ‘relative colour learning’ either. The results of Kinoshita and Arikawa (2000) are a conclusive proof of colour constancy in this species as well. Honeybees do not learn relative colours, otherwise it would have been impossible to obtain their wavelength discrimination curve (Helvesen, 1972; Menzel, 1979).

#### Learning of illumination colours

The total number of visits made by the moths changed when

the illumination differed from the illumination used during training. The moths noticed the changed illumination and reacted to it as a contextual cue. Such a cue modulates which behaviour the animal performs but does not control it directly (Mackintosh, 1983).

On the first day that the moths saw the new illumination, they showed an orienting reaction to the changed environment and thus increased exploration (Gray, 1975). As a consequence, they made fewer choices although the choices were still correct. The orienting reaction to the changed illumination interfered with the trained behaviour. After some days, the moths habituated to the yellow illumination, and the number of visits to the patches increased. During this phase, there was no significant difference between the behaviour of the moths in white and yellow illumination. Finally, the moths learned to discriminate between the context of the white illumination, where they were rewarded during training, and the yellow illumination, where they were never rewarded. Tests under yellow illumination were generally performed before tests under white illumination, and decreased motivation can therefore not account for the result. We therefore propose that moths learned that they were never rewarded under yellow illumination and therefore did not approach the stimuli.

The intensity remained almost the same under white and yellow illumination. In earlier experiments, moths did not respond to much larger intensity changes (2–3 log units), although they stopped flying when it became too dark (A.K., unpublished). We cannot exclude that they learned to react to the small change in intensity instead of the changed spectrum but, even if this is the case, they still treated this as a context change.

#### Colour constancy mechanisms

Either of two mechanisms, receptor adaptation or lateral interaction, can explain the colour constancy in the experiments. However, the moths can react to the colour of the illumination even though they have colour constancy. There are two possible explanations of this behaviour. One is that the colour constancy mechanism does not work instantly, which allows the animals to react to the colour change while adapting to it. This strongly implicates receptor adaptation that works slowly as the underlying colour constancy mechanism. Lateral interactions are assumed to work instantly and can therefore be ruled out. Another possible explanation is that colour constancy involves higher-level mechanisms that can distinguish between the colour of the illumination and the colour of an object.

Studies with human infants have shown that colour constancy is not an inborn property and that humans show individual differences. Children develop colour constancy between the ages of two and four months (Dannemiller, 1989). In our experiments, the moths were tested after a very short training period where the illumination never changed, which indicates an innate mechanism for colour constancy. Receptor adaptation most certainly works innately and without learning. Higher cognitive levels of colour constancy in humans are

probably learned, but it is difficult to test if animals also have these abilities.

#### Conclusions

Colour constancy is necessary if colour vision should be used to recognise objects solely by their colour. It has been suggested that all colour vision systems have colour constancy and that colour constancy could have been the driving pressure for the evolution of colour vision (Campenhausen, 1986). Receptor adaptation most probably existed in animals even before colour vision evolved. Our results prove that *D. elpenor* and *M. stellatarum* have colour constancy and this fits well with this picture.

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