

COLOUR CONSTANCY OF THE SWALLOWTAIL BUTTERFLY *PAPILIO XUTHUS*

MICHIYO KINOSHITA¹ AND KENTARO ARIKAWA^{1,2,*}

¹Graduate School of Integrated Science, Yokohama City University, Yokohama, Japan and

²PRESTO, Japan Science and Technology Corporation, Japan

*Author for correspondence (e-mail: arikawa@yokohama-cu.ac.jp)

Accepted 29 August; published on WWW 2 November 2000

Summary

We have recently shown that the Japanese yellow swallowtail butterfly *Papilio xuthus* uses colour vision when searching for food. In the field, these butterflies feed on nectar provided by flowers of various colours not only in direct sunlight but also in shaded places and on cloudy days, suggesting that they have colour constancy. Here, we tested this hypothesis. We trained newly emerged *Papilio xuthus* to feed on sucrose solution on a paper patch of a certain colour under white illumination. The butterflies were then tested under both white and coloured illumination. Under white illumination, yellow- and red-trained butterflies selected the correctly coloured patch from a four-colour pattern and from a colour Mondrian

collage. Under four different colours of illumination, we obtained results that were fundamentally similar to those under white illumination. Moreover, we performed critical tests using sets of two similar colours, which were also correctly discriminated by trained butterflies under coloured illumination. Taken together, we conclude that the butterfly *Papilio xuthus* exhibits some degree of colour constancy when searching for food.

Key words: colour vision, foraging, behaviour, insect, visual system, compound eye, ommatidium, photoreceptor, training, learning, memory, butterfly, *Papilio xuthus*.

Introduction

Colour vision is the ability to discriminate visual stimuli on the basis of their chromatic content regardless of their brightness (Goldsmith, 1990). The reliability of colour vision is reinforced by the phenomenon of colour constancy, which enables animals to recognize an object's colour irrespective of the spectral content of the illuminating light (Land, 1977). For example, to a human observer, a red apple appears red both in sunshine and in fluorescent light, although the irradiation spectra are distinctly different. This phenomenon is the colour constancy of human vision. Colour constancy has been demonstrated in some other animals, including an insect, the honeybee *Apis mellifera* (Neumeyer, 1981; Werner et al., 1988). Is colour constancy unique to honeybees or is it shared by other insects?

In the course of our studies of butterfly vision, we have demonstrated that the retina of the Japanese yellow swallowtail butterfly *Papilio xuthus* has at least five different types of spectral receptor, which are randomly distributed over the retina (Arikawa et al., 1987, 1999a,b; Arikawa and Stavenga, 1997). Presumably, these spectral receptors form the physiological basis of colour vision (Arikawa and Stavenga, 1997).

Many butterflies feed on the nectar of a variety of flowers, each displaying different colours. Many researchers in the past few decades have investigated this behaviour to examine the possibility of colour vision in butterflies (Goulson and Cory, 1993; Horridge et al., 1984; Kandori and Ohsaki, 1996, 1998;

Scherer and Kolb, 1987a,b; Weiss, 1991, 1995, 1997). However, it is only recently that true colour vision in butterflies has been demonstrated convincingly in physiological terms (Kelber and Pfaff, 1999; Kinoshita et al., 1999). We used an indoor experimental arrangement to demonstrate that *Papilio xuthus* memorizes the colour of a food source and can subsequently recognize this colour within a complex pattern (Kinoshita et al., 1999). Presumably, colour vision is essential for recognising suitable flowers for feeding in the field. However, butterflies visit flowers not only in bright sunlight but also in shaded places or even on cloudy days, suggesting that they can recognize colours under different spectral illuminations; i.e. they are probably colour-constant.

To test this hypothesis, we trained *Papilio xuthus* to feed on a glass-covered paper patch of a certain colour under white illumination. After having confirmed that the trained butterfly recognized the trained colour under the same white illumination, we modified the spectral content of the illumination using colour filters and then tested whether the butterfly could still recognize the correct colour. The results strongly indicate that *Papilio xuthus* has colour constancy.

Materials and methods

Animals

We used spring-form males of *Papilio xuthus* L. from a

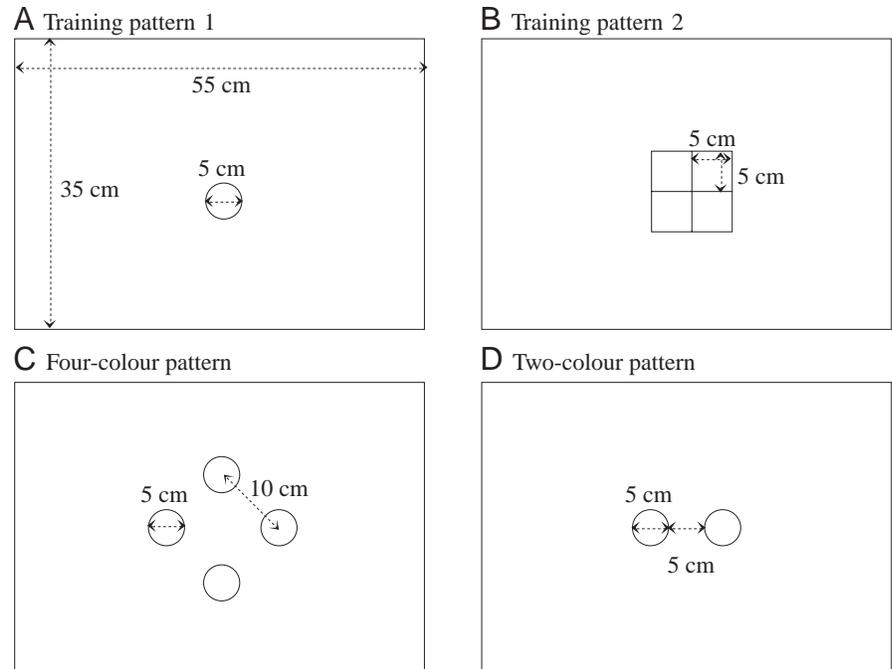


Fig. 1. Patterns presented on the cage floor. (A) Training pattern 1. A blue, yellow or red disk was placed on a black sheet. (B) Training pattern 2. Juxtaposed rectangular patches of blue, green, yellow and red. (C) The four-colour pattern used in testing. Four patches of different colour papers (blue, green, yellow and red) were presented. (D) The two-colour pattern used in experiment 2.

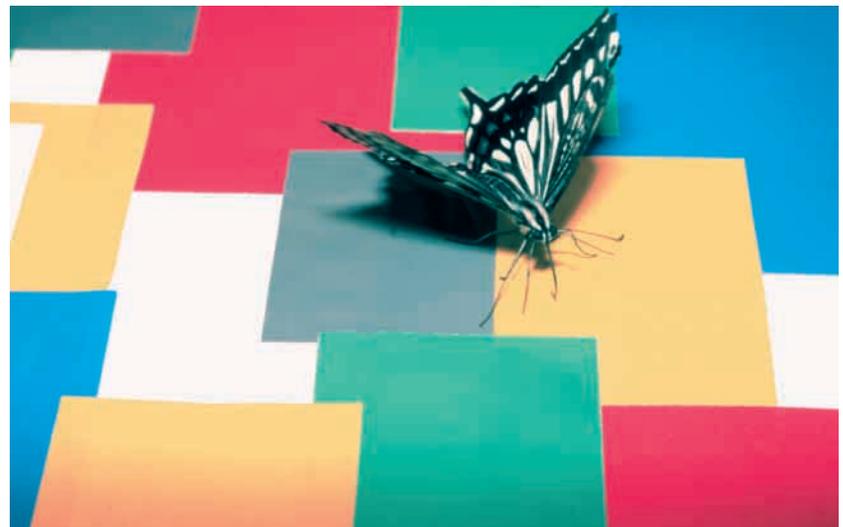


Fig. 2. A yellow-trained butterfly on a yellow portion of the Mondrian.

laboratory stock culture that had been derived from eggs laid by females caught in the field. The hatched larvae were reared on fresh citrus leaves at 25 °C under a 8h:16h light:dark regime. The pupae were chill-treated at 4 °C for at least 3 months and were then allowed to emerge at 27 °C in a plastic box covered with gauze. The day of emergence was defined as post-emergence day 1.

Stimuli and illumination

All experiments were carried out in an indoor cage measuring 80 cm×60 cm×45 cm (Kinoshita et al., 1999) at 27 °C. The floor of the cage was covered with black cardboard. Each butterfly was released into the cage to test its preference for colour stimuli. The stimuli were colour papers (Nihon

Shikisai Training Colour 200; 1997 version) placed on the floor of the cage. The reflectance spectra of the colour papers used in this study (see Fig. 3A) were measured with a spectrometer (S2000, Ocean Optics Inc., USA). For efficient presentation of the stimuli, we prepared four patterns (Fig. 1). Each pattern had certain numbers and shapes of colour papers (blue, blue-green, emerald-green, green, yellow, orange and/or red) on a black cardboard background (55 cm×35 cm). We also prepared a Mondrian collage (Fig. 2) that contained blue, green, yellow, red, grey and white (Fig. 3A). The patterns were covered with anti-reflection glass when presented.

The cage was placed in a room equipped with eight 500 W halogen lamps, with glass light diffusers attached, hanging from the ceiling (Fig. 3B inset). To minimize the effect of heat

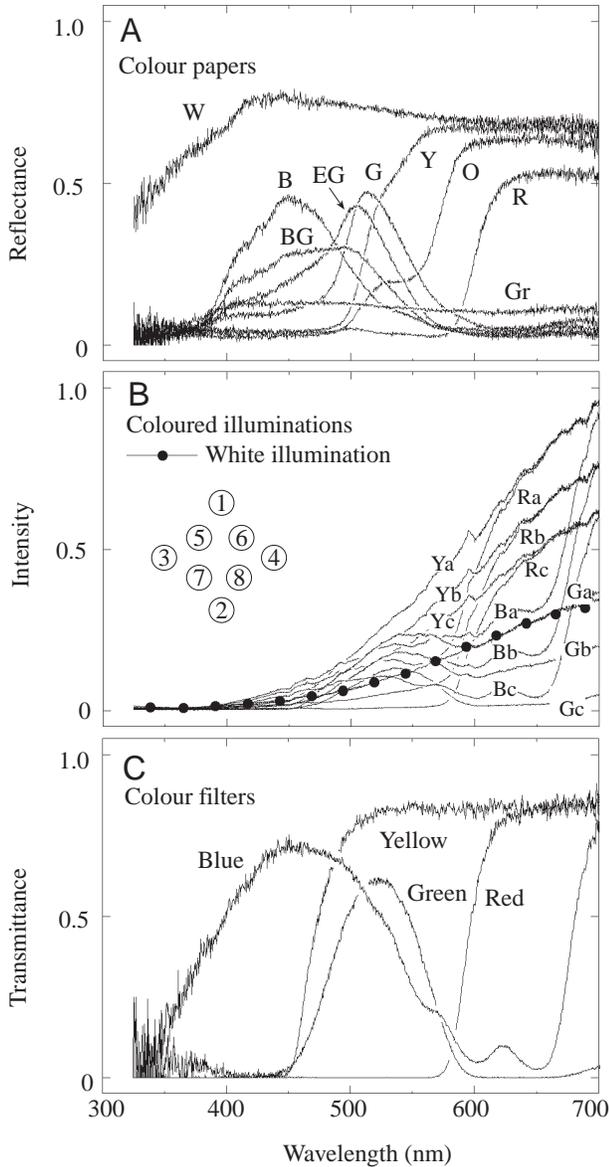


Fig. 3. The colour papers and filters used in the present experiments. (A) Reflectance spectra of the colour papers relative to a MgO-coated surface. B, blue; BG, blue-green; EG, emerald-green; G, green; Y, yellow; R, red; W, white; Gr, grey. (B) Spectral power distribution of the illuminating light (B, bluish; G, greenish; Y, yellowish; R, reddish). The spectra were measured as the amount of light reflected from MgO-coated surface. The attached letters, a, b and c, indicate the degree of colour saturation. For example, Ba, Bb and Bc indicate bluish illumination that was weakly (Ba), moderately (Bb) or strongly (Bc) saturated. Maximal intensity (1.0) indicates the intensity of light when all eight lamps were turned on without any filters. Inset, layout of the illuminating eight halogen lamps on the ceiling. To avoid excess heat in the experimental arena, a heat-absorbing glass filter (6 mm thick) was positioned between the lamps and the cage. The cage was placed directly under lamps 5, 6, 7 and 8. The peak intensity of unfiltered (white) light with all eight lamps were turned on was set as 1.0. (C) Transmittance spectra of the colour filters used to change the chromatic content of the illuminating lights. 0.5 transmittance indicates that half the incident light is transmitted.

produced by the lamps, a 6 mm thick heat-blocking glass filter was placed between the lamps and the cage. The emission of light at wavelengths shorter than 400 nm became negligible under this experimental condition (Fig. 3B). For simplicity, however, we will hereafter refer to the halogen light as ‘white’ illumination, although *Papilio xuthus* is distinctly sensitive to ultraviolet light (Arikawa et al., 1987). The required illumination was achieved by switching on different combination of lamps and by placing colour filters above the cage. For example, four lamps (no. 1–4) were switched on for white illumination, resulting in 2700 lx at the floor of the cage.

The spectral content of the illumination was changed using colour filters. Fig. 3C shows the transmittance of the filters. For simplicity, we hereafter refer to these filters as blue (B), green (G), yellow (Y) and red (R) colour filters, according to their appearance to human observers. The filters were always placed in front of lamps 5–8, which were placed directly above the cage. When only the filtered lamps were turned on, the arena appeared strongly coloured to a human observer. We attenuated the saturation of the coloured illumination by turning on additional, unfiltered lamps. To make the illumination moderately saturated, we turned on unfiltered lamps 1 and 2 in addition to filtered lamps 5–8. For weakly saturated illumination, we also turned on the lamps 3 and 4. The resulting spectral content of each illumination measured as the reflection from a MgO-coated surface is shown in Fig. 3B. For example, Yc corresponds to the illumination from four yellow-filtered lamps 5–8. For Yb, unfiltered lamps 1 and 4 were switched on in addition to the filtered lamps 5–8. For Yc, four unfiltered lamps 1, 2, 3 and 4 were switched on.

Behavioural experiments

We conducted two sets of behavioural experiments. In experiment 1, we used a four-colour pattern and a colour Mondrian, which is generally used to demonstrate colour

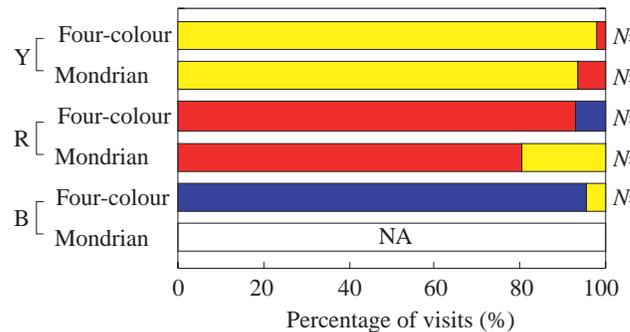


Fig. 4. Results after 7 days of training under white illumination. The percentage of total visits to each colour on the four-colour pattern (see Fig. 1C) and on the Mondrian is plotted on the abscissa. Blue-trained butterflies did not land on the Mondrian, and therefore no data were available (NA). N, number of visiting individuals. Each individual was allowed to make five visits. The total number of visits was, therefore, 5N (e.g. 5×9=45 for yellow- and blue-trained butterflies). B, blue-trained butterflies; R, red-trained butterflies; Y, yellow-trained butterflies,

constancy. In experiment 2, we used a two-colour pattern containing two similar colours for the critical test of colour constancy.

Experiment 1

Training. Training was carried out under white illumination. Newly emerged butterflies were kept in a plastic box without food for 1 day. On post-emergence day 2, we released a naive and hungry butterfly into the cage. We trained the butterfly for a few minutes to feed on a drop of 5% sucrose solution placed on a blue, yellow or red paper within the training pattern (Fig. 1A,B). During the training period, the butterflies landed and took off repeatedly, approximately 10 times on average. During training, only one butterfly was released into the cage at a time. We removed possible odour and visual cues left by previous animals in the cage by carefully wiping the anti-reflection glass with a wet cloth as frequently as possible.

We repeated the training once a day for 7 days. On the first 3 days of training, we presented training pattern 1 (Fig. 1A), which had only one patch of the training colour. On the following 4 days of training, we presented training pattern 2 (Fig. 1B), which had four juxtaposed rectangles of blue, green, yellow and red. In most training sessions, the butterfly landed on the correct training colour and fed on the sucrose solution immediately after being released into the cage, but in some cases it did not. When a butterfly did not land on the training colour by itself, we held its wings and extended its proboscis using a needle so that it fed on the sucrose solution on the correct training colour.

Testing. We tested trained butterflies by examining their foraging behaviour in the cage. We defined a 'visit' as a positive feeding response when a butterfly landed and extended its proboscis towards a colour patch on the floor.

The trained butterflies were tested on post-emergence day 9. We presented either a four-colour pattern (Fig. 1C) or a Mondrian (Fig. 2) under white illumination. For each test pattern, we let the butterfly visit colour patches five times and recorded the number of visits to each patch. The array of colours on the pattern was changed after the butterfly had visited three patches to avoid the effect of the butterfly learning the positions of the patches. After the tests, we fed the butterfly with 5% sucrose solution until it spontaneously recoiled its proboscis.

On post-emergence days 10, 11, 12 and 13, trained butterflies were tested on the four-colour pattern and on the Mondrian under different coloured illumination (Fig. 3B). On a single day, we tested the butterfly under only one colour of illumination. Different illumination colours were used on subsequent days.

The test was carried out as follows. Before presenting stimuli under coloured illumination, we confirmed that the butterfly could correctly select the trained colour with training pattern 1 (Fig. 1A) under white illumination. We then changed the colour of illumination. First, we presented the four-colour pattern (Fig. 1C) under weakly saturated illumination (e.g. Ya for yellow filter; Fig. 3B), and recorded the colours of patches visited in the first five visits. The pattern was then changed to the Mondrian, and we recorded

the colours of the first five visits. The tests were repeated under moderately and strongly saturated illumination (e.g. Yb and Yc; Fig. 3B). When the butterfly did not visit any colour patch for 5 min under coloured illumination, we presented the four-colour pattern again under white illumination. If the butterfly correctly selected the trained colour in this task, we concluded that the butterfly had not visited the correct patch in the previous task because of the coloured illumination and not because of loss of motivation.

Experiment 2

Training. On post-emergence day 2, we released a naive and hungry butterfly into the cage under white illumination. There, we trained the butterfly for a few minutes to feed on a drop of 5% sucrose solution on blue-green, emerald-green, orange or red paper placed within training pattern 1 (Fig. 1A) or the two-colour pattern (Fig. 1D). We prepared two types of two-colour pattern, one with the blue-green and emerald-green papers, and the other with orange and red papers.

We repeated the training once a day for 10 days. On the first 3 days of the training, we presented training pattern 1 (Fig. 1A). On the following 7 days, we presented the two-colour pattern (Fig. 1D). While presenting the two-colour pattern, sucrose solution was placed only on the training colour.

Testing. The design of the tests here was similar to that in experiment 1. Briefly, on post-emergence day 12, trained butterflies were tested on the two-colour pattern under white and coloured illumination. Butterflies trained to either blue-green or emerald-green were tested under both white and greenish illuminations. Butterflies trained to either orange or red were tested under both white and reddish illumination. We let the butterfly visit colour patches 10 times, and recorded the number of visits to each patch. After the fifth visit, the position of the colours was changed to avoid the butterfly learning the patch positions.

Calculation of the butterfly-specific colour

We calculated the effects of the illumination on the reflection spectra of the visual system of *Papilio xuthus*. The retina of *Papilio xuthus* contains at least five spectral types of photoreceptor, including ultraviolet and violet photoreceptors that are maximally sensitive to wavelengths shorter than 400 nm. However, the illumination used in this study contained hardly any light whose wavelength was shorter than 400 nm. We assumed, therefore, that only the blue, green and red receptors were functional in the experimental conditions, and constructed a presumptive three-dimensional colour space for *Papilio xuthus* accordingly. We calculated the colour loci of colour paper i under illumination x using the equations;

$$X = \int_{400}^{700} 1.00S_B(\lambda)I_x(\lambda)R_i(\lambda)d\lambda, \quad (1)$$

$$Y = \int_{400}^{700} 0.89S_G(\lambda)I_x(\lambda)R_i(\lambda)d\lambda, \quad (2)$$

$$Z = \int_{400}^{700} 0.29S_R(\lambda)I_x(\lambda)R_i(\lambda)d\lambda, \quad (3)$$

where $I_x(\lambda)$ is the spectral power distribution of illumination x , $R_i(\lambda)$ is the reflectance spectrum of colour paper i , $S(\lambda)$ is the spectral sensitivity of the blue (B), green (G) and red (R) receptors of *Papilio xuthus*, as determined by intracellular recording, and λ is the wavelength. The numbers represent the relative sensitivity calculated from the maxima at blue (460 nm), green (540 nm) and red (600 nm) in the sensitivity spectrum determined from an electroretinographic (ERG) recording (data not shown). With $x=X/(X+Y+Z)$, $y=Y/(X+Y+Z)$, $z=Z/(X+Y+Z)$, the coordinates for a two-dimensional plot of the colour triangle were obtained.

Results

Experiment 1

We trained butterflies to feed on one of the colour patches, yellow, red or blue, using training patterns 1 and 2 (Fig. 1A,B). The butterflies were then tested to select the training patch from a four-colour pattern (Fig. 1C) and a Mondrian (Fig. 2). Fig. 4 shows the results under white illumination. We tested whether trained butterflies selected the trained colour correctly. The yellow- and red-trained butterflies successfully selected the trained colours either from a four-colour pattern or from a Mondrian ($P < 0.001$, Mann–Whitney U -test). In preliminary experiments, where we tested butterflies after training them only with training pattern 1, the butterflies were unable to discriminate colours in a Mondrian. However, the butterflies mastered this ability after being trained with training pattern 2. The blue-trained butterflies visited blue from a four-colour pattern, but did not visit any colour patch in a Mondrian even after training with training pattern 2.

The results of the tests under four different colours of illumination (blue, green, yellow and red) are shown in Fig. 5. The left- and right-hand columns show the results using the four-colour pattern and the Mondrian, respectively. For each colour of illumination, the butterflies were tested under three different levels of saturation (a, b and c, see Fig. 3B).

The trained butterflies selected the correct patch under most conditions of illumination. Only strongly saturated coloured light sometimes prevented butterflies from selecting the correct patches. For example, under the strong greenish illumination, Gc, no butterfly landed on the floor, and most butterflies did not even take off.

Experiment 2

Butterflies trained to blue-green, emerald-green, orange or red were tested to see whether they could select the correct colour from the two-colour pattern under both white light and coloured illumination. The results are shown in Fig. 6. Under white illumination, all the trained butterflies correctly

discriminated the trained colour from another colour when presented in the two-colour pattern (Fig. 6). Under coloured illumination, fewer selections were correct, especially when the illumination light was strongly saturated. Only orange-trained butterflies could select the trained colour under strongly saturated illumination (Fig. 6D). Butterflies trained to emerald-green, blue-green or red became non-selective under these conditions. Emerald-green-trained butterflies were even non-selective under moderately saturated greenish illumination (Fig. 6A).

Discussion

Colour constancy

The present series of colour discrimination tests under white illumination reinforces our previous findings that foraging *Papilio xuthus* possess colour vision (Kinoshita et al., 1999). More importantly, the results under coloured illumination indicate that foraging *Papilio xuthus* have colour constancy.

The trained butterflies selected the correct colour even under coloured illumination (Figs 5, 6), which drastically changes the reflection spectra of the colour papers to which the butterflies had been trained. This phenomenon indicates that the reflection spectrum of the coloured patch, which stimulates the spectral receptors in the ommatidia viewing the patch, does not in itself directly determine colour recognition. The butterflies must somehow convert wavelength information at the retina into 'colour'.

Brightness

Because we already know that foraging *Papilio xuthus* do not use brightness as a cue when they select colour under white illumination (Kinoshita et al., 1999), we did not adjust the brightness of the colour papers in the present experiments. However, it remained possible that the butterflies used brightness as a cue under coloured illumination. Therefore, we calculated the butterfly-subjective brightness (B_{ix}) of colour paper i under illumination x using the equation:

$$B_{ix} = \int_{325}^{700} I_x(\lambda)R_i(\lambda)S(\lambda)d\lambda, \quad (4)$$

where $I_x(\lambda)$ is the spectral power distribution of illumination x , $R_i(\lambda)$ is the reflectance spectrum of colour paper i , $S(\lambda)$ is the spectral sensitivity of *Papilio xuthus* as determined by ERG recording, and λ is the wavelength (see also Kinoshita et al., 1999; Arikawa et al., 1987). Note that the ERG-determined value of $S(\lambda)$ may be quite different from $S(\lambda)$ determined from foraging behaviour (Neumeyer, 1980; von Helversen, 1972). However, we used the ERG-determined value of $S(\lambda)$ as a first approximation because a behaviourally determined value of $S(\lambda)$ for *Papilio xuthus* is not available. We then compared the brightness of the colour papers under different illumination to determine whether the selection behaviour of the butterflies was dependent on the brightness. Table 1 shows the order of

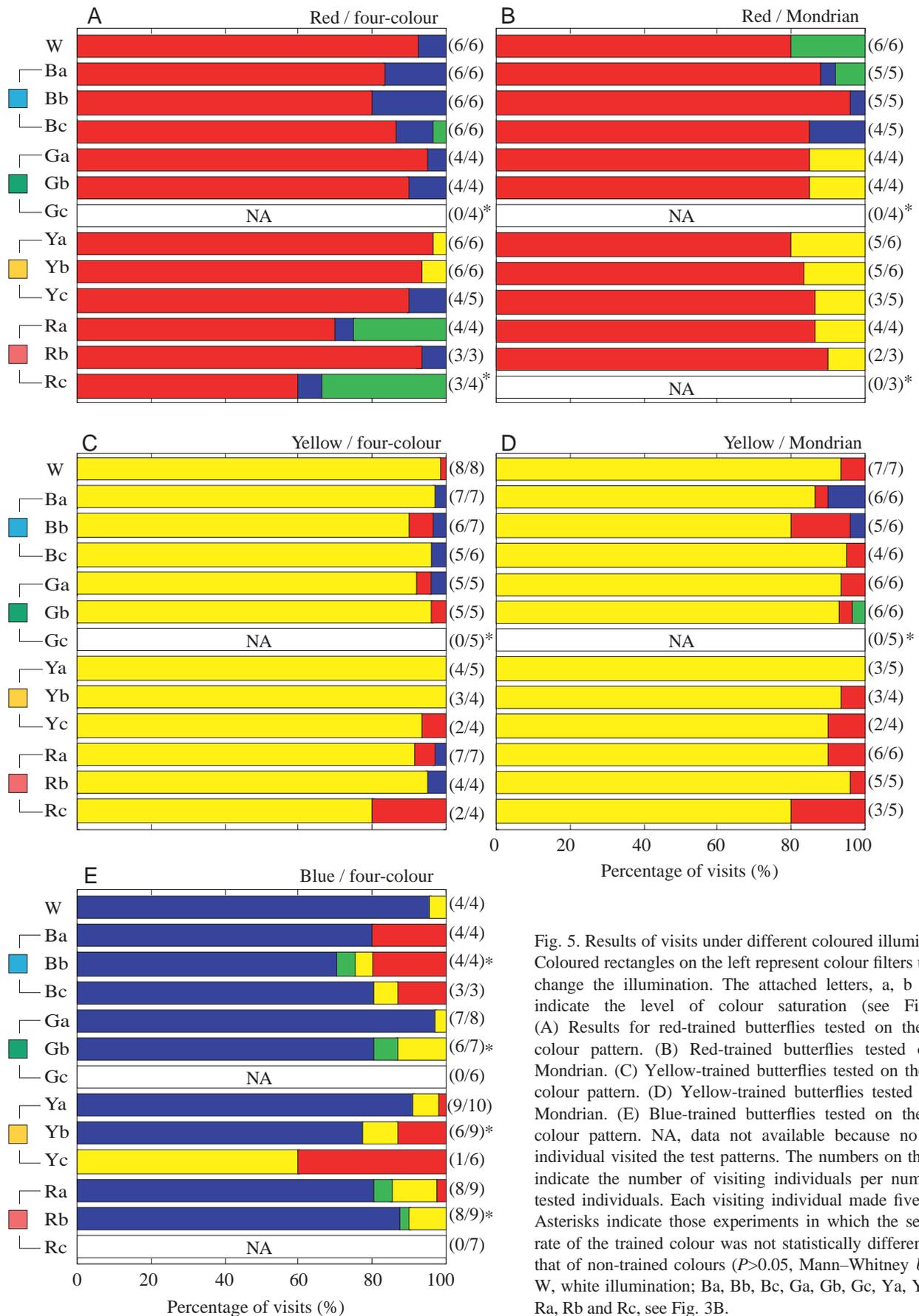
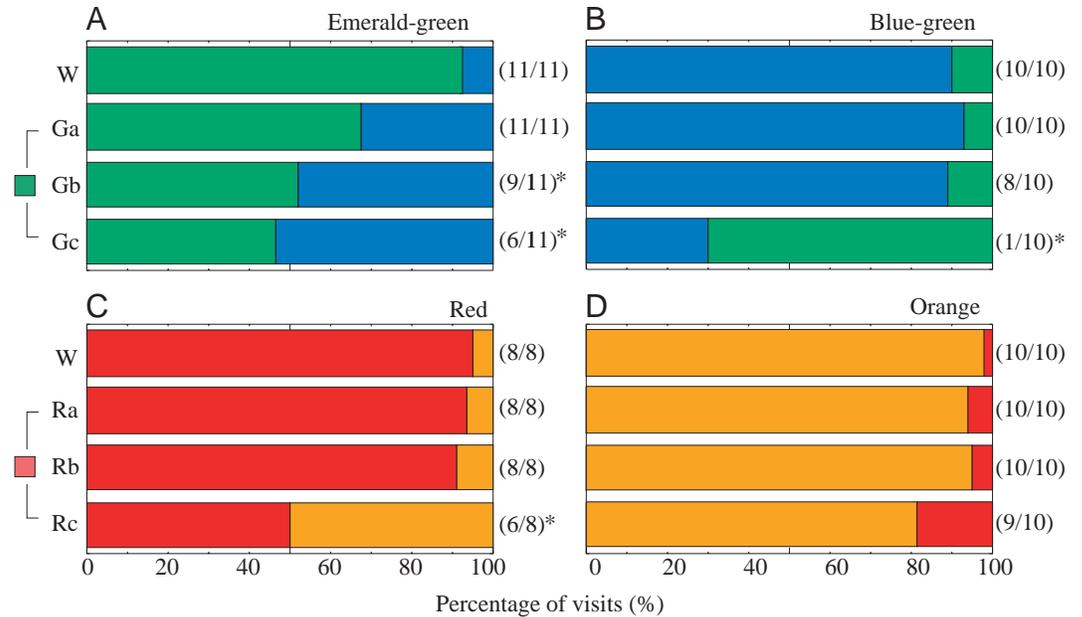


Fig. 5. Results of visits under different coloured illumination. Coloured rectangles on the left represent colour filters used to change the illumination. The attached letters, a, b and c, indicate the level of colour saturation (see Fig. 3B). (A) Results for red-trained butterflies tested on the four-colour pattern. (B) Red-trained butterflies tested on the Mondrian. (C) Yellow-trained butterflies tested on the four-colour pattern. (D) Yellow-trained butterflies tested on the Mondrian. (E) Blue-trained butterflies tested on the four-colour pattern. NA, data not available because no tested individual visited the test patterns. The numbers on the right indicate the number of visiting individuals per number of tested individuals. Each visiting individual made five visits. Asterisks indicate those experiments in which the selection rate of the trained colour was not statistically different from that of non-trained colours ($P > 0.05$, Mann-Whitney U -test). W, white illumination; Ba, Bb, Bc, Ga, Gb, Gc, Ya, Yb, Yc, Ra, Rb and Rc, see Fig. 3B.

Fig. 6. Results of experiment 2. The coloured rectangles on the left represent the colour filters used to change the illumination. (A) Results for emerald-green-trained butterflies on the two-colour pattern of blue-green and emerald-green under white light and greenish illumination. (B) Blue-green-trained butterflies tested under the same condition as for A. (C) Red-trained butterflies on the two-colour pattern of orange and red under white light and reddish illumination. (D) Orange-trained butterflies tested under the same conditions as for C. The numbers on the right indicate the number of visiting individuals per number of tested individuals. Each visiting individual made 10 visits. Asterisks indicate those experiments in which the selection rate of the trained colour was not statistically different from that of non-trained colours ($P > 0.05$, Mann-Whitney U -test). W, white illumination; Ga, Gb, Gc, Ra, Rb and Rc, see Fig. 3B.



the subjective brightness of colour papers (from the brightest, 1, to the dimmest, 6) under each illumination.

The order of brightness depends on the colour of illumination. For example, although the blue paper should appear dimmer to *Papilio xuthus* than the yellow paper under white illumination, it should appear brighter than the yellow paper under strong bluish illumination (Bc, Fig. 3B). The butterflies might have used the brightness as a cue but, because the yellow-trained butterflies correctly selected the yellow paper, the second brightest under the Bc illumination, this was apparently not the case. The same conclusion was drawn for other cases, such as for the green paper under greenish or yellowish illuminations and the red paper under reddish illuminations. All our results strongly indicate that the butterflies do not use the brightness difference when they make decisions. The order of the subjective brightness of the coloured papers used in experiment 2 did not change under any colour and saturation of illumination (data not shown).

Mondrian

Using a Mondrian under differently coloured illuminations is an approved method of demonstrating colour constancy (Land, 1977). In the present experiments, the trained butterflies could usually select the correct colour in the Mondrian as well as from the four-colour pattern. However, in preliminary experiments, we noticed that it was necessary to train the butterflies to select the correct colour from a specialized four-colour pattern in which four differently coloured rectangles were juxtaposed (training pattern 2, Fig. 1B) before they became able to land on a Mondrian. Otherwise, most butterflies did not land on the appropriate patch in the Mondrian: they

approached the correct colour and extended their proboscis, but did not land. The butterflies probably learned how to land on a specific portion of the Mondrian through the training process that used training pattern 2. We assume that is similar to the

Table 1. The *Papilio xuthus*-subjective intensity of illumination (P_x) and brightness (B_{ix}) of colour papers under these illuminations

Illumination	P_x	Ranking of B_{ix} of each colour paper					
		1	2	3	4	5	6
White	1.00	W	Y	B	G	R	Gr
Ba	1.69	W	Y	B	G	Gr	R
Bb	1.32	W	Y	B	G	Gr	R
Bc	0.89	W	B	Y	G	Gr	R
Ga	1.28	W	Y	G	B	Gr	R
Gb	0.88	W	Y	G	B	Gr	R
Gc	0.49	W	Y	G	B	Gr	R
Ya	2.03	W	Y	G	R	B	Gr
Yb	1.55	W	Y	G	R	B	Gr
Yc	1.20	W	Y	G	R	B	Gr
Ra	1.34	W	Y	R	B	G	Gr
Rb	0.89	W	Y	R	B	G	Gr
Rc	0.50	W	Y	R	Gr	B	G

For each illumination, the symbols for each colour paper (see Fig. 3A) are arranged along a row from the brightest (1) to the dimmest (6).

Training colours: W, white; Y, yellow; B, blue; G, green; R, red; Gr, grey.

P_x and B_{ix} were calculated using equations 5 and 4, respectively.

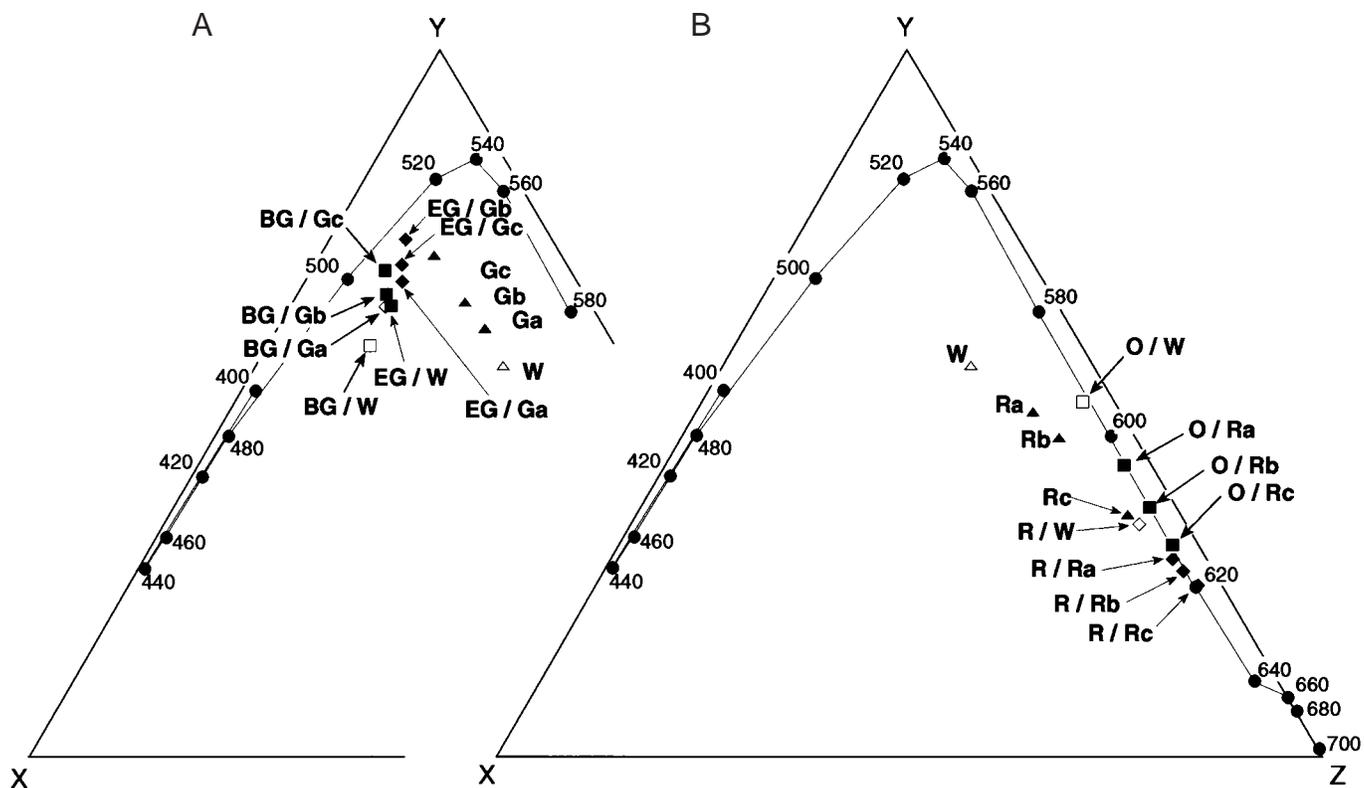


Fig. 7. The colour loci of colour papers under various types of coloured illumination on the presumptive three-dimensional colour space of *Papilio xuthus*. The outer lines indicate the loci of monochromatic light from 400 nm to 700 nm. (A) The colour loci of blue-green (BG) and emerald-green (EG) papers under white (W) and greenish illumination of different saturations (Ga, Gb and Gc). (B) The colour loci of orange (O) and red (R) papers under white (W) and reddish illumination of different saturations (Ra, Rb and Rc).

case of honeybee workers trained to recognize a pattern by motion parallax (Zhang and Srinivasan, 1994).

Training with pattern 2 was effective when the target patch was yellow or red but not when it was blue (Fig. 5). In fact, the blue-trained butterflies did not land on blue even when presented within training pattern 2. The butterflies only landed on the blue patch in training pattern 2 when the co-existing yellow patch, which was the brightest, was covered with a neutral density filter of 40% transmittance (data not shown). Apparently, landing on the blue patch within training pattern 2 was inhibited by brighter patches of other colours.

Similar colours

The reflection spectra of the blue, green, yellow and red papers used in experiment 1 were very different from each other, so it is possible that the trained butterflies simply selected a colour because it looked similar to the trained colour. We therefore conducted experiment 2, in which we used two similar colours whose reflection spectra can be identical under certain illumination conditions.

Fig. 7 shows the colour loci of the illuminations and the colour papers used in experiment 2. The loci of monochromatic lights are also shown. The loci of the blue-green paper under white illumination (BG/W) and the emerald-green paper under white illumination (EG/W) differ distinctly. However, the

locus of the blue-green paper under weakly saturated greenish illumination (BG/Ga) occupies almost the same locus as EG/W (Fig. 7A). Therefore, a butterfly trained on emerald-green under white light should select the blue-green paper under Ga illumination if there is no colour constancy. However, as shown in Fig. 6A, the butterflies correctly selected emerald-green, the trained colour, at a level of approximately 70%, indicating that they are colour-constant. In the reverse case, in which butterflies were trained on blue-green, the blue-green paper is always less green than the emerald-green paper irrespective of illumination. Therefore, the butterflies could perform as if they were colour-constant simply by selecting the most blue of two colours under all illuminations. Under Gc illumination, the blue-green paper probably became too greenish to be selected.

The same holds for the experiments using orange and red papers. The locus of the orange paper under moderately saturated reddish illumination (O/Rb) is very close to the locus of the red paper under white illumination (R/W) (Fig. 7B). Here, the decisive experiment is the one in which we trained butterflies on red paper. The red-trained butterflies selected not orange paper but red paper under Rb illumination: they were colour-constant (Fig. 6C).

Taken together, we conclude that foraging *Papilio xuthus* are, at least to some extent, colour-constant.

Limitation of colour constancy

We were unable to acquire behavioural data under some strongly saturated illuminations (Gc, Rc). Under these illuminations, only a few butterflies flew within the cage. Most settled on the cage net immediately after release. This did not appear to be due to a lack of motivation to fly because, when the illumination changed to white, they promptly started to fly in the cage in a normal manner. What factor inhibited flight under these strongly saturated colours of illumination? To determine whether the overall brightness of illumination is important to release foraging behaviour, we calculated the butterfly-subjective intensity of illumination x (P_x) using the equation:

$$P_x = \int_{325}^{700} I_x(\lambda)S(\lambda)d\lambda . \quad (5)$$

The P_x values were normalized by taking the white illumination (2700 lux, see Materials and methods) as 1.0 (Table 1). Some coloured illuminations were brighter than the white illumination, because of the additional unfiltered halogen lamps used to reduce the saturation of coloured illumination (see Materials and methods).

The P_x values of the strongly saturated greenish and reddish illuminations, Gc and Rc, were approximately 0.5, the smallest used in this study. The butterflies probably needed a certain intensity of illumination before they would fly and forage. In fact, they never flew under dim white illumination of approximately 200 lx. However, they did perform the task under white illumination of 1100 lx ($P_x=0.41$, data not shown), which was even dimmer than the Gc and Rc illuminations. These observations suggest that the overall intensity of illumination is an important factor, but that the spectral content of the illumination is crucial to performing the task. Under strongly saturated illumination, the butterfly's colour constancy system does not function properly.

Interestingly, orange-trained butterflies clearly discriminated the orange paper from the red paper under strongly saturated reddish illumination (Fig. 6D). However, red-trained butterflies could not select red under the same condition (Fig. 6C). Both groups of butterflies were, of course, subjected to identical visual stimuli. The only difference was in their training. The same was true for the emerald-green- and blue-green-trained butterflies under moderately saturated greenish illumination (Fig. 6A,B). This phenomenon indicates that colour discrimination under different illuminations is limited not only by the lighting conditions and some innate property of the visual system but also by the search image that the butterflies have learned through the training process.

Perspectives

Foraging *Papilio xuthus* have been shown to have colour constancy. As we discuss above, recognition of an object's colour does not depend simply on the wavelength information received by the ommatidia viewing the object. The information must be converted into a recognized 'colour'. For this

conversion, light received by surrounding ommatidia must be precisely analyzed. In *Papilio xuthus*, there are at least four types of secondary neuron (large monopolar neurons, L1–L4) in the first optic ganglion (lamina). The lamina of *Papilio xuthus* consists of units termed cartridges each derived from a single ommatidium. The dendrites of L1 and L3 are restricted to a single cartridge, whereas L2 and L4 send dendrites to at least seven neighbouring cartridges (Ribi, 1987). L2 and L4 could therefore enable wavelength information from surrounding ommatidia to be integrated. We recently showed that the retina of *Papilio xuthus* is a random mesh of three types of ommatidia, each having a different spectral set of photoreceptors (Arikawa et al., 1999a,b; Arikawa and Stavenga, 1997; Kitamoto et al., 1998). An electrophysiological study focusing on how wavelength signals in these different types of ommatidia are processed by L2 and L4 would be a good starting point to determine the neuronal mechanisms underlying the colour constancy demonstrated by this behavioural study.

As in most other insects that have been studied (Goldsmith, 1990), the retina of *Papilio xuthus* is furnished with ultraviolet-sensitive photoreceptors (Arikawa et al., 1987). In honeybees, ultraviolet is involved in colour vision (Menzel and Backhaus, 1989) and colour constancy (Werner et al., 1988). In the present study, we only investigated colour constancy for wavelengths longer than 400 nm. Whether colour constancy in *Papilio xuthus* also holds for wavelengths shorter than 400 nm has yet to be investigated.

We thank Dr D. G. Stavenga for critical reading of the manuscript. This work was supported by the JSPS Research Fellowship to M.K. and by Research Grants from the Ministry of Education, Science, and Culture of Japan to K.A.

References

- Arikawa, K., Inokuma, K. and Eguchi, E. (1987). Pentachromatic visual system in a butterfly. *Naturwissenschaften* **74**, 297–298.
- Arikawa, K., Mizuno, S., Scholten, D. G. W., Kinoshita, M., Seki, T., Kitamoto, J. and Stavenga, D. G. (1999a). An ultraviolet absorbing pigment causes a narrow-band violet receptor and a single-peaked green receptor in the eye of the butterfly *Papilio*. *Vision Res.* **39**, 1–8.
- Arikawa, K., Scholten, D. G. S., Kinoshita, M. and Stavenga, D. G. (1999b). Tuning of photoreceptor spectral sensitivities by red and yellow pigments in the butterfly *Papilio xuthus*. *Zool. Sci.* **16**, 17–24.
- Arikawa, K. and Stavenga, D. G. (1997). Random array of colour filters in the eyes of butterflies. *J. Exp. Biol.* **200**, 2501–2506.
- Goldsmith, T. H. (1990). Optimization, constraint and history in the evolution of eyes. *Q. Rev. Biol.* **65**, 281–322.
- Goulson, D. and Cory, J. S. (1993). Flower constancy and learning in foraging preferences of the green-veined white butterfly *Pieris napi*. *Ecol. Ent.* **18**, 315–320.
- Horridge, G. A., Marcelja, L. and Jahnke, R. (1984). Color vision in butterflies. I. Single colour experiments. *J. Comp. Physiol. A* **155**, 529–542.
- Kandori, I. and Ohsaki, N. (1996). The learning abilities of the white

- cabbage butterfly, *Pieris rapae*, foraging for flowers. *Res. Popul. Ecol.* **38**, 111–117.
- Kandori, I. and Ohsaki, N.** (1998). Effect of experience on foraging behavior towards artificial nectar guide in the cabbage butterfly, *Pieris rapae crucivora* (Lepidoptera: Pieridae). *Appl. Ent. Zool.* **33**, 35–42.
- Kelber, A. and Pfaff, M.** (1999). True colour vision in the orchard butterfly, *Papilio aegaeus*. *Naturwissenschaften* **86**, 221–224.
- Kinoshita, M., Shimada, N. and Arikawa, K.** (1999). Colour vision of the foraging swallowtail butterfly *Papilio xuthus*. *J. Exp. Biol.* **202**, 95–102.
- Kitamoto, J., Sakamoto, K., Ozaki, K., Mishina, Y. and Arikawa, K.** (1998). Two visual pigments in a single photoreceptor cell: identification and histological localization of three mRNAs encoding visual pigment opsins in the retina of the butterfly *Papilio xuthus*. *J. Exp. Biol.* **201**, 1255–1261.
- Land, E. H.** (1977). The retinex theory of color vision. *Scient. Am.* **237**, 108–128.
- Menzel, R. and Backhaus, W.** (1989). Color vision in honey bees: Phenomena and physiological mechanisms. In *Facets of Vision* (ed. D. G. Stavenga and R. C. Hardie), pp. 281–297. Berlin, Heidelberg, New York, London, Paris, Tokyo: Springer-Verlag.
- Neumeyer, C.** (1980). Simultaneous color contrast in the honeybee. *J. Comp. Physiol. A* **139**, 165–176.
- Neumeyer, C.** (1981). Chromatic adaptation in honeybee: successive color contrast and color constancy. *J. Comp. Physiol. A* **144**, 543–553.
- Ribi, W. A.** (1987). Anatomical identification of spectral receptor types in the retina and lamina of the Australian orchard butterfly, *Papilio aegaeus aegaeus* D. *Cell Tissue Res.* **247**, 393–407.
- Scherer, C. and Kolb, G.** (1987a). Behavioral experiments on the visual processing of color stimuli in *Pieris brassicae* L. (Lepidoptera). *J. Comp. Physiol. A* **160**, 645–656.
- Scherer, C. and Kolb, G.** (1987b). The influence of color stimuli on visually controlled behavior in *Aglais urticae* L. and *Pararge aegeria* L. (Lepidoptera). *J. Comp. Physiol. A* **161**, 891–898.
- von Helversen, O.** (1972). Zur spektralen Unterschiedsempfindlichkeit der Honigbiene. *J. Comp. Physiol.* **80**, 439–472.
- Weiss, M. R.** (1991). Floral colour changes as cues for pollinators. *Nature* **354**, 227–229.
- Weiss, M. R.** (1995). Associative colour learning in a nymphalid butterfly. *Ecol. Ent.* **20**, 298–301.
- Weiss, M. R.** (1997). Innate colour preferences and flexible colour learning in the pipivine swallowtail. *Anim. Behav.* **53**, 1043–1052.
- Werner, A., Menzel, R. and Wehrhahn, C.** (1988). Color constancy in the honeybee. *J. Neurosci.* **8**, 156–159.
- Zhang, S. W. and Srinivasan, M. V.** (1994). Prior experience enhances pattern discrimination in insect vision. *Nature* **368**, 330–332.